Supporting Information

Novel 2-Substituted 7-Azaindole and 7-Azaindazole Analogs as Potential Anti-Viral agents for the Treatment of Influenza.

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General information

All commercially available reagents and anhydrous solvents were used without further purification. Purity assessment for final compounds was based on analytical HPLC: 4.6 x 50 mm Waters YMC Pro-C18 column, 5 μ m, 120 Å. Mobile phases are as follows: A, H_2O with 0.2% formic acid; B, CH_3CN with 0.2% formic acid; gradient, 10−90% B in A over 3 min at a flow rate of 1.5 mL/min. Unless specified otherwise, all compounds were ≥95% pure. Mass samples were analyzed on a Micro Mass ZQ, ZMD, Quattro LC, or Quatro II mass spectrometer operated in a single MS mode with electrospray ionization. Samples were introduced into the mass spectrometer using flow injection

(FIA) or chromatography. The mobile phase for all mass analysis consisted of CH_3CN −water mixtures with either 0.2% formic acid or ammonium formate. High-resolution mass spectra (HRMS) were collected by direct infusion on a Thermo QExactive. The samples were dissolved in MeOH at a concentration of approximately 0.2 mg/mL and infused with a flow rate of 5 μL/min. Electrospray ionization in positive ion mode was employed with a spray voltage of 4.0 kV. The mass resolution was set to 35,000. ^1H NMR spectra were recorded using either a Bruker Avance 400 (400 MHz) or a Bruker Avance II-300 (300 MHz) instrument. Preparative column chromatography was performed using Teledyne ISCO RediSep normal phase (35−70 μm) or RediSep Gold normal phase (25−40 μm) silica flash columns using a Teledyne ISCO Combiflash Companion or Combiflash Rf purification system. Preparative HPLC was performed on a Gilson HPLC system equipped a UV−VIS 156 Gilson detector. Separations were accomplished on an Agilent Zorbax SB-C18 column (21.2 x 100 mm) eluted with a linear gradient of CH_3CN in H_2O over 10 min (0.1% TFA) at a flow rate of 20 mL/min as indicated.

Abbreviations

t-Bu = *tert*-butyl

dppf = 1,1'-Bis(diphenylphosphino)ferrocene

DCM = dichloromethane

DPPA= diphenylphosphoryl azide

HLM = human liver microsomes

NIS = N-iodoscucinimide

PK = Pharmacokinetic

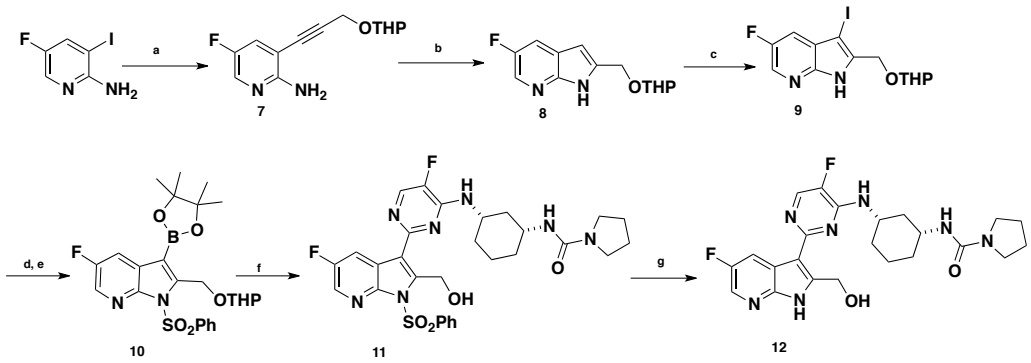
THP = tetrahydropyranyl acetal

Tr = trityl

X-phos = 2-Dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl

Synthesis of Target Compounds

Scheme 1. Synthesis of Compound 12



Reagents and conditions: (a) ≡—OTHP, $Pd(PPh_3)_2Cl_2$, CuI, THF, Et_3N, 80 ºC, 1 h, 82%; (b) *t*-BuOK, THF, 85 ºC, 2 h, 64%; (c) NIS, DMF, DCM, RT, 1 h, 99%;(d) i. NaH, THF, RT, 30 min; ii. $PhSO_2Cl$, 1 h, 97%; (e) bis(pinacolato)diboron, $PdCl_2(dppf)$·DCM, KOAc, DMF, 85 ºC, 3 h, 60%; (f) i. **14**, $Pd(PPh_3)_4$, THF, aq. Na_2CO_3, 80 ºC, 18 h; ii. TFA, MeOH, H_2O, 1 h, 80%; (g) 4N HCl, 1,4-dioxane, CH_3CN, 65 ºC, 1 h, 68%.

5-Fluoro-3-(3-((tetrahydro-2*H*-pyran-2-yl)oxy)prop-1-yn-1-yl)pyridin-2-amine (7)

A solution of 5-fluoro-3-iodo-pyridin-2-amine (2 g, 8.40 mmol) and 2-prop-2-ynoxytetrahydropyran (1.4 mL, 10 mmol) in THF (10 mL) and Et_3N (10 mL) was purged with nitrogen for 10 min. $Pd(PPh_3)_2Cl_2$ (0.3 g, 0.43 mmol) and CuI (10.16 g, 0.84 mmol) were added. The solution was heated at 80 ºC for 2 h and the reaction mixture was cooled to room temperature. The solvent was removed under reduced pressure and the resulting dark solution was dissolved in ethyl acetate (150 mL) and washed with water (200 mL). The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel chromatography (5-85% EtOAc/hexanes gradient) to afford compound **7** (1.7 g, 81%) as a brown oil: LCMS Rt = 2.76 min, m/z = 251 ($M+H^+$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.92 (d, *J* = 3.0 Hz, 1H),

7.32 (m, 1H), 4.95 (s, 2H), 4.89 (m, 1H), 4.54 (AB q, *J*= 18 Hz, 2H), 3.86-3.90 (m, 1H), 3.56-3.63 (m,1H), 1.57-1.91(m, 6H).

5-Fluoro-2-(((tetrahydro-2*H*-pyran-2-yl)oxy)methyl)-1*H*-pyrrolo[2,3-*b*]pyridine (8).

Potassium *tert*-butoxide (1.52 g, 13.6 mmol) was added to a stirred solution compound **7** (1.70 g, 6.79 mmol) in DMF (10 mL) and the solution was heated at 105 °C for 1 h. The reaction mixture was cooled to room temperature and poured into water (200 mL). The aqueous layer was extracted with ethyl acetate (3x50 mL). The organic layers were dried over Na_2SO_4 and concentrated to afford compound **8** as a pale brown solid (1.15 g, 68%): LCMS Rt = 2.80 min, m/z = 251.3 ($M+H^+$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.0 (s, 1H), 8.12 (s, 1H), 7.47-7.51(m, 1H), 6.29(s, 1H), 4.72 (AB q, *J* = 21 Hz, 2H), 4.63- 4.65(m, 1H), 3.86-3.90 (m, 1H), 3.55-3.48 (m, 1H), 1.46-1.81(m, 6H).

5-Fluoro-3-iodo-2-(((tetrahydro-2*H*-pyran-2-yl)oxy)methyl)-1*H*-pyrrolo[2,3-*b*]pyridine (9).

N-iodo succinimide (1.14 g, 5.05 mmol) was added to a stirred solution of compound **8** (1.15 g, 4.59 mmol) in DCM (25 mL) and the solution was stirred at room temperature for 1 h to form a precipitate. The solvent was removed under reduced pressure. The residue was diluted with water (100 mL). The aqueous layer was extracted with ethyl acetate (2x50 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure to afford compound **9** (1.35 g, 78%) as a brown solid: LCMS Rt = 3.37 min, m/z = 377 ($M+H^+$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.0 (s, 1 H), 8.22 (s, 1H), 7.42-7.46 (m, 1H), 4.89 (AB q, *J* = 15 Hz, 2H), 4.73- 4.76 (m, 1H), 3.97- 4.06 (m, 1H), 3.59-3.66 (m, 1H), 1.79-1.88 (m, 2H),1.61-1.63 (m, 4H).

5-Fluoro-1-(phenylsulfonyl)-2-(((tetrahydro-2*H*-pyran-2-yl)oxy)methyl)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (10).

Sodium hydride (0.18 g, 7.31 mmol) was added to a stirred solution of compound **9** (1.10 g, 2.92 mmol) in THF (30 mL) and the solution was stirred at room temperature for 20 min. Benzenesulfonyl chloride (0.47 mL, 3.65 mmol) was then added and the solution was stirred for 30 min. The solvent was removed under reduced pressure and residue was

diluted with water (20 mL) and the aqueous layer was extracted with EtOAc (3x25 mL). The organic layers were dried and concentrated. The crude product was purified by silica gel chromatography (5-40% gradient of EtOAc in hexane) to afford 5-fluoro-3-iodo-1-(phenylsulfonyl)-2-(((tetrahydro-2*H*-pyran-2-yl)oxy)methyl)-1*H*-pyrrolo[2,3-*b*]pyridine (1.16 g, 77%) as a white solid. A solution of 5-fluoro-3-iodo-1-(phenylsulfonyl)-2-(((tetrahydro-2*H*-pyran-2-yl)oxy)methyl)-1*H*-pyrrolo[2,3-*b*]pyridine (1.16 g, 2.24 mmol), potassium acetate (0.66 g, 6.74 mmol). bis(pinacol)diboron (0.09 g, 3.37 mmol) and Pd (dppf)Cl_2.DCM (0.18 g, 0.23 mmol) in dry DMF (10 mL) was purged nitrogen for 30 min and the reaction mixture was heated in a pressure vial (Q-tube) at 100 °C for 3 h. The reaction mixture was cooled to room temperature and the resulting dark solution was diluted withe water (20 mL). The aqueous phase was extracted with EtOAc (2x25 mL), the organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel chromatography (0-30% EtOAc/hexanes gradient) to afford compound **10** (0.56 g, 48%) as a white foam; LCMS Rt= 4.26 min, m/z = 517 ($M+H^+$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (d, *J* = 6.0 Hz, 2H), 7.49 (m, 1H), 7.46 (t, *J* = 6 Hz, 2H), 5.51(d, *J* =12 Hz, 1H), 5.17 (d, *J* = 12 Hz, 1H), 4.96 (s, 1H), 4.02-4.07(m, 1H), 3.59-3.63 (m, 1H), 1.54-1.85(m, 6H). 1.37 (s, 12H)

_N_-((1*R*,3*S*)-3-((2-(5-Fluoro-2-(hydroxymethyl)-1-(phenylsulfonyl)-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyrimidin-4-yl)amino)cyclohexyl)pyrrolidine-1-carboxamide (11)

A mixture of compound **14** (0.16 g, 0.47 mmol) and compound **10** (0.30 g, 0.58 mmol) dissolved in THF (5 mL) and Na_2CO_3 (0.7 mL of 2 M, 1.4 mmol) in a pressure vial (Q-tube) was purged with nitrogen for 30 min. $Pd(PPh_3)_4$ (0.054 g, 0.05 mmol) was then added and the solution was purged with nitrogen for 15 min. The mixture was heated at 85 °C for 5 h and the solvent was removed under reduced pressure. The crude product was purified by silica gel chromatography (0-100% gradient of EtOAc in hexane) to afford compound *N*-((1*R*,3*S*)-3-((2-(5-fluoro-1-(phenylsulfonyl)-2-(((tetrahydro-2*H*-pyran-2-yl)oxy)methyl)-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyrimidin-4-yl)amino)cyclohexyl)pyrrolidine-1-carboxamide (0.17 g, 48%) as a white foam which was dissolved in MeOH (5 mL). TFA (1 mL) was added and the solution was stirred at room temperature for 1 h. The solvent was removed under reduced pressure and the

crude product was dissolved in EtOAc (25 mL). The organic layer was washed with sat. $NaHCO_3$, dried over Na_2SO_4 and the solvent was removed under reduced pressure in to afford compound **11**: LCMS Rt= 3.18 min, m/z = 612 ($M+H^+$); ^{1}H NMR (300 MHz, MeOD) δ 8.51-8.43 (m, 1H), 8.31-8.24 (m, 3H), 8.15 (d, *J* = 3.9Hz, 1H), 7.67 -7.51 (m, 5H), 5.57 (ABq *J* = 15 Hz, 2H), 4.20 (t, *J* = 11.9 Hz, 1H), 3.71 (d, *J* = 11.6 Hz, 1H), 3.42 - 3.21 (m, 4H), 2.26 -2.30 (m, 1H), 2.08-2.12 (m, 1H), 2.00-1.80 (m, 6H), 1.56 -1.16 (m, 4H).

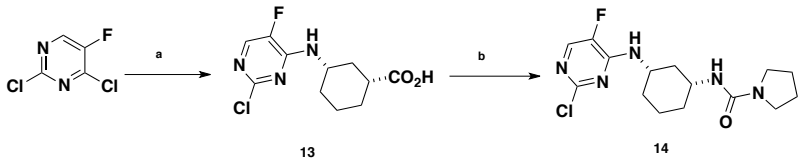
***N*-((1*R*,3*S*)-3-((5-Fluoro-2-(5-fluoro-2-(hydroxymethyl)-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyrimidin-4-yl)amino)cyclohexyl)pyrrolidine-1-carboxamide (12)**

HCl (1 mL of 4 M, 4.0 mmol) in 1,4-dioxane was added to compound **11** (0.45 g, 0.06 mmol) in CH_3CN (1 mL). The solution was heated at 65 °C for 2 h and the solvent was removed under reduced pressure. The crude product was purified by reverse phase chromatography (10-70% gradient of CH_3CN in H_2O (0.1%TFA) over 15 min) to afford compound **12** (24 mg, 74%) as a white solid: LCMS Rt= 2.06 min, m/z = 472 ($M+H^+$); ^{1}H NMR (300 MHz, MeOD) δ 8.55 (dd, *J* = 9.8, 2.7 Hz, 1H), 8.08 (m, 2H), 5.12 (m, 2H), 4.19 (m, 1H), 3.80 (m, 1H), 3.31(m,4H), 2.33 (m, 1H), 2.17 (m, 1H), 2.06 – 1.77 (m, 6H), 1.69 – 1.21 (m, 4H**);** HRMS: m/z calcd for $C_{23}H_{27}F_2N_7O_2$ $[M + H]^+$ 472.2267; found, 472.2273; [α] = -85.0 (C= 0.5, MeOH)

(*R*)-3-Fluoro-N-((1*R*,3*S*)-3-((5-fluoro-2-(5-fluoro-2-(hydroxymethyl)-1*H*-pyrrolo[2,3-b]pyridin-3-yl)pyrimidin-4-yl)amino)cyclohexyl)pyrrolidine-1-carboxamide (15). Compound **22** was synthesized adapting the procedure described for compound **12** by Suzuki coupling of boronate **10** with *N*-((1*R*,3*S*)-3-((2-chloro-5-fluoropyrimidin-4-yl)amino)cyclohexyl) (2)-(*R)* -pyrrolidine-1-carboxamide. LCMS Rt= 1.99 min, m/z = 490 ($M+H^+$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.09 (s, 1H), 8.11 (s, 1H), 8.05 (m, 1H), 7.86 (d, *J* = 3.5 Hz, 1H), 5.32 - 4.78 (m, 4H), 4.14 (d, *J* = 8.0 Hz, 1H), 3.84 (s, 1H), 3.74 - 3.18 (m, 6H), 2.67 (d, *J* = 11.7 Hz, 1H), 2.33 - 1.75 (m, 7H), 1.48 - 1.27 (m, 2H), 1.26 - 0.95 (m, 4H); HRMS: m/z calcd for $C_{23}H_{26}F_3N_7O_2$ $[M + H]^+$ 490.2172; found, 490.2178.

;[α] = -118.0 (C= 0.5, MeOH).

Scheme 2. Synthesis of compound 14



Reagents and conditions: (a) i. Ethyl (1*R*, 3*S*)-3-aminocyclohexanecarboxylate, THF, reflux, 18 h, 92%; ii LiOH, THF, water, 95° C, 1 h, 97%; (b) diphenyl phosphoryl azide (DPPA), Et_3N, pyrrolidine, Et_3N, THF, RT, 85° C, 2h, 85%.

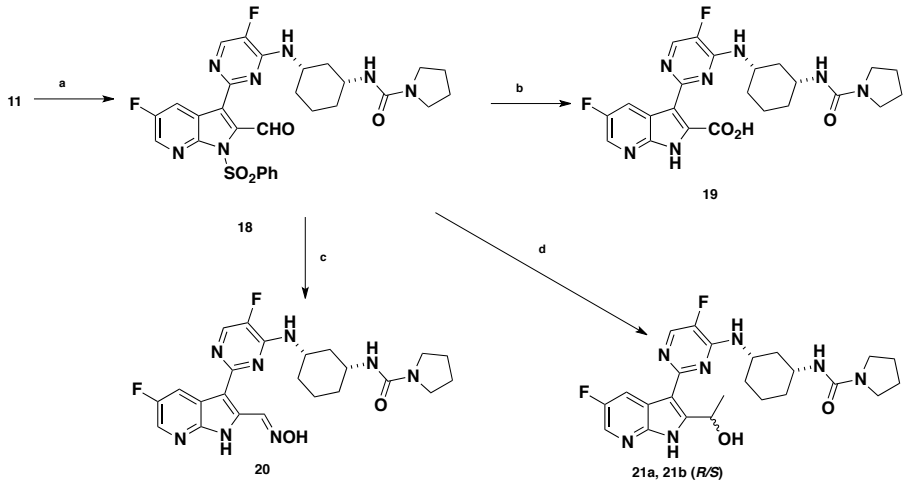
(1*R*,3*S*)-3-((2-Chloro-5-fluoropyrimidin-4-yl)amino)cyclohexane-1-carboxylic acid (13)

A solution of ethyl (1*R*,3*S*)-3-aminocyclohexanecarboxylate.HCl salt (5.1 g, 24.1 mmol), 2,4-dichloro-5-fluoro-pyrimidine (6.0 g, 36.1 mmol) and DIPEA (9.6 mL, 55.4 mmol) in THF (60 mL) was refluxed overnight and cooled to room temperature. The solvent was removed under reduced pressure and water (200 mL) was added. The aqueous phase was extracted with EtOAc (2x100 mL) and the combined organic layers were dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by silica gel chromatography (0-40% gradient of EtOAc in hexane) to afford ethyl (1*R*,3*S*)-3-[(2-chloro-5-fluoro-pyrimidin-4-yl)amino]cyclohexane carboxylate (6.7 g, 92%) as a white solid. LiOH (833 mg, 34.8 mmol) was added to a stirred solution of the resulting ester (3.5 g, 11.6 mmol) in THF (20 mL) and water (20 mL). The solution was heated at 95 °C for 1 h. The solvent was evaporated under reduced pressure and the aqueous layer was acidified with 6N HCl to form a white precipitate. The precipitate was dissolved in EtOAc (100 mL) and washed with water. Organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure to afford compound **13** (3.1 g, 97%) a white solid: LCMS Rt= 2.33 min, m/z = 274 ($M+H^+$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.91 (d, *J* = 2.7 Hz, 2H), 5.24 (d, *J* = 7.3 Hz, 2H), 4.19 – 4.03 (m, 3H), 3.84 – 3.68(m, 3H), 2.59 (ddd, *J* = 11.5, 8.2, 3.6 Hz, 2H), 2.38 (d, *J* = 12.4 Hz, 2H), 2.08 (d, *J* = 9.6 Hz, 6H), 1.99 -1.76 (m, 5H), 1.63 -1.34 (m, 6H), 1.32 - 1.15 (m, 4H).

***N*-((1*R*,3*S*)-3-((2-Chloro-5-fluoropyrimidin-4-yl)amino)cyclohexyl)pyrrolidine-1-carboxamide (14)**

A solution of compound **13** (3.1 g, 11.3 mmol), diphenylphosphoryl azide (DPPA) (3 mL, 17.0 mmol) and Et_3N (2.2 mL, 15.9 mmol) in THF (75 mL) was purged with nitrogen for 10 min and heated at 85 °C for 1.5 hr. Pyrrolidine (2.8 mL, 34.0 mmol) was added. Heating was continued for 30 min and the reaction mixture was cooled to room temperature. The solvent was removed under reduced pressure and water (100 mL) was added. The aqueous layer was extracted with EtOAc (3x100 mL). The combined organic extracts were dried and concentrated under reduced pressure to afford a white solid. The solid was suspended in ether (50 mL) and filtered and washed with ether (100 mL) to afford compound **14** (3.3 g, 85%) as a white solid: LCMS Rt= 2.21 min, m/z = 342 ($M+H^+$); ^1H NMR (300 MHz, CD_3OD) δ 7.85 (d, *J* = 6.0 Hz, 1H), 4.10-4.02 (m, 1H), 3.72-3.68 (m, 1H), 3.40-3.28 (m, 4H), 2.20-2.16 (m, 1H), 1.92-1.86 (m, 6H), 1.51-1.20 (m, 4H); [α] = -88.5 (C= 0.5, MeOH)

Scheme 3. Synthesis of compounds 19, 20 and 21



Reagents and conditions: (a) $(COCl)_2$, DMSO, Et_3N,-78 ºC, 2 h, 86%; (b) i. Na_2HPO_4, $NaClO_2$, 2-butene, *t*-BuOH, RT, 18 h , 88% ii. 4N HCl, 1,4-dioxane, CH_3CN, 65 ºC, 1 h, 68%; (c) i. NH_2OH, EtOH, reflux, 3 h; ii. 4N HCl, 1,4-dioxane, CH_3CN, 65 ºC, 1 h, 68%; (d) i. CH_3MgBr, THF, RT, 1 h; ii. 4N HCl, 1,4-dioxane, CH_3CN, 65 ºC, 1 h, 68%.

_N_-((1_R_,3_S_)-3-((5-Fluoro-2-(5-fluoro-2-formyl-1-(phenylsulfonyl)-1_H_-pyrrolo[2,3-_b_]pyridin-3-yl)pyrimidin-4-yl)amino)cyclohexyl)pyrrolidine-1-carboxamide (18)

DMSO (0.29 mL, 4.12 mmol) was added to a stirred solution of 2M oxalyl chloride in DCM (1.03 mL, 2.06 mmol) in DCM (10 mL) at -78 °C and the solution was stirred for 15 min. Compound **11** (0.84 g, 1.37 mmol) in DCM (10 mL) was added dropwise and the solution was stirred at -78 °C. for 1 hr. Triethylamine (1.15 mL, 8.24 mmol) was added slowly and the reaction mixture was warmed to room temperature (1.5 h). Water (50 mL) was added and the organic phase was separated. The aqueous layer was extracted with DCM (2x 25 mL), the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to afford compound **18** as a white solid. (640 mg): LCMS Rt= 3.5 min, m/z = 610 ($M+H^+$); ^1H NMR (300 MHz, $CDCl_3$) δ 10.77 (s, 1H), 8.58 (d, *J* = 2.9 Hz, 1H), 8.38-8.44 (m, 2H), 8.09 (d, *J* = 3.1 Hz, 1H), 7.46-7.72 (m, 8H), 4.06-4.10 (m, 2H), 3.92 – 3.72 (m, 1H), 3.32-3.36 (m, 4H), 2.50-2.54 (m, 1H), 2.23 - 2.04 (m, 2H), 1.92-1.96 (m, 4H), 1.33 -1.01 (m, 4H)

5-Fluoro-3-(5-fluoro-4-(((1_S_,3_R_)-3-(pyrrolidine-1 carboxamido)cyclohexyl)amino)pyrimidin-2-yl)-1_H_-pyrrolo[2,3-_b_]pyridine-2-carboxylic acid (19)

A solution of sodium chlorite (0.42 g, 4.60 mmol) and Na_2HPO_4 (0.65 g, 4.60 mmol) in water (0.2 mL) was added to a stirred solution of compound **18** (0.7 g, 1.15 mmol) in tertiary butanol (12.5 mL). 2-Methylbut-2-ene (1.9 mL, 17.8 mmol) was then added. The solution was stirred at room temperature for 18 h and concentrated under reduced pressure. Water (50 mL) was added and aqueous phase was extracted with EtOAc (3x50 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to afford carboxylic acid-sulfonate (0.77 g, 97%) as a yellow solid. A solution of HCl (g) in 1,4-dioxane (1 mL of 4 M, 4 mmol) was added to the carboxylic acid-sulfonate solid (0.065 g, 0.105 mmol) in CH_3CN (2 mL). The solution was heated at 65 °C for 1 hr, cooled to room temperature and concentrated under reduced pressure to give a brown solid. The solid was washed with EtOAc (25 mL) to remove contaminated sulfonyl chloride. The solid was dried in high vacuum to afford compound **19** as a HCl

salt (0.08 g, 73%): LCMS Rt= 2.03 min, m/z = 486 ($M+H^+$); ^{1}H NMR (300 MHz, DMSO) δ 8.30 - 8.05 (m, 1H), 7.60 (dd, *J* = 7.3, 2.4 Hz, 1H), 7.31 (dd, *J* = 5.0, 2.1 Hz, 1H), 4.09 (m, 1H), 3.57 (m, 1H), 3.17 (m, 4H), 1.99 (m, 2H), 1.77 (m, Hz, 4H), 1.56 - 1.10 (m, 3H); HRMS: m/z calcd for $C_{23}H_{25}F_2N_7O3$ [M + H^+] 486.2060; found, 486.2068.

_N_-((1_R_,3_S_)-3-((5-Fluoro-2-(5-fluoro-2-((_E_)-(hydroxyimino)methyl)-1_H_-pyrrolo[2,3-_b_]pyridin-3-yl)pyrimidin-4-yl)amino)cyclohexyl)pyrrolidine-1-carboxamide (20)

A mixture of compound **18** (0.050 g, 0.082 mmol), $NH_2OH.HCl$ (0.017 g, 0.24 mmol) and Et_3N (0.035 mL, 0.25 mmol) in ethanol (2 mL) was refluxed for 3 h and then cooled to room temperature. The solvent was removed under reduced pressure. The residue was dissolved in water (5 mL). The aqueous phase extracted with EtOAc (3x 10 mL), the combined organic extracts were dried over Na_2SO_4. The solvent was removed under reduced pressure to afford oxime-sulfonate (52 mg) as a white solid. A solution of HCl in 1,4-dioxane (1 mL of 4 M, 4.00 mmol) was added to the oxime-sulfonate (0.050 g) in CH_3CN (2 mL). The solution was heated at 65 ºC for 1 hr, cooled to room temperature and concentrated under reduced pressure to give a solid. The solid was washed with EtOAc (25 mL) to remove the sulfonyl chloride contaminant. The solid was dried under high vacuum to afford compound **20** as a HCl salt (0.012 g, 29%): LCMS Rt= 2.34 min, m/z = 485 ($M+H^+$); ^{1}H NMR (300 MHz, DMSO) δ 9.35 (s, 1H), 8.52 (d, *J* = 8.1 Hz, 1H), 8.28 (s, 1H), 8.21 (s,1H), 7.58 (brs, 1H), 5.82 (d, *J* = 7.8 Hz, 1H), 4.05 (brs, 1H), 3.57 (brs, 1H), 3.31 (brs, 4H),1.09-2.09 (brm, 2H), 1.76 (m, 6H), 1.46-1.52 (m, 2H), 1.15-1.30 (m, 2H); HRMS: m/z calcd for $C_{23}H_{26}F_2N_8O_2$ [M + H^+] 485.2219; found, 485.2211.

_N_-((1_R_,3_S_)-3-((5-Fluoro-2-(5-fluoro-2-(1-hydroxyethyl)-1_H_-pyrrolo[2,3-_b_]pyridin-3-yl)pyrimidin-4-yl)amino)cyclohexyl)pyrrolidine-1-carboxamide (21a, 21b)

Methyl magnesium bromide (0.49 mL of 1 M, 0.5 mmol) was added to stirred solution of compound **18** (0.1 g, 0.164 mmol) in THF (2 mL) at room temperature. The solution was stirred at room temperature for 1 h. Aqueous saturated NH_4Cl solution (5 mL) was added, followed by EtOAc (5 mL). The organic layer was separated, dried over Na_2SO_4 and concentrated to afford an inseparable diasteroemeric mixture of as a yellow solid.

HCl (1 mL of 4 M solution in 1,4-dioxane) was added to the alcohol-sulfonate (50 mg) in THF (2 mL) and water (2 mL). The solution was heated at 85 °C for 2 h and concentrated under reduced pressure. Water (5 mL) was added and the aqueous layer was extracted with EtOAc (3x5 mL). The combined organic layers were dried and the solvent was removed under reduced pressure. The crude product was purified by reverse phase preparative chromatography (gradient of 10-80% CH_3CN in H_2O (0.1% TFA) over 15 min). The diastereomeric alcohols were isolated in 1:1 ratio**:** diastereomer **21a**
LCMS Rt= 1.99 min, m/z = 486 ($M+H^+$), ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.33 (brs, 1H), 8.34 (d, *J* = 6 Hz, 1H), 8.22 (s, 1H), 8.03 (d, *J* = 3.4 Hz, 1H), 5.64 (q, *J* = 6.4 Hz, 1H), 5.01 (d, *J* = 4.8 Hz, 1H), 4.15 (d, *J* = 8.0 Hz,1H), 3.94-3.98 (m, 1H), 3.74 -3.77 (m, 1H), 3.37 (m, 4H), 2.61-2.65 (m, 1H), 2.20-2.24 (m,1H), 1.94-2.07 (m, 4H), 1.60 (d, *J* = 9 Hz, 3H), 1.12-1.27(m, 4H); HRMS: m/z calcd for $C_{24}H_{29}F_2N_7O_2$ [M + H^+] 486.2423; found, 486.2429.
diastereomer **21b**. LCMS Rt= 2.14 min, m/z = 486 ($M+H^+$); ^{1}HNMR (300 MHz, $CDCl_3$) δ 10.49 (brs, 1H), 8.35 (d, *J* = 7.7 Hz, 1H), 8.13 (s, 1H), 7.94 (d, *J* = 3.3 Hz, 1H), 5.89 (brs, 1H), 4.87 (d, *J* = 4.4 Hz, 1H), 4.05 (d, *J* = 7.8 Hz, 1H), 3.80-3.85 (m, 1H), 3.53-3.70 (m, 1H), 3.23 (m, 4H), 2.31 (t, *J* = 8.1 Hz, 1H), 2.08 – 1.67 (m, 5H), 1.62 (d, *J* = 6.4 Hz, 3H), 0.75-1.27 – 1.08 (m, 4H); HRMS: m/z calcd for $C_{24}H_{29}F_2N_7O_2$ [M + H^+] 486.2423; found, 486.2432.

***N*-((1*R*,3*S*)-3-((5-Fluoro-2-(5-fluoro-2-methyl-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyrimidin-4-yl)amino)cyclohexyl)pyrrolidine-1-carboxamide (16).**
Title compound **16** with methyl substitution at 2-position of the azaindole ring was synthesized adapting the procedure described for compound **13** starting from prop-1-yne as a Sonagashira coupling partner.

LCMS Rt= 2.67 min, m/z = 456 ($M+H^+$), ^{1}H NMR (300 MHz, CD_3OD) δ 8.46 (dd, *J* = 10.0, 2.8 Hz, 1H), 8.17 - 7.91 (m, 2H), 4.28 - 4.08 (m, 1H), 3.88 - 3.71 (m, 1H), 3.62 (d, *J* = 5.1 Hz, 3H), 3.46 - 3.32 (m, 4H), 2.87 (s, 3H), 2.32 (d, *J* = 11.9 Hz, 1H), 2.15 (d, *J* = 12.9 Hz, 1H), 2.03 - 1.85 (m, 2H), 1.67 - 1.20 (m, 4H), HRMS: m/z calcd for $C_{23}H_{27}F_2N_7O$ [M + H^+] 456.2318; found, 456.2324. HRMS: m/z calcd for $C_{23}H_{27}F_2N_7O$

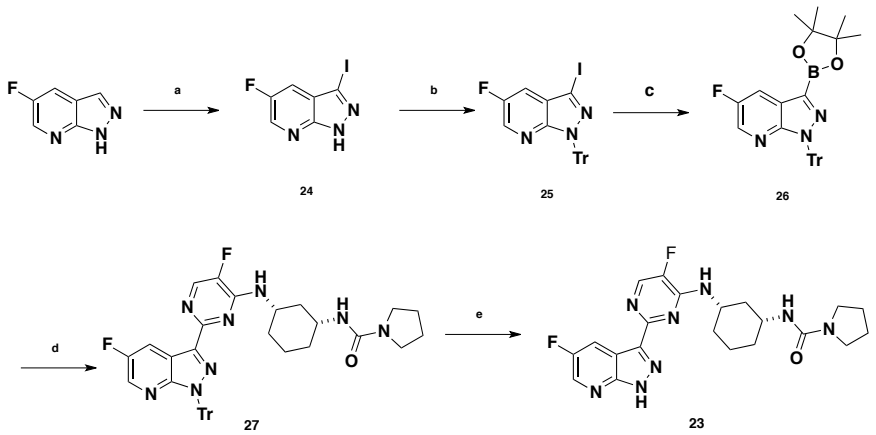
[M + H^+] 456.2318; found, 456.2324.

***N*-((1*R*,3*S*)-3-((2-(2-Cyclopropyl-5-fluoro-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-5-fluoropyrimidin-4-yl)amino)cyclohexyl)pyrrolidine-1-carboxamide (17).**

Title compound **17** with methyl substitution at 2-position of the azaindole ring was synthesized adapting the procedure described for compound **13** starting from ethynylcyclopropane as a Sonagashira coupling partner.

LCMS Rt= 2.69 min, m/z = 482($M+H^+$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.71 (s, 1H), 8.53 (d, *J* = 9.9 Hz, 1H), 8.20 (s, 1H), 8.11 (d, *J* = 2.9 Hz, 1H), 4.87(d, *J* = 6.9 Hz, 1H), 4.29 (d, *J* = 6.9 Hz, 1H), 4.14 (m, 1H), 3.82 (m, 4H), 3.34 (m, 4H), 2.56 (s, 1H), 2.30 (m, 1H), 2.05 (m, 1H), 1.91 (m, 1H), 1.81 - 1.42 (m, 2H), 1.41 - 0.87 (m, 7H); HRMS: m/z calcd for $C_{25}H_{29}F_2N_7O$ [M + H^+] 482.2472; found, 482.2479.

Scheme 4. **Synthesis of compound 23**



Reagents and conditions: (a) NIS, DCM, RT, 100 ºC, 2 h, 80%; (b) trityl chloride, K_2CO_3, DMF, RT, 18 h, 81%; (c) bis(pinacolato)diboron, $PdCl_2$(dppf).DCM, KOAc, DMF, 95 ºC, 1 h, 100%; (d) **14**, $Pd_2(dba)_3$, X-Phos, K_3PO_4, 2-methylTHF, H_2O,120 ºC, 2 h, 48%; (e) Et_3SiH, 30 mol-equiv TFA, DCM, RT, 30 min, 92%.

5-Fluoro-3-iodo-1*H*-pyrazolo[3,4-*b*]pyridine (24).

A solution of 5-fluoro-1*H*-pyrazolo[3,4-b]pyridine (1.95 g, 14.2 mmol) in dichloromethane (100 mL) was added to a solution of NIS (4.48 g, 19.91 mmol) in

dichloromethane (200 mL). The reaction suspension was stirred at 100 ^{0} C for 2 hr and the reaction mixture was washed with brine (100 mL). The solvent was removed under reduced pressure and the crude product was purified by silica gel chromatography (gradient 20-85% EtOAc in hexanes) to afford compound **24** (3.0 g, 81%) as a brown oil: LCMS Rt= 3.86 min, m/z = 685.1 ($M+H^+$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.56 (dd, *J* = 2.6, 1.8 Hz, 1H), 7.59 (dd, *J* = 7.4, 2.6 Hz, 1H).

5-Fluoro-3-iodo-1-trityl-1*H*-pyrazolo[3,4-*b*]pyridine (25)

A solution of compound **24** (3.0 g, 11.4 mmol) was added K_2CO_3(4.73 g, 34.23 mmol) and trityl chloride (3.8 g, 13.7 mmol) in DMF (16 mL) was stirred at room temperature for over night. The reaction mixture was diluted with EtOAc (100 mL) and brine (100 mL). The organic phase was separated and dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude product was purified by silica gel chromatography (gradient 10-85% EtOAc in hexanes) to afford compound **25** (4.7 g, 81%) as a brown oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (dd, *J* = 2.7, 1.5 Hz, 1H), 7.40 (dd, *J* = 7.5, 2.8 Hz, 1H), 7.26 (s, 13H**).**

5-Fluoro-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-trityl-1*H*-pyrazolo[3,4-*b*]pyridine (26).

A solution of compound **25** (4.60 g, 9.10 mmol), KOAc (2.68 g, 27.31 mmol) and bis (pinacolato)diboron (3.46 g, 13.65 mmol) in DMF (40 mL) was degassed with N_2 for 40 min. $Pd(dppf)_2.Cl_2.DCM$ (742 mg, 0.91 mmol) was added and the reaction mixture was heated at 95 ^{0} C for 75 min. The reaction mixture was filtered through celite pad and the filtrate was diluted with ether (50 mL) and brine (50 mL). The organic phase was dried over $MgSO_4$ and the solvent was removed under reduced pressure to give compound **25** (5.3 g). The material was used in the next step without further purification: LCMS Rt = 3.03 min, m/z = 528 ($M+H^+$).

***N*-((1*R*,3*S*)-3-((5-Fluoro-2-(5-fluoro-1-trityl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)pyrimidin-4-yl)amino)cyclohexyl)pyrrolidine-1-carboxamide (27).**

A solution of compound **26** (4.20 g, 8.31 mmol), **15** (2.37 g, 6.92 mmol), $Pd_2(dba)_4$ (0.16 mg, 0.17 mmol), K_3PO_4 (5.88 g, 27.70 mmol) and X-Phos (0.4 mg, 0.83 mmol) in 2 methyl THF (120 mL) and water (15 mL) was de gassed for 30 min. The reaction mixture was heated at 120 °C in a pressure tube for 1h. The aqueous phase was removed and the organic phase was filtered through a celite pad. The solvent was evaporated under reduced pressure and the crude product was purified by silica gel chromatography (0-20% MeOH/DCM gradient) to afford compound **27** (2.3 g, 48%): LCMS Rt= 3.86 min, m/z = 685.1 ($M+H^+$); ^1H NMR (300 MHz, CD_3OD) δ 8.45 (dd, *J* = 8.6, 2.9 Hz, 1H), 8.13 (d, *J* = 1.6 Hz, 1H), 8.04 (d, *J* = 3.9 Hz, 1H), 7.32 (dd, *J* = 8.0, 1.8 Hz, 4H), 7.27 – 7.17 (m, 6 H), 4.23 (s, 1H), 3.76 (s, 1H), 3.27 (s, 3H), 2.24 (s, 1H), 2.13 (s, 1H), 2.04 – 1.94 (m, 1H), 1.89 (t, *J* = 6.5 Hz, 4H), 1.46 (dt, *J* = 11.9, 9.5 Hz, 2H).

_N_-((1_R_,3_S_)-3-((5-Fluoro-2-(5-fluoro-1_H_-pyrazolo[3,4-_b_]pyridin-3-yl)pyrimidin-4-yl)amino)cyclohexyl)pyrrolidine-1-carboxamide (23).

Triethylsilane (8.1 mL, 50.4 mmol)) was added to a solution of compound **27** (2.30 g, 3.36 mmol) in DCM (115 mL) and followed by TFA (7.8 mL, 101 mmol) at room temperature and the reaction mixture was stirred at room temperature for 30 min. The reaction mixture was diluted DCM (100 mL) and saturated aqueous Na_2CO_3 (50 mL). The organic phase was separated and washed with brine and dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude product was purified by silica gel chromatography (gradient 0-20% MeOH in DCM) to afford compound **23** (0.85 g, 56%): LCMS Rt= 2.33 min, m/z = 443.6 ($M+H^+$); ^1H NMR (300 MHz, $CDCl_3$) δ 14.05 (s, 1 H), 8.57 - 8.33 (m, 1H), 7.97 (d, *J* = 3.2 Hz, 1H), 7.58 (t, *J* = 12.6 Hz, 1H), 5.38 (brs, 1H), 4.26 (d, *J* = 7.8 Hz, 1H), 3.90-3.92 (m, 1H), 3.40 (m, 3H), 3.22 (brs, 1H), 2.84-2.88 (m, 1H), 1.82-2.25 (m, 6H), 1.00-1.40 (m, 4H); HRMS: m/z calcd for $C_{21}H_{24}F_2N_8O$ [M + H^+] 443.2114; found, 443.2120.

_N_-((1_R_,3_S_)-3-((5-Fluoro-2-(5-fluoro-1_H_-pyrazolo[3,4-_b_]pyridin-3-yl)pyrimidin-4-yl)amino)cyclohexyl)morpholine-4-carboxamide (28).

The title compound **28**, was synthesized adapting the procedure described for compound

23 by Suzuki coupling of **26** with *N*-((1*R*,3*S*)-3-((2-chloro-5-fluoropyrimidin-4-yl)amino)cyclohexyl)morpholine-4-carboxamide.
LCMS Rt= 1.76 min, m/z = 459 ($M+H^+$); ^{1}H NMR (300 MHz, CD_3OD) δ 8.46 (s, H), 8.30 (dd, *J* = 2.7, 8.5 Hz, 1H), 8.04 (d, *J* = 3.8 Hz, 1H), 4.12-4.14 (m, 1H), 3.87 - 3.78 (m, 1H), 3.65 - 3.62 (m, 4H), 3.38 - 3.30 (m, 4H), 2.30-2.34 (m, 1H), 2.15-2.17 (m, 1H), 1.98 - 1.88 (m, 2H), 1.54 -1.21 (m, 4H); HRMS: m/z calcd for $C_{21}H_{24}F_2N_8O_2$ $[M + H]^+$ 459.2063; found, 459.2071.

(*R*)-3-Fluoro-*N*-((1*R*,3*S*)-3-((5-fluoro-2-(5-fluoro-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)pyrimidin-4-yl)amino)cyclohexyl)pyrrolidine-1-carboxamide (29).

The title compound **29,** was synthesized adapting the procedure described for compound **23** by Suzuki coupling of **26** with (*R*)-*N*-((1*R*,3*S*)-3-((2-chloro-5-fluoropyrimidin-4-yl)amino)cyclohexyl)-3-fluoropyrrolidine-1-carboxamide.
LCMS Rt= 1.87 min, m/z = 461 ($M+H^+$); ^{1}H NMR (300.0 MHz, CD_3OD) δ 8.46 (s, 1H), 8.34 (dd, *J* = 2.8, 8.5 Hz, 1H), 8.07 - 8.04 (m, 1H),4.15-4.25 (m,1H), 3.80-3.90 (m,1H), 3.30-3.70(m,6H), 1.90-2.40(m,5H), 1.25-1.60(m, 4H); HRMS: m/z calcd for $C_{21}H_{23}F_2N_8O$ $[M+H^+]\backslash$ 461.2019; found, 461.2013.

***N*-((1*R*,3*S*)-3-((5-Fluoro-2-(5-fluoro-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)pyrimidin-4-yl)amino)cyclohexyl)-1-methyl-1*H*-imidazole-4-carboxamide (30).**

The title compound **30** was synthesized adapting the procedure described for compound **23** by Suzuki coupling of **26** with *N*-((1*R*,3*S*)-3-((2-chloro-5-fluoropyrimidin-4-yl)amino)cyclohexyl)-1-methyl-1*H*-imidazole-4-carboxamide.
LCMS Rt= 1.61 min, m/z = 454 ($M+H^+$); ^{1}H NMR (400 MHz, MeOD) δ 8.86 (s, 1H), 8.68 – 8.601 (d, 1H), 8.41 (dd, *J* = 8.0, 2.6 Hz, 1H), 8.36 (d, *J* = 5.3 Hz, 1H), 8.06 (s, 1H), 4.50-4.56 (m, 1H), 4.12-4.18 (m, 1H), 3.96 (s, 3H), 2.51 (d, *J* = 11.9 Hz, 1H), 2.24 (d, *J* = 12.2 Hz, 1H), 2.06-2.13 m, 2H), 1.73 -1.44 (m, 4H); HRMS: m/z calcd for $C_{21}H_{21}F_2N_9O$ $[M + H^+]$ 454.1920; found, 454.1920.

_N_-((1_R_,3_S_)-3-((5-Fluoro-2-(5-fluoro-1_H_-pyrazolo[3,4-_b_]pyridin-3-yl)pyrimidin-4-yl)amino)cyclohexyl)thiophene-3-carboxamide (31).

The title compound **31**,was synthesized adapting the procedure described for compound **23** Suzuki coupling of **26** with _N_-((1_R_,3_S_)-3-((2-chloro-5-fluoropyrimidin-4-yl)amino)cyclohexyl)thiophene-3-carboxamide.
LCMS Rt= 2.07 min, m/z = 457 ($M+H^+$); ^{1}H NMR (400 MHz) δ 8.63 (s, 1H), 8.40 (dd, J = 7.9, 2.6 Hz, 1H), 8.29 (d, J = 4.5 Hz, 1H), 8.04 (dd, J = 2.9, 1.3 Hz, 1H), 7.49 (dd, J = 5.1, 1.3 Hz, 1H), 7.45 (dd, J = 5.1, 3.0 Hz, 1H), 4.53- 4.43 (m, 1H), 4.27 -4.00 (m, 1H), 2.47-2.49 (m, 1H), 2.22-2.25 (m, 1H), 2.06-2.10 (m, 2H), 1.20-1.70 (m, 4H); HRMS: m/z calcd for $C_{21}H_{19}F_2N_7OS$ [$M + H^+$] 456.1412; found, 456.1422.

(2_S_,3_S_)-2-(((1_R_,3_S_)-3-((5-Fluoro-2-(5-fluoro-1_H_-pyrazolo[3,4-_b_]pyridin-3-yl)pyrimidin-4-yl)amino)cyclohexyl)carbamoyl)bicyclo[3.2.1]octane-3-carboxylic acid (32).

The title compound **32**, was synthesized adapting the procedure described for compound **23** by Suzuki coupling of **26** with (2_S_,3_S_)-2-(((1_R_,3_S_)-3-((2-chloro-5-fluoropyrimidin-4-yl)amino)cyclohexyl)carbamoyl)bicyclo[3.2.1]octane-3-carboxylic acid.

LCMS Rt= 2.23 min, m/z = 401 ($M+H^+$); ^{1}H NMR (400 MHz, DMSO) δ 14.17 (s, 1H), 12.36 (s, 1H), 8.64 (s, 1H), 8.51 (d, J = 7.8 Hz, 1H), 8.29 (d, J = 3.5 Hz, 1H), 7.84 (d, J = 6.7 Hz, 1H), 4.75 (m, 1H), 2.89 (d, J = 6.5 Hz, 1H), 2.03 (s, 1H), 1.94 (s, 1H), 1.20-1.78 (m, 7H); HRMS: m/z calcd for $C_{19}H_{18}F_2N_6O_2$ [$M + H^+$] 401.1539; found, 401.1532.

Experimentals for described assays.

Materials Used Madin-Darby Canine Kidney (MDCK, CCL-34) cells obtained from American Type Culture Collection (ATCC) were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 2 mM L-glutamine, 1,000U/ml penicillin, 1,000 ug/mL streptomycin, 10 mM HEPES, and 10% fetal bovine medium. Antiviral assays were performed in supplemented DMEM with no FBS. Influenza virus strain A/PR/8/34 (tissue culture adapted) was obtained from ATCC (VR-1469). Low-passage virus stocks were prepared in MDCK cells using standard methods (WHO Manual on Animal Influenza Diagnosis and Surveillance, 2002).

In vitro **antiviral assays.** Details are presented in the SI appendix. MDCK cell protection and cytotoxicity assays were performed using a modification of standard methods, essentially the 3-day CPE-based approach employing an ATP cell viability endpoint. Replication of influenza virus RNA, either positive-strand or negative-strand polarity, was measured using strand-specific probes using the bDNA Quantigene method (Affymetrix), as previously described. Influenza virus sensitivity to the neuraminidase inhibitors oseltamivir carboxylate and zanamivir was determined by the chemiluminescent neuraminidase inhibitor assay using the NA-XTD kit (Applied Biosystems; Foster City, CA) per the manufacture's recommendations.

Influenza Antiviral Assay: bDNA assay

A cell-based antiviral assay was developed that depends on the multiplication of virus-specific RNA molecules in the infected cells, with negative strand RNA levels being directly measured using the branched-chain DNA (bDNA), hybridization method (Wagaman et al, J. Virol Meth, 105:105-114, 2002). Cells were initially infected at an MOI of 0.2 in 96-well microtiter plates and incubated in the presence of test compound for approximately 20 hours. Viral replication was quantified by determination of negative

strand HA RNA levels by bDNA assay. Dose response curves were analyzed using 4-parameter curve fitting methods. The concentration of test compound resulting in viral RNA levels equal to that of 50% of the control wells were reported as EC_{50}.

Human Cytosol stability assay

The incubations are carried out in triplicate with 1 mg/mL cytosol protein in 100 mM at pH 7.4 PBS, a final concentration of 1 mM of test compounds with and without 10 mM Raloxifene as an inhibitor. The experiment is carried out over 4 hours in a 37 ºC water bath, with time points taken at 0, 30, 60, 120 & 240 minutes. Each 50 mL aliquot is quenched in 200 mL acetonitrile containing 0.05 mg/mL internal standard (N-(1H-indazol-3-yl)-2-[2-(trifluoromethyl)phenyl]quinazolin-4-amine), centrifuged at the end of the experiment, and the resulting supernatant is analyzed by MS/MS.

X-Ray Crystal Structures

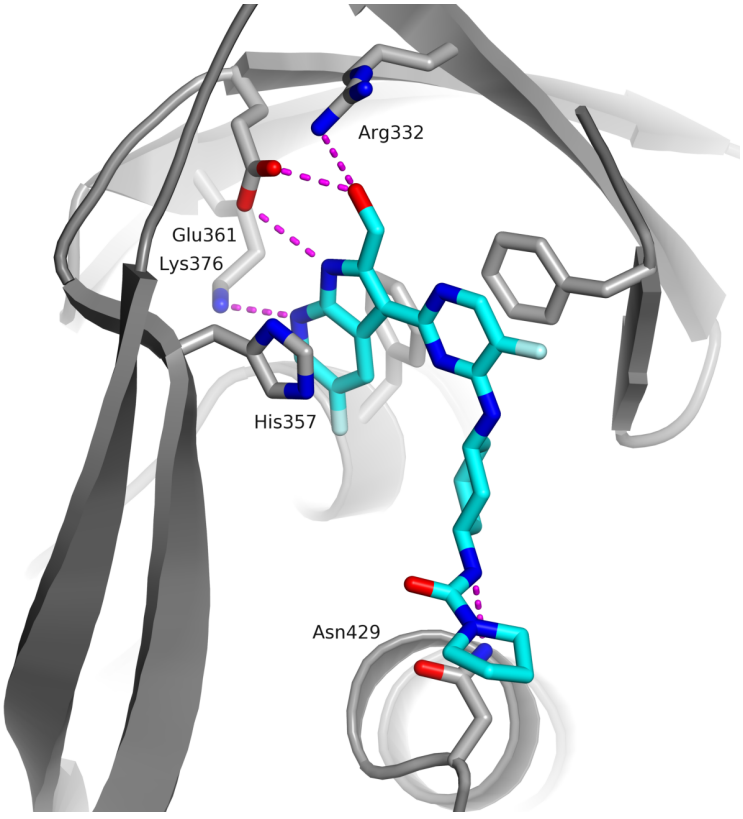


Figure 3. X-ray crystal structure of **12** bound to PB2 (PDB ID: 5BUH)

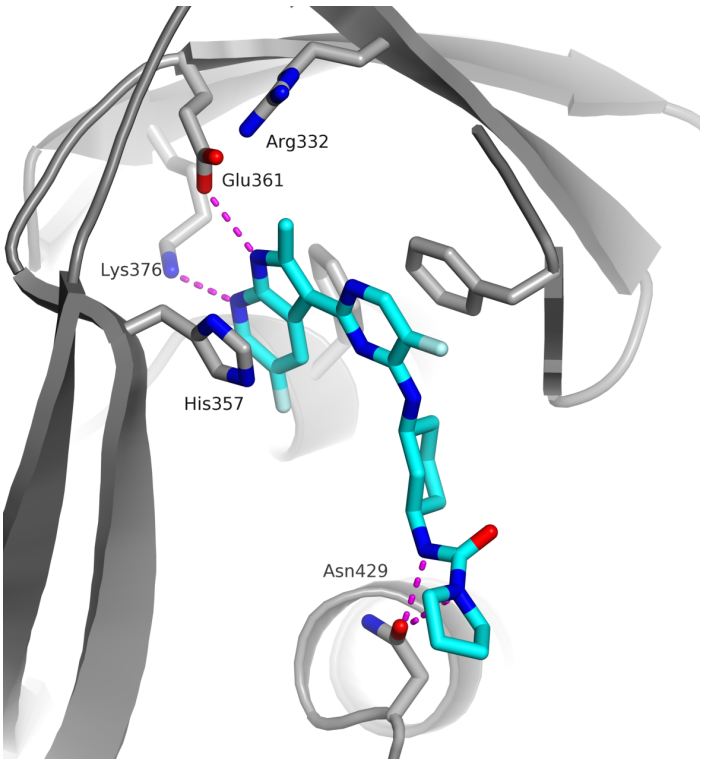


Figure 4. X-ray crystal structure of **16** bound to PB2 (PBD ID: 5F79)

Crystallization and X-ray Analysis

Crystal of the PB2 cap-binding domain (residues R318-M483) were grown by the vapor diffusion method at approximately 20 ÅãC. A mixture of 1 µL protein solution (2.8 mg/ml protein, 50 mM Tris buffer pH 8, 200 mM sodium chloride, 2 mM dithiothreitol, 1 mM anthraquinone-2,6-disulfonic acid disodium salt, 7.5 mM GTP) and 0.4 µL well solution (approximately 1.5 M sodium formate, 100 mM sodium citrate buffer pH 4.7, 10 mM dithiothreitol) was suspended over 1 mL of well solution. The crystals were transferred to a soaking solution (3.25 M sodium formate, 100 mM sodium citrate buffer pH 4.7) containing 1 mM compound **12**. Crystals were incubated approximately 15 hours at room temperature, and then transferred to a cryo-preservative solution (soaking solution with 25 % v/v glycerol) prior to freezing in liquid nitrogen. X-ray data were collected at the Advanced Light Source (beamline 5.0.1) at Berkeley. National Laboratories on an ADSC Q210 CCD detector. The intensities were integrated and scaled using auto PROC (Global Phasing Inc, Cambridge, UK). The protein model was built using COOT (CCP4) and refined using Refmac5 (CCP4) and auto BUSTER (Global

Phasing Inc, Cambridge, UK).