Supplemental Material for the Manuscript "Design and Synthesis of Orally Bioavailable Benzimidazole reverseamides as Raf Kinase Inhibitors"

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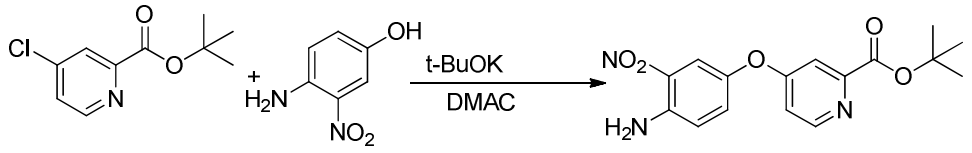
Contents

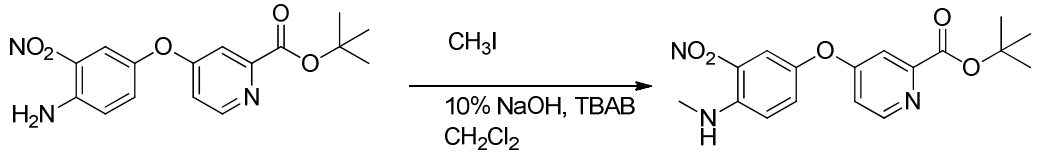
General Methods. All reagents and solvents were of commercial quality and used without further purification. Column chromatography was performed using merck silica gel 60 (230-400 mesh). The compounds and/or intermediates were characterized by high performance liquid chromatography (HPLC) using a Waters Millenium chromatography system with a 2695 Separation Module (Milford, MA). The analytical columns were reversed phase Phenomenex Luna C18 -5 μ, 4.6 x 50 mm, from Alltech (Deerfield, IL). A gradient elution was used (flow 2.5 mL/min), typically starting with 5% acetonitrile/95% water and progressing to 100% acetonitrile over a period of 10 minutes. All solvents contained 0.1% trifluoroacetic acid (TFA). Mass spectrometric analysis was performed according to two different liquid chromatography / mass spectroscopy (LCMS) methods. Method A employed a Waters System (Alliance HT HPLC and a Micromass ZQ mass spectrometer for the LCMS instrument, an Eclipse XDB-C18, 2.1 x 50 mm for the chromatography column, and a solvent system that was a 5-95% gradient of acetonitrile in water with 0.05% TFA over a 4 min period (flow rate 0.8 mL/min molecular weight range 200-1500; cone Voltage 20 V; column temperature 40°C). Method B employed a Hewlett Packard System (Series 1100 HPLC and a Micromass ZQ mass spectrometer for the LCMS instrument, an Eclipse XDB-C18, 2.1 x 50 mm for the chromatography column, and a solvent system that was a 5-95% gradient of acetonitrile in water with 0.05% TFA over a 4 min period (flow rate 0.8 mL/min molecular weight range 150-850; cone Voltage 50 V; column temperature 30^{o}C). All masses were reported as those of the

protonated parent ions. HR-MS data employed a Waters Synapt G2 QTof Mass spectrometry. Ionization mode: ESI positive, cone voltage: 25V, capillary voltage: 3.0 kV, desolvation temperature: 350°C, acquisition range: 100 to 2000 m/z, Scan time: 0.2s. Gas chromatography / mass spectroscopy (GCMS) analysis was performed on a Hewlett Packard instrument (HP6890 Series gas chromatograph with a Mass Selective Detector 5973; injector volume: 1 μL; initial column temperature: 50°C; final column temperature: 250°C; ramp time: 20 minutes; gas flow rate: 1 mL/min; column: 5% phenyl methyl siloxane, Model No. HP 190915-443, dimensions: 30.0 m x 25 m x 0.25 m).^{1}H and ^{13}C NMR spectra of all compounds were recorded at 300 and 75 MHz, respectively. ^{1}H shifts are referenced to the residual protonated solvent signal (δ 7.25 for $CDCl_3$), and ^{13}C shifts are referenced to the deuterated solvent signal (δ 77 for $CDCl_3$).



4 **3** **26**

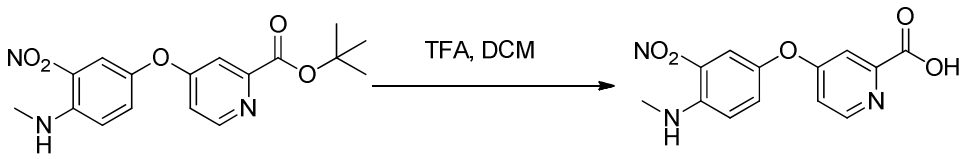
tert-butyl 4-(4-amino-3-nitrophenoxy)picolinate (26). A mixture containing 4-amino-3-nitrophenol **3** (36g, 250mmol) and potassium-t-butoxide (25g, 250mmol) was stirred in DMAc (500ml) for 2h at RT. To this mixture was added tert-butyl 4-chloropicolinate **4** (50g, 230mol) and potassium carbonate (30.77g, 230mmol) and stirred at 80°C overnight. The excess DMAc was removed by distillation in vacuum to give tert-butyl 4-(4-amino-3-nitrophenoxy)picolinate, **26,** which was continuosly, extracted with ethyl acetate for 48h using a soxlet extractor. The yellow solid obtained on concentration was recrystallized from ethyl acetate to give (25g, 75mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.5 (s, 9H), 7.0-7.6 (m, 6H), 7.8 (s, 1H), 8.5 (s, 1H); ^{13}CNMR ($CDCl_3$, 75MHz) δ 28 82 112 114 116 122 132 152 130 142 144 152 164 166; LC/MS m/z: 332 (MH^+).



27 **28**

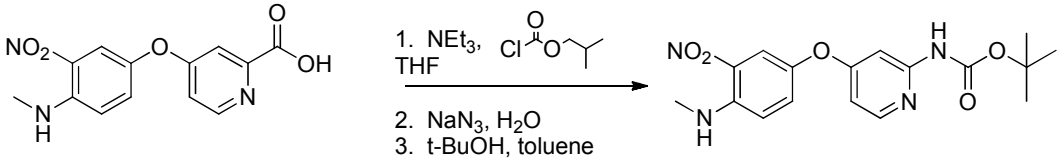
tert-butyl 4-(4-(methylamino)-3-nitrophenoxy)picolinate (28). To a stirred solution of tert-butyl 4-(4-amino-3-nitrophenoxy)picolinate **27** (10g, 7mmol), 10% aqueous sodium hydroxide and TBAB (2.5g, 7mmol) in dichloromethane was added methyl iodide (12.5g, 8mmol) and the mixture was stirred at RT for 8h. The reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with brine, and dried (Na_2SO_4) and concentrated. The

crude was purified by silica gel chromatography using 40% CH_2Cl_2 in hexane as the eluent to yield 5g of tert-butyl 4-(4-(methylamino)-3-nitrophenoxy)picolinate, **28** in 47% yield; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.6 (s,9H), 3.0 (d,3H), 7.0–8.6 (m, 6H), 8.2(s,1H); ^{13}C NMR ($CDCl_3$, 75 MHz*)*, δ 166, 164, 152, 144, 142, 132, 11, 116, 114, 112, 82, 30. 28; ; melting point 195°C; LC/MS m/z: 346.4 (MH^+)



28 **5**

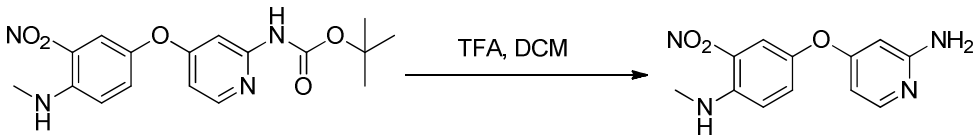
4-(4-(methylamino)-3-nitrophenoxy)pyridine-2-carboxylic acid (5). A solution of tert-butyl 4-(4-(methylamino)-3-nitrophenoxy)pyridine-2-carboxylate **28** (10g, 0.029mol) in methylene chloride (50ml) is treated with trifluoroacetic acid (20ml) and stirred at room temperature for 16 hours. The reaction is then concentrated and dried under vacuum for several hours to give 4-(4-(methylamino)-3-nitrophenoxy)pyridine-2-carboxylic acid **5**.



5 **6**

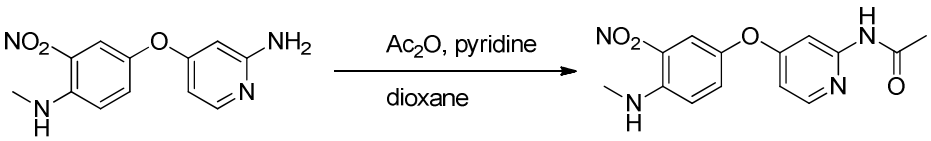
tert-butyl (4-(4-(methylamino)-3-nitrophenoxy)pyridin-2-yl)carbamate (6). Triethylamine (5 mL, 35.6 mmol) was added to a stirring suspension of acid **5** (3.34 g, 11.6 mmol) in dry THF (44 mL) at 0°C. The reaction was maintained at 0°C for 1 h, after which a solution of isobutylchloroformate (1.8 mL, 13.9 mmol) in dry THF (14 mL) was added dropwise. After 1 h at 0°C, a solution of sodium azide (2.28 g, 35.1 mmol) in water (8 mL) was added and the resulting reaction was maintained at 0°C for 45 min. The reaction solution was concentrated into an aqueous slurry and partitioned between saturated aqueous $NaHCO_3$ solution and CH_2Cl_2. The phases were separated and the aqueous portion was extracted with CH_2Cl_2 (3 X). The combined organics were washed with brine and the combined aqueous portions were further extracted with CH_2Cl_2. The combined organic phases were dried ($MgSO_4$) and concentrated to give 2.71 g (8.6 mmol, 75%) of an orange solid as crude acyl azide. A suspension of acyl azide (322 mg, 1.02 mol) and t-butanol (0.2 mL, 2.09 mmol) in dry toluene (12 mL) was heated to 100°C and maintained at that temperature for 1.5 h. The reaction was allowed to cool to RT and then partitioned between saturated aqueous Na_2CO_3 solution and CH_2Cl_2. The phases were separated and the aqueous portion was extracted with CH_2Cl_2 (3 X). The combined organic portions were

washed with saturated aqueous Na_2C0_3 solution (2 X) and brine, dried ($MgSO_4$), and adsorbed onto SiO_2. Purification by flash chromatography (9 : 1, 4: 1, 2: 1 hexanes-EtOAc) afforded 131 mg (0.36 mmol, 36%) of an orange solid as **6**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18 (br s, 1 H), 8.12 (d, *J*= 5.8 Hz, 1 H), 8.04 (br dd, 1 H), 7.95 (d, *J*= 2.8 Hz, 1 H), 7.53 (d, *J*= 2.2, 1 H), 7.29 (dd, *J*= 2.8,9.1 Hz, 1 H), 6.91 (d, *J*= 9.3 Hz, 1 H), 6.47 (dd, *J*= 2.5,5.8 Hz, 1 H), 3.07 (d, *J*= 5.2 Hz, 3 H), 1.49 (s, 9 H).



6 **7**

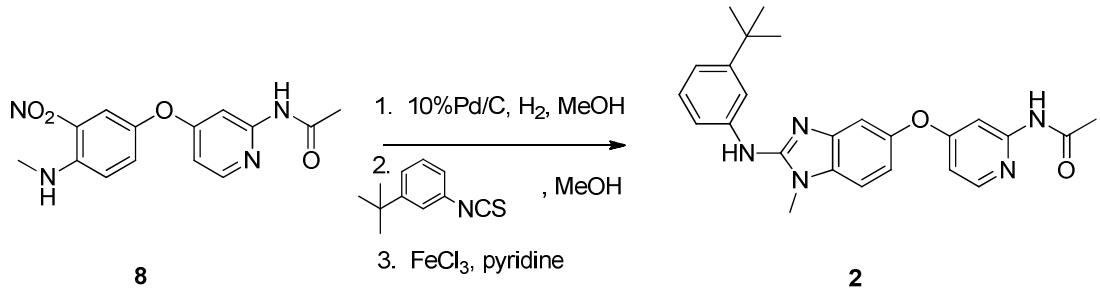
4-(4-(methylamino)-3-nitrophenoxy)pyridin-2-amine (7). Trifluoroacetic acid (4 mL) was added to a stirring suspension of BOC carbamate **6** (181 mg, 0.5 mmol) in CH_2Cl_2 (4 mL). The resulting reaction was maintained at RT for 3.5 hr and was then concentrated. The crude residue was suspended in saturated aqueous Na_2CO_3 and extracted with CH_2Cl_2 (3 X). The combined organic portions were concentrated and the resulting residue was absorbed onto SiO_2. Purification by flash chromatography (0.5 : 99.5, 0.75 : 99.25, 1 : 99, 2 : 98, 5 : 95 methanol-CH_2Cl_2) gave 94 mg (0.36 mmol, 72%) of a bright orange solid as **7**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (br d, *J*= 3.3 Hz, 1 H), 7.93 (d, *J*= 2.8 Hz, 1 H), 7.92 (d, *J*= 5.8 Hz, 1 H), 7.27 (dd, *J*=2.8, 9.4 Hz, 1 H), 6.89 (d, *J*= 9.3 Hz, 1 H), 6.24 (dd, *J*= 2.2,6.0 Hz, 1 H), 5.92 (d, *J*= 2.2 Hz, 1 H), 4.44 (br s, 2 H), 3.05 (d, *J*= 5.0 Hz, 3 H).



7 **8**

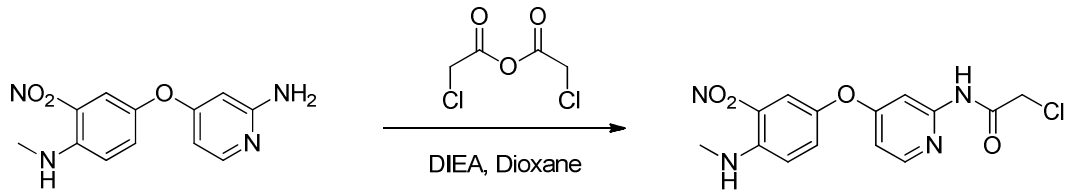
N-(4-(4-(methylamino)-3-nitrophenoxy)pyridin-2-yl)acetamide (8). Pyridine (0.08 mL, 0.99 nmol) and.acetic anhydride (0.04 mL, 0.42 mmol) was added to suspension of 2-aminopyridine **7** (94 mg, 0.36 nunol) in dry dioxane (1.7 mL). The resulting reaction mixture was heated to and maintained at 85°C for 2 h. The reaction was allowed to cool to RT and was then partitioned between EtOAc and saturated aqueous Na_2CO_3. The layers were separated and the aqueous layer was extracted with EtOAc (3 X). The combined organic portions were washed with brine, dried ($MgSO_4$),and absorbed onto SiO_2. Purification by flash chromatography (2: 1, 1:1, 1:2, 1:3 hexanes-EtOAc) provided 75 mg (0.25 mmol, 69 %) of an orange solid as **8:** ^{1}HNMR (300 MHz, $CDCl_3$) δ 8.35 (br s, 1 H), 8.10 (d, *J*= 5.8 Hz, 1 H), 8.05 (br d, *J*= 4.4 Hz, 1H), 7.95 (d, *J*= 2.8

Hz, 1 H), 7.76 (br d, *J*= 1.7 Hz 1 H), 7.30 (dd, *J*= 2.7,9.1 Hz, 1 H), 6.91 (d, *J*= 9.3 Hz, 1 H), 6.60 (dd, *J*= 2.5,5.8 Hz, 1 H), 3.05 (d, *J*= 5.2 Hz, 3 H), 2.16 (s,3 H).



8 **2**

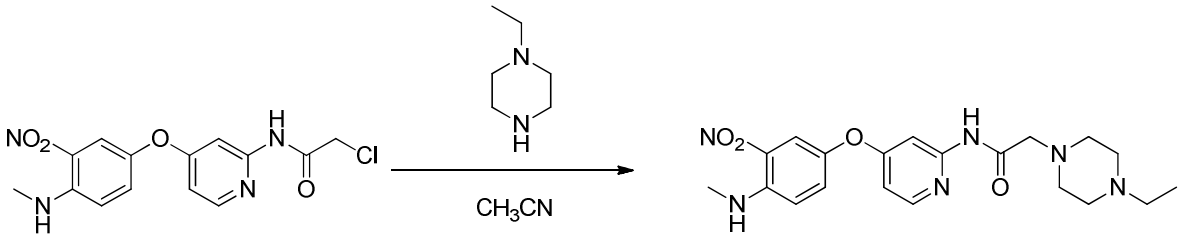
N-(4-((2-((3-(tert-butyl)phenyl)amino)-1-methyl-1H-benzo[d]imidazol-5-yl)oxy)pyridin-2-yl)acetamide (2). A suspension of acetamide **8** (75 mg, 0.25 mmol) and 10% Pd/C (30 mg, 0.03 mmol) in methanol (5 mL) was charged with H_2 and the resulting reaction mixture was maintained under a H_2 atmosphere for 1 hr at RT. The mixture was filtered and the remaining solids washed thoroughly with EtOAc and methanol. The combined organic portions were evaporated to afford 60 mg (0.22 mmol, 88%) of a brown residue as the phenelene diamine, which was carried forward without further purification. The above diamine (60 mg, 0.22 mmol) was dissolved in methanol (3 mL) and a solution of *3-tert-butyl* phenylthioisocyanate (62 mg, 0.32mmol) in methanol was added. The reaction was maintained for 16 hr. Pyridine (0.06 mL, 0.74 mmol) was added to the reaction, followed by ferric chloride (45 mg, 0.28 mmol). The resulting dark reaction mixture was maintained at RT for 16 hr. Reaction mixture was then suspended in saturated aqueous Na_2CO_3 solution and filtered thru Celite. The remaining solids were washed with EtOAc and the combined. The filtrate was partitioned and separated. The aqueous portion was extracted with EtOAc (3 X) and the combined organic portions were washed with brine, dried ($MgSO_4$), and evaporated. Purification by semi-prep HPLC gave **2** as the TFA salt which was neutralized with saturated aqueous Na_2CO_3 solution and extracted with EtOAc (3 X). The combined organic portions were washed with water and brine, dried ($MgSO_4$), and evaporated. The resulting residue was reconstituted as the mono citrate salt: LCMS *m/z* 430.3 (MH^+), t_R =2.24 min.



7 **29**

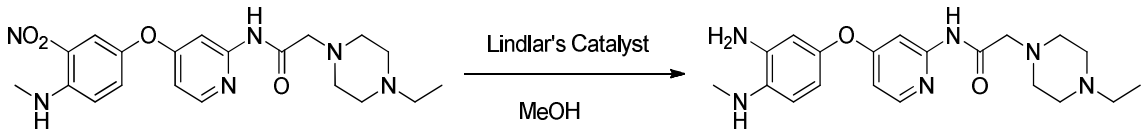
N -(4-(4-(methylamino)-3-nitrophenoxy)pyridin-2-yl)-2-chloroacetamide (29). A two liter round bottom flask was fitted with a mechanical stir bar, sealed, evacuated and flame dried. The

flask was allowed to cool to room temperature while purged with nitrogen. The flask was then charged with 4-(4-(methylamino)-3-nitrophenoxy)pyridin-2-amine **7** (40g, 0.154mol), diisopropylethylamine (26.9ml, 0.154mol), and dioxane (700ml). To this solution was added dropwise chloroacetic anhydride (39.5g, 0.230mol) in dioxane (110ml) over a 30 minute period. After the addition the resulting solution was allowed to stir at room temperature for 2 hours, at which time reaction became homogenous and was determined complete by LC/MS. Water 20 mL) were added to quench excess anhydride and the dioxane was evaporated. Water (800ml) and dichloromethane (800ml) were added and the layers were separated. The aqueous layer was extracted two times with dichloromethane. The organic layers were combined, washed with brine and dried over sodium sulfate. The solution was filtered and evaporated to give orange, solid crude product. To this solid was added isopropanol (400ml) which was heated to a boil, allowed to cool to room temperature and the solid collected via filtration to give the pure product N-(4-(4-(methylamino)-3-nitrophenoxy)pyridin-2-yl)-2-chloroacetamide **29** (48.5g, 91% yield). LCMS *m/z* 337 (MH^+), t_R =3.82 min.



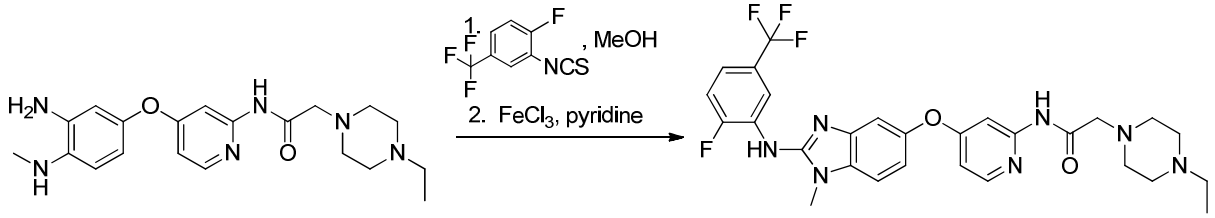
30 **31**

N-(4-(4-(methylamino)-3-nitrophenoxy)pyridin-2-yl)-2-(4-ethylpiperazin-1-yl)acetamide (31). N-ethylpiperazine, (97.5mL, 0.766) was added to a mixture of 2-chloro-N-{4-[4-(methylamino)-2-nitrophenoxy](2-pyridyl)}acetamide **30** (86.0g, 0.255mmol), and 1.0 L CH_3CN. The resulting mixture was brought to 60°C and stirred for 1 hr. The orange slurry was filtered at 50°C. The solid was washed with CH_3CN, slurried in CH_3CN (250 mL) and filtered, washed with CH_3CN. The solids were slurried in water (250 mL) and filtered, washed with water. The orange crystalline solid was dried under suction for 4 hrs and further dried in 40°C vacuum oven overnight. Recovered 81.0g (77% yield) orange crystalline solids of N-(4-(4-(methylamino)-3-nitrophenoxy)pyridin-2-yl)-2-(4-ethylpiperazin-1-yl)acetamide **31**. LCMS *m/z* 415 (MH^+), t_R =3.26 min.



31 32

N-(4-(3-amino-4-(methylamino)phenoxy)pyridin-2-yl)-2-(4-ethylpiperazin-1-yl)acetamide (32). A 1L round bottom flask was charged with 25g (0.0603 mole) of 2-(4-ethylpiperazinyl)-N-{4-[4-(methylamino)-3-nitro](2-pyridyl)}acetamide **31** and 500 mL of methanol. To it was then passed a stream of nitrogen gas while adding catalytic amounts of Lindlar's catalyst. The resulting mixture was then hydrogenated over night until disappearance of yellow color. The mixture was then filtered under vacuum to remove the catalyst. The filter paper was then washed with methanol until the solution filtering through was colorless. The filtrate was concentrated to afford N-{4-[3-amino-4-(methylamino) phenoxy](2-pyridyl)}-2-(4-ethylpiperazinyl)acetamide **32** as a brown oil. LCMS *m/z* 385 (MH^+), t_R =2.00 min.



32 25

N-(4-(2-(2-fluoro-5-(trifluoromethyl)phenylamino)-1-methyl-1H-benzo[d]imidazol-5-yloxy)pyridin-2-yl)-2-(4-ethylpiperazin-1-yl)acetamide (25). N-(4-(2-(2-fluoro-5-(trifluoromethyl)phenylamino)-1-methyl-1H-benzo[d]imidazol-5-yloxy)pyridin-2-yl)-2-(4-ethylpiperazin-1-yl)acetamide **32** was dissolved in MeOH (500 mL). To this solution was then added 13.35g (0.061 mole) of 2-fluoro-5-(trifluromethyl)benzeneisothiocyanate and the resulting mixture was stirred at ambient temperature for 4hr. The flask was then charged with 14.5g (0.09 mole) of anhydrous ferric chloride and the reaction mixture was stirred at ambient temperature for 24 hr. The reaction mixture was then concentrated. Saturated sodium carbonate solution was added to the concentrated mixture and solution was adjusted to pH=12-13. The aqueous solution was sonicated for 10 minutes and filtered under vacuum and the filtered residue was washed with ethyl acetate. The filtrate was then partitioned between ethyl acetate and water. The combined organic layer was washed with water, followed by saturated sodium chloride solution. It was then dried with potassium carbonate and the organic layer was filtered through a frit funnel. The ethyl acetate was concentrated on a rotary evaporator to a dry solid and vacuum dried to yield 28g of crude product. To 28g of the crude product was added of ethyl ether (450 mL) and of

ethyl acetate (150 mL) and the mixture was heated with a heat gun until the liquid boiled. The mixture was set-aside until it cooled down to ambient temperature. The mixture was then filtered and the solid was washed with ether until the filtrate was colorless. The solid was then dried under vacuum to yield 12.9g (37.3%) of N-(4-(2-(2-fluoro-5-(trifluoromethyl)phenylamino)-1-methyl-1H-benzo[d]imidazol-5-yloxy)pyridin-2-yl)-2-(4-ethylpiperazin-1-yl)acetamide **25.** LCMS *m/z* 572 (MH^+), t_R =3.40 min.

HRMS Analysis

Compound	Molecular Formula	Calculated Mass	Mass
2	$C_{25}H_{28}N_5O_2$	430.2243	430.2245
9	$C_{23}H_{24}N_5O_2$	402.1930	402.1933
10	$C_{24}H_{26}N_5O_2$	416.2087	416.2083
11	$C_{22}H_{19}N_5O_3F_3$	458.1440	458.1443
12	$C_{22}H_{19}N_5O_2F_3$	442.1491	442.1493
13	$C_{25}H_{28}N_5O_2$	430.2243	430.2246
14	$C_{23}H_{24}N_5O_2$	402.1930	402.1932
17	$C_{22}H_{19}N_5O_2F_3$	442.1491	442.1493
18	$C_{22}H_{18}N_5O_2F_4$	460.1397	460.1401
20	$C_{29}H_{35}N_6O_2$	499.2821	499.2822
22	$C_{32}H_{41}N_6O_2$	541.3291	541.3292
23	$C_{29}H_{31}N_6O_2F_4$	571.2445	571.2449
24	$C_{29}H_{32}N_7O_2F_4$	586.2554	586.2558
25	$C_{28}H_{30}N_7O_2F_4$	572.2397	572.2399

Ionization Method – Electrospray Positive Mode
Mass Detector Type – Q-Time of Flight Mass Spectrometry