Supporting Information

Imidazopyridazine Hepatitis C Virus Polymerase Inhibitors. Structure-Activity Relationship Studies and the Discovery of a Novel, Traceless Prodrug Mechanism

Martin Leivers, John F. Miller, Stephanie A. Chan, Ryan Lauchli, Sebastian Liehr, Wenyan Mo, Tony Ton, Elizabeth M. Turner, Michael Youngman, J. Greg Falls, Susan Long, Amanda Mathis and Jill Walker*

GlaxoSmithKline Research & Development, 5 Moore Drive, Research Triangle Park, NC 27709, USA

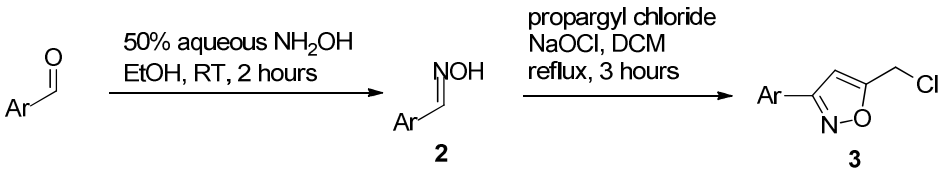
Contents

1. Experimental Details for Compound Syntheses

All commercially obtained solvents and reagents were used as received. 1H NMR spectra were taken on a Varian (Agilent) Inova 400 NMR spectrometer. Chemical shifts are reported in parts per million (ppm, δ) using the residual solvent line as a reference. Splitting patterns are designated using the following abbreviations: s, singlet; d, doublet; t, triplet; dd, doublet of doublet; m, multiplet; br, broad. Coupling constants (J) are reported in Hertz (Hz). Mass spectrometric analyses and compound purity determinations were conducted on a Waters Acquity UPLC system (Phenomenex Kinetex column at 40 °C, mobile phase of water with 0.2% v/v formic acid and acetonitrile with 0.15% v/v formic acid) and Waters Acquity SQD with alternating positive/negative electrospray ionization scanning from 125 to 1000 amu, with a scan time of 105 ms and an interscan delay of 20 ms. All final target compounds were found to have purities of ≥95%. High resolution mass spectrometric analysis was

performed on a Waters qTOF Premiere mass spectrometer using flow injection operating in W mode. Reverse phase HPLC purifications were performed on an Agilent 1100 preparative system.

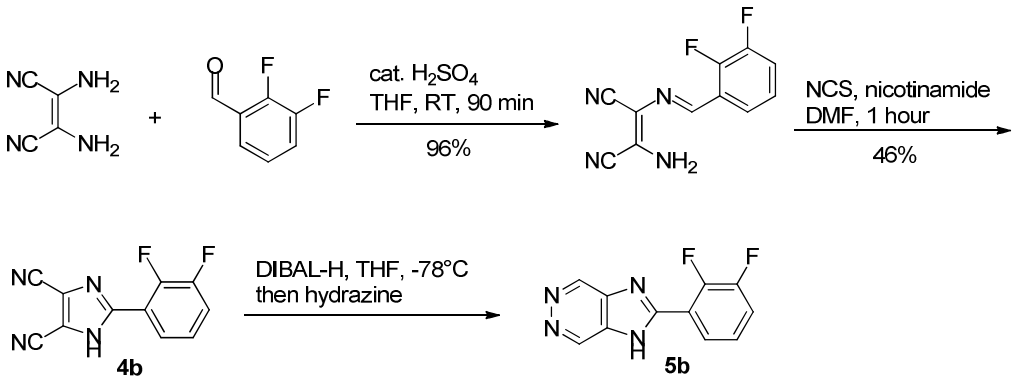
General Procedure for the Synthesis of 3-aryl-5-(chloromethyl)isoxazole Intermediates (3) .



*(A) Arylaldehyde oxime (**2**).* A solution of the aldehyde starting material in EtOH (1.3 M) was treated with 50% aqueous hydroxylamine (2.3 equiv). After stirring at RT for 2 hours, the solution was concentrated to dryness at reduced pressure to give the arylaldehyde oxime (**2**) which was used in the next step without purification.

*(B) 3-Aryl-5-(chloromethyl)isoxazole (**3**).* A solution of the arylaldehyde oxime in DCM (1.0 M) was cooled to 0 °C and treated with propargyl chloride (1.0 equiv). The solution was then treated with 6.5% aqueous NaOCl (2.0 equiv) by dropwise addition. After stirring at 0 °C for 15 minutes, the solution was heated to reflux for 3 hours. After cooling to RT, the mixture was partitioned between DCM and water and the phases separated. The aqueous phase was extracted with DCM (3x). The combined DCM extracts were washed with saturated brine, dried over $MgSO_4$, and concentrated to dryness at reduced pressure to afford the crude 3-aryl-5-(chloromethyl)isoxazole (**3**) which was used without purification.

2-(2,3-Difluorophenyl)-5H-imidazo[4,5-d]pyridazine (5b).

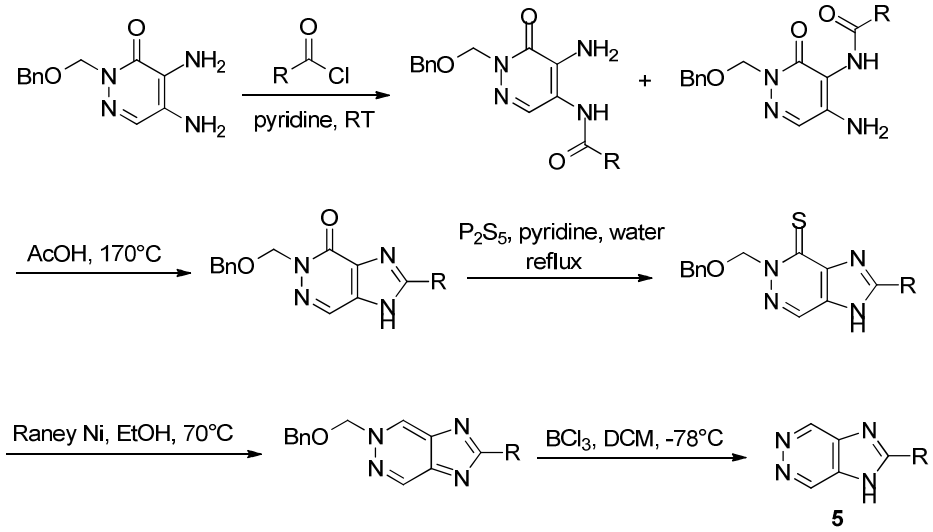


(A) 2-Amino-3-((E)-(2,3-difluorobenzylidene)amino)maleonitrile. To a solution of diaminomaleonitrile (15.0 g, 139 mmol) was added 2,3-difluorobenzaldehyde (19.8 g, 139 mmol) followed by catalytic sulfuric acid (4 drops). After stirring at RT for 1.5 hours the solvent was evaporated at reduced pressure. The solid was triturated with 1:1 ether/hexanes, collected by filtration, and dried *in vacuo* to afford the title compound (31.3 g, 96%).

*(B) 2-(2,3-Difluorophenyl)-1H-imidazole-4,5-dicarbonitrile (**4b**).* A stirred solution of 2-amino-3-((*E*)-(2,3-difluorobenzylidene)amino)maleonitrile (30.8 g,133 mmol) in DMF (400 mL) was treated with NCS (26.5 g, 199 mmol) followed by nicotimamide (24.3 g, 199 mmol). After 1 hour the precipitated nicotimamide hydrochloride was removed by filtration and the filtrate concentrated at reduced pressure. The residue was partitioned between EtOAc and water and the phases separated. The EtOAc solution was washed with saturated brine (1x), dried over $MgSO_4$, and concentrated to afford a black oil. This material was dissolved in DCM and filtered through a silica gel plug washing with 4:1 DCM/MeOH. The resulting filtrate was concentrated to dryness at reduced pressure to give **4b** (14.1 g, 46%). ES-LCMS *m/z*: 231 (M+1).

*(C) 2-(2,3-Difluorophenyl)-5H-imidazo[4,5-d]pyridazine (**5b**).* A solution of 2-(2,3-difluorophenyl)-1H-imidazole-4,5-dicarbonitrile (14.1 g, 61.3 mmol) in THF (80 mL) was cooled to -78 °C and treated with 1M DIBAL-H/THF (400 mL, 400 mmol) by rapid dropwise addition. The solution was then quenched by careful addition of water until gas evolution ceased. Hydrazine hydrate (5.77 mL, 184 mmol) was added and the solution was allowed to warm to RT. The solution was treated with MeOH (61 mL) and the solid aluminum salts removed by filtration. The filter cake was washed with an additional portion (50 mL) of MeOH. The filtrate was concentrated to dryness at reduced pressure and the residue subjected to flash chromatography [silica gel, 10-30% MeOH (containing 10% NH_4OH)/DCM] to give **5b** as a solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.58 (s, 2 H), 8.11 (m, 1 H), 7.58 (m, 1 H), 7.37 (m, 1 H). ES-LCMS *m/z*: 233 (M+1).

Alternate General Procedure for the Synthesis of 2-Substituted 5H-imidazo[4,5-d]pyridazines (5).



(A) 5-Acylamino-4-amino-2-((benzyloxy)methyl)pyridazin-3(2H)-one and 4-Acylamino-5-amino-2-((benzyloxy)methyl)pyridazin-3(2H)-one regioisomer mixture. 4,5-Diamino-2-benzyloxymethyl-2H-pyridazine-3-one (*J. Het. Chem.* 21, 481, **1984**) was dissolved in pyridine (0.8 M) and the solution was treated with the acid chloride

(1.1 equiv) by dropwise addition at RT. After 2 hours the solvent was removed at reduced pressure to give the amide as a mixture of regioisomers.

(B) 2-Substituted 5-((benzyloxy)methyl -1H-imidazo[4,5-d]pyridazin-4(5H)-one. The amide regioisomer mixture was dissolved in glacial AcOH (0.5 M) and the solution heated to 170 °C for 30 minutes. The AcOH was evaporated at reduced pressure and the residue triturated with MeOH to afford the title compound.

(C) 2-Substituted 5-((benzyloxy)methyl)-1H-imidazo[4,5-d]pyridazine-4(5H)-thione. A solution of the 2-substituted 5-((benzyloxy)methyl-1H-imidazo[4,5-d]pyridazin-4(5H)-one in pyridine containing 0.75% water (0.83M) was treated with P_2S_5 (4.5 equiv) and the reaction mixture stirred at reflux overnight. Additional P_2S_5 was added if the reaction was still incomplete. Once complete, the mixture was cooled to RT, filtered to remove solids, and the filter cake washed with hot pyridine. The filtrate was concentrated at reduced pressure. The oily residue was partitioned between $CHCl_3$ and saturated aqueous $NaHCO_3$ and the phases separated. The organic solution was dried over Na_2SO_4 and concentrated to dryness at reduced pressure. The residue was subjected to flash chromatography (silica gel, DCM/MeOH) to give the title compound.

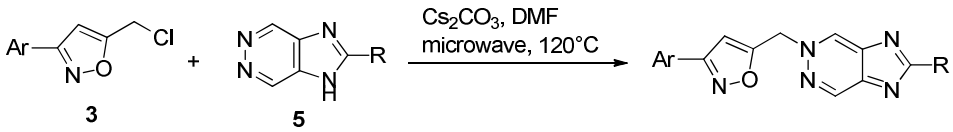
(D) 2-Substituted 5-((benzyloxy)methyl)-5H-imidazo[4,5-d]pyridazine.

The 2-substituted 5-((benzyloxy)methyl)-1H-imidazo[4,5-d]pyridazine-4(5H)-thione was dissolved in EtOH (0.14 M) and the solution treated with Raney nickel (unwashed, 0.40 g/mmol of thione) and the mixture heated to 70 °C. After 1 hour additional Raney nickel was added if the reaction was incomplete. Once complete, the mixture was cooled to RT. The catalyst was removed by filtration and washed thoroughly with hot EtOH. The filtrate was concentrated to dryness at reduced pressure affording the title compound.

*(E) 2-Substituted-5H-imidazo[4,5-d]pyridazine (**5**).*

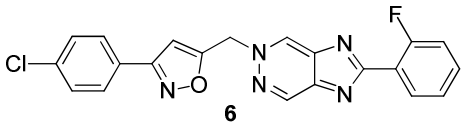
A solution of the 2-substituted 5-((benzyloxy)methyl)-5H-imidazo[4,5-d]pyridazine in DCM (0.03 M) was cooled to -78 °C and treated with 1 M BCl_3/DCM (8 equiv). After 30 minutes, MeOH was added and the mixture warmed to RT. The solvents were removed at reduced pressure and the residue triturated with MeOH, filtered, and dried to give the 2-substituted-5H-imidazo[4,5-d]pyridazine (**5**).

General procedure for the alkylation of 2-substituted 5H-imidazo[4,5-d]pyridazines (5) with 3-aryl-5-(chloromethyl)isoxazoles (3) to give compounds 6, 7, 9, 12, 13, 16, and 18.



A mixture of 3-aryl-5-(chloromethyl)isoxazole (**3**), 2-substituted 5H-imidazo[4,5-d]pyridazine (**5**) (1.0 equiv) and Cs_2CO_3 (3.0 equiv.) in DMF (0.04 M) was subjected to microwave heating at 120 °C for 10 minutes. The reaction mixture was filtered to remove solids and the filtrate subjected to RP-HPLC purification (C18, MeCN/water with 0.1% TFA) followed by treatment with 1N aqueous HCl to afford the desired compound as the hydrochloride salt.

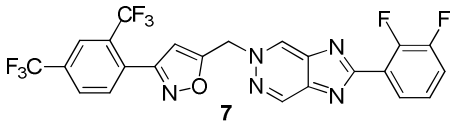
3-(4-Chlorophenyl)-5-((2-(2-fluorophenyl)-5H-imidazo[4,5-d]pyridazin-5-yl)methyl)isoxazole hydrochloride (6).



6

^{1}H NMR (300 MHz, DMSO-d_6): δ 10.40 (s, 1 H), 9.71 (s, 1 H), 8.33 (m, 1 H), 7.84 (dd, *J* = 7.5 Hz, 1.4 Hz, 2 H), 7.73 (m, 1 H), 7.44 – 7.63 (m, 2 H), 7.42 (dd, *J* = 7.5 Hz, 1.3 Hz, 2 H), 7.26 (s, 1 H), 6.30 (s, 2 H). HRMS *m/z* calcd for $C_{23}H_{13}ClFN_5O$ (M+1): 406.0865; found: 406.0870.

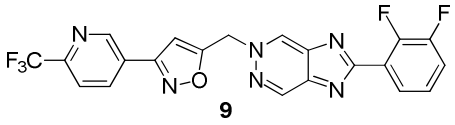
3-(2,4-Bis(trifluoromethyl)phenyl)-5-((2-(2,3-difluorophenyl)-5H-imidazo[4,5-d]pyridazin-5-yl)methyl)isoxazole hydrochloride (7).



7

^{1}H NMR (300 MHz, DMSO-d_6): δ 10.17 (s, 1 H), 9.55 (s, 1 H), 8.12 – 8.26 (m, 3 H), 7.93 (d, *J* = 7.5 Hz, 1 H), 7.54 – 7.65 (m, 1 H), 7.33 – 7.41 (m, 1 H), 7.06 (s, 1 H), 6.26 (s, 2 H). HRMS *m/z* calcd for $C_{23}H_{11}F_8N_5O$ (M+1): 526.0909; found: 526.0915.

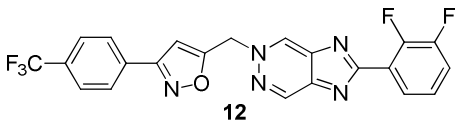
5-((2-(2,3-Difluorophenyl)-5H-imidazo[4,5-d]pyridazin-5-yl)methyl)-3-(6-(trifluoromethyl)pyridin-3-yl)isoxazole hydrochloride (9).



9

^{1}H NMR (300 MHz, DMSO-d_6): δ 10.24 (s, 1 H), 9.58 (s, 1 H), 9.24 (s, 1 H), 8.50 – 8.57 (m, 1 H), 8.11 – 8.19 (m, 1 H), 8.02 – 8.09 (m, 1 H), 7.56 – 7.68 (m, 1 H), 7.34 – 7.44 (m, 2 H), 6.29 (s, 2 H). HRMS *m/z* calcd for $C_{21}H_{11}F_5N_6O$ (M+1): 459.0987; found: 459.0990.

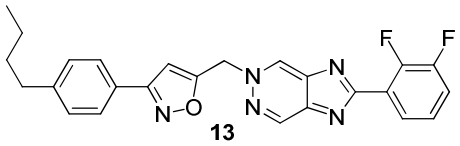
5-((2-(2,3-Difluorophenyl)-5H-imidazo[4,5-d]pyridazin-5-yl)methyl)-3-(4-(trifluoromethyl)phenyl)isoxazole hydrochloride (12).



12

^{1}H NMR (300 MHz, DMSO-d_6): δ 10.46 (s, 1 H), 9.73 (s, 1 H), 8.04 – 8.20 (m, 3 H), 7.84 – 7.91 (m, 2 H), 7.64 – 7.77 (m, 1 H), 7.34 – 7.50 (m, 2 H), 6.35 (s, 2 H). HRMS *m/z* calcd for $C_{22}H_{12}F_5N_5O$ (M+1): 458.1035; found: 458.1038.

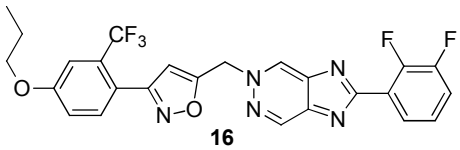
3-(4-Butylphenyl)-5-((2-(2,3-difluorophenyl)-5H-imidazo[4,5-d]pyridazin-5-yl)methyl)isoxazole hydrochloride (13).



13

^{1}H NMR (300 MHz, DMSO-d_6): δ 10.26 (s, 1 H), 9.60 (s, 1 H), 8.11 – 8.20 (m, 1 H), 7.74 (dd, *J* = 7.5 Hz, 1.4 Hz, 2 H), 7.55 – 7.69 (m, 1 H), 7.27 – 7.45 (m, 3 H), 7.18 (m, 1 H), 6.23 (s, 2 H), 2.62 (t, *J* = 7.1 Hz, 2 H), 1.48 – 1.63 (m, 2 H), 1.21 – 1.36 (m, 2 H), 0.90 (t, *J* = 7.9 Hz, 3 H). HRMS *m/z* calcd for $C_{25}H_{21}F_2N_5O$ (M+1): 446.1787; found: 446.1790.

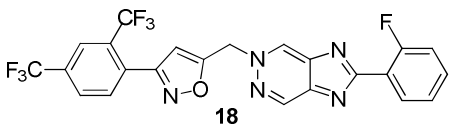
5-((2-(2,3-Difluorophenyl)-5H-imidazo[4,5-d]pyridazin-5-yl)methyl)-3-(4-propoxy-2-(trifluoromethyl)phenyl)isoxazole hydrochloride (16).



16

^{1}H NMR (400 MHz, DMSO-d_6): δ 10.11 (s, 1 H), 9.50 (s, 1 H), 8.08 – 8.24 (m, 1 H), 7.48 – 7.65 (m, 2 H), 7.27 – 7.43 (m, 3 H), 6.91 (s, 1 H), 6.20 (s, 2 H), 4.07 (t, *J* = 7.1 Hz, 2 H), 1.64 – 1.85 (m, 2 H), 0.98 (t, *J* = 7.9 Hz, 3 H). ES-LCMS *m/z*: 516 (M+1).

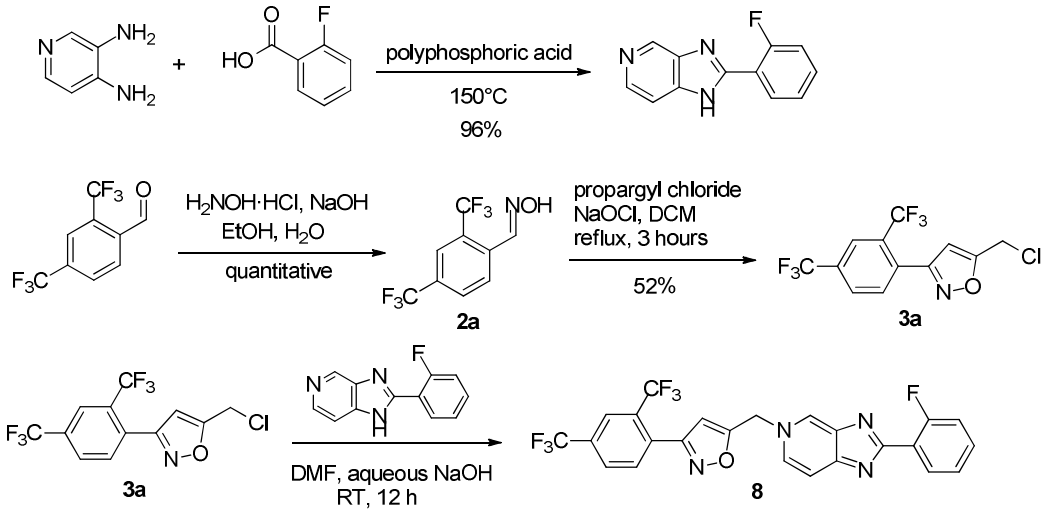
3-(2,4-Bis(trifluoromethyl)phenyl)-5-((2-(2-fluorophenyl)-5H-imidazo[4,5-d]pyridazin-5-yl)methyl)isoxazole hydrochloride (18).



18

^{1}H NMR (300 MHz, DMSO-d_6): δ 10.30 (s, 1 H), 9.60 (s, 1 H), 8.30 (m, 1 H), 8.20 (m, 2 H), 7.90 (d, *J* = 7.4 Hz, 1 H), 7.60 (m, 1 H), 7.40 (m, 2 H), 7.00 (s, 1 H), 6.30 (s, 2 H). ES-LCMS *m/z*: 508 (M+1).

3-(2,4-Bis(trifluoromethyl)phenyl)-5-((2-(2-fluorophenyl)-5H-imidazo[4,5-c]pyridin-5-yl)methyl)isoxazole (8).



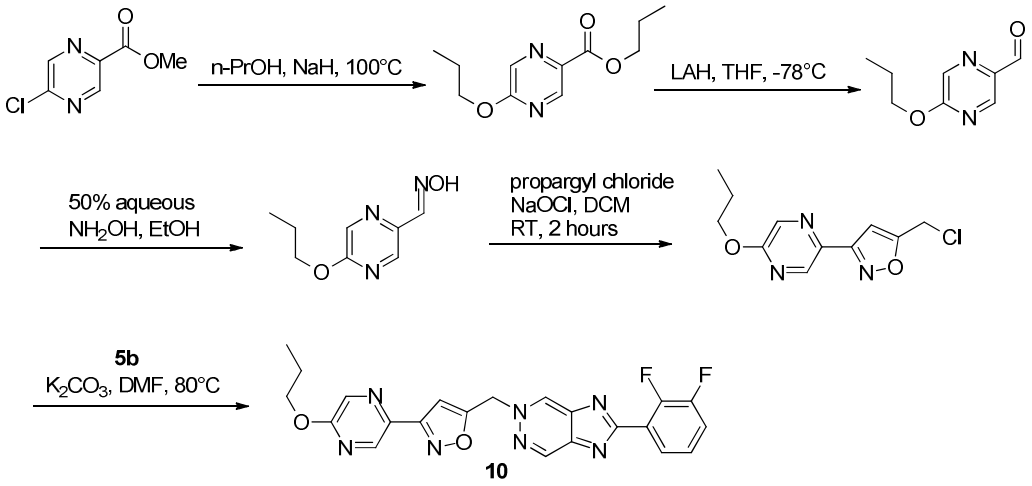
(A) 2-(2-Fluorophenyl)-5H-imidazo[4,5-c]pyridine. A mixture of pyridine-3,4-diamine (2.18 g, 20.0 mmol) and 2-fluorobenzoic acid (2.80 g, 20.0 mmol) in polyphosphoric acid (10.0 mL) was heated to 150 °C. After 18 hours the light brown solution was cooled to RT and diluted with water (100 mL). The pH was adjusted to 13 by addition of aqueous NaOH and the resulting mixture extracted with EtOAc (3x50 mL). The combined EtOAc extracts were washed once with saturated brine, dried over Na_2SO_4, and concentrated to dryness at reduced pressure to give the title compound (4.10 g, 96%) as a white solid.

*(B) 2,4-Bis(trifluoromethyl)benzaldehyde oxime (**2a**).* To a stirred suspension of 2,4-bis(trifluoromethyl)benzaldehyde (5.08 g, 21.0 mmol) in 1:2 EtOH/H_2O (230 mL) was added hydroxylamine hydrochloride (1.59 g, 23.0 mmol). The mixture was cooled to 4 °C and treated with 50% aqueous NaOH (4.13 mL, 52.0 mmol) by dropwise addition. After warming to RT and stirring for 1.5 hours, the reaction mixture was acidified with 2N aqueous HCl and extracted with DCM (3x50 mL). The combined DCM extracts were washed with saturated brine (1x), dried over Na_2SO_4, and concentrated to dryness at reduced pressure to afford **2a** (5.30 g, quantitative). The crude material was used in the next step without purification.

*(C) 3-(2,4-Bis(trifluoromethyl)phenyl)-5-(chloromethyl)isoxazole (**3a**).* A stirred suspension of **2a** (9.75 g, 37.9 mmol) and propargyl chloride (2.72 mL, 37.9 mmol) in DCM (45 mL) was cooled to 4 °C. The mixture was treated with aqueous NaOCl (10-13% free chlorine, 37.6 mL, 61.0 mmol) by dropwise addition. After stirring at 4 °C for 15 minutes, the mixture was heated to reflux. After 3 hours the solution was cooled to RT, partitioned between water and DCM, and the phases separated. The DCM solution was washed with saturated brine (1x), dried over Na_2SO_4, and concentrated to dryness at reduced pressure. The crude product was purified by flash chromatography (silica gel, 10% DCM/hexanes) to afford **3a** (6.50 g, 52%).

*(D) 3-(2,4-Bis(trifluoromethyl)phenyl)-5-((2-(2-fluorophenyl)-5H-imidazo[4,5-c]pyridin-5-yl)methyl)isoxazole (**8**).* A stirred suspension of 2-(2-fluorophenyl)-5H-imidazo[4,5-c]pyridine (14.3 g, 67.0 mmol) in DMF (40 mL) was treated with 10% aqueous NaOH (32.2 mL, 80.0 mmol). To the resulting mixture was added a solution of **3a** (26.3 g, 80.0 mmol) in DMF (16 mL). After 12 hours, the solvents were evaporated at reduced pressure to give a tan solid. This material was recrystallized from MeOH/H_2O to afford **8**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.2 (s, 1 H), 8.2 – 8.4 (m, 4 H), 7.9 – 8.0 (d, 1 H), 7.8 – 7.9 (d, 1 H), 7.4 – 7.5 (m, 1 H), 7.3 (t, 2 H), 7.0 (s, 1 H), 6.1 (s, 2 H). HRMS *m/z* calcd for $C_{24}H_{13}F_7N4O$ (M+1): 507.1050; found: 507.1056.

5-((2-(2,3-Difluorophenyl)-5H-imidazo[4,5-d]pyridazin-5-yl)methyl)-3-(5-propoxypyrazin-2-yl)isoxazole hydrochloride (10).



(A) Propyl 5-propoxypyrazine-2-carboxylate. A solution of methyl 5-chloropyrazine-2-carboxylate (1.00 g, 5.70 mmol) in n-PrOH (10 mL) was treated with 60% NaH (mineral oil dispersion, 0.230 g, 5.70 mmol) and heated to 100 °C for 15 minutes. The reaction mixture was cooled to RT, diluted with water, and extracted with DCM (2x). The combined DCM extracts were washed with brine, dried over Na_2SO_4, and concentrated to dryness at reduced pressure. The crude material was subjected to silica gel flash chromatography to afford the title compound. ES-LCMS *m/z*: 225 (M+1).

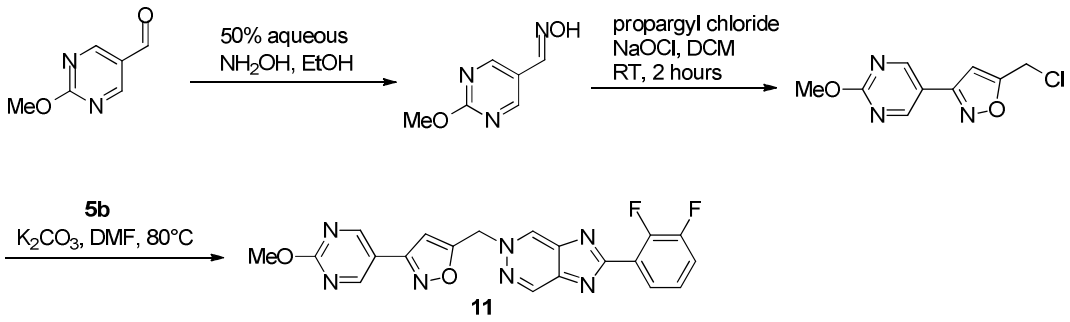
(B) 5-Propoxypyrazine-2-carbaldehyde. A solution of propyl 5-propoxypyrazine-2-carboxylate (0.770 g, 3.43 mmol) in THF (20 mL) was cooled to -78 °C and treated with 2M LAH/THF (0.858 mL, 1.72 mmol). After stirring at -78 °C for 1 hour, the solution was quenched with AcOH (0.500 mL) and warmed to RT. The mixture was diluted with water (10 mL) and extracted with DCM (2x). The combined DCM extracts were washed with brine (1x), dried over Na_2SO_4, and concentrated to dryness at reduced pressure. The residue was subjected to flash chromatography (silica gel, EtOAc/hexanes) to afford the title compound. ES-LCMS *m/z*: 167 (M+1).

(C) 5-Propoxypyrazine-2-carbaldehyde oxime. The title compound was prepared from 5-propoxypyrazine-2-carbaldehyde according to the general procedure for the synthesis of 3-aryl-5-(chloromethyl)isoxazole intermediates (**3**, step A), and was used in the next step without purification.

(D) 5-(Chloromethyl)-3-(5-propoxypyrazin-2-yl)isoxazole. The title compound was prepared from 5-propoxypyrazine-2-carbaldehyde oxime according to the general procedure for the synthesis of 3-aryl-5-(chloromethyl)isoxazole intermediates, (**3**, step B), and was used in the next step without purification.

*(E) 5-((2-(2,3-Difluorophenyl)-5H-imidazo[4,5-d]pyridazin-5-yl)methyl)-3-(5-propoxypyrazin-2-yl)isoxazole (**10**).* Compound **10** was prepared from 5-(chloromethyl)-3-(5-propoxypyrazin-2-yl)isoxazole and **5b** according to procedure described herein for the synthesis of **29**, step C. The crude product was purified by RP-HPLC (C18, MeCN/water with 0.1% TFA) followed by treatment with 2N aqueous HCl to afford **10** as the hydrochloride salt. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.45 (s, 1 H), 9.71 (s, 1 H), 8.79 (d, J = 1.5 Hz, 1 H), 8.42 (d, J = 1.5 Hz, 1 H), 8.12 – 8.22 (m, 1 H), 7.65 – 7.78 (m, 1 H), 7.40 – 7.51 (m, 1 H), 7.24 (s, 1 H), 6.34 (s, 2 H), 4.32 (t, J = 7.0 Hz, 2 H), 1.69 – 1.82 (m, 2 H), 0.98 (t, J = 7.2 Hz, 3 H). HRMS m/z calcd for $C_{22}H_{17}F_2N_7O_2$ (M+1): 450.1485; found: 450.1487.

5-((2-(2,3-Difluorophenyl)-5H-imidazo[4,5-d]pyridazin-5-yl)methyl)-3-(2-methoxypyrimidin-5-yl)isoxazole (11).



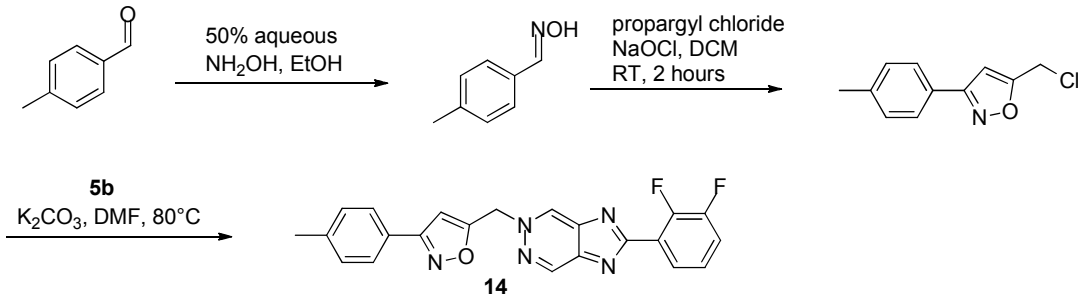
(A) 2-Methoxypyrimidine-5-carbaldehyde oxime. The title compound was prepared from 2-methoxypyrimidine-5-carbaldehyde according to the general procedure for the synthesis of 3-aryl-5-(chloromethyl)isoxazole intermediates (**3**, step A), and was used in the next step without purification.

(B) 5-(Chloromethyl)-3-(2-methoxypyrimidin-5-yl)isoxazole. The title compound was prepared from 2-methoxypyrimidine-5-carbaldehyde oxime according to the general procedure for the synthesis of 3-aryl-5-(chloromethyl)isoxazole intermediates (**3**, step B), and was used in the next step without purification.

*(C) 5-((2-(2,3-Difluorophenyl)-5H-imidazo[4,5-d]pyridazin-5-yl)methyl)-3-(2-methoxypyrimidin-5-yl)isoxazole (**11**).* Compound **11** was prepared from 5-(chloromethyl)-3-(2-methoxypyrimidin-5-yl)isoxazole and **5b** according to procedure described herein for the synthesis of **29**, step C. The crude product was purified by RP-HPLC (C18, MeCN/water with 0.1% TFA). Following RP-HPLC purification the product was passed through an ion exchange

column to remove TFA thereby affording **11** as the free base. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.13 (s, 1 H), 9.51 (s, 1 H), 9.07 (s, 2 H), 8.13 – 8.22 (m, 1 H), 7.50 – 7.64 (m, 1 H), 7.30 – 7.41 (m, 1 H), 7.27 (s, 1 H), 6.22 (s, 2 H), 3.97 (s, 3 H). HRMS *m/z* calcd for $C_{22}H_{13}F_2N_7O$ (M+1): 422.1172; found: 422.1173.

5-((2-(2,3-Difluorophenyl)-5H-imidazo[4,5-d]pyridazin-5-yl)methyl)-3-(p-tolyl)isoxazole (14).

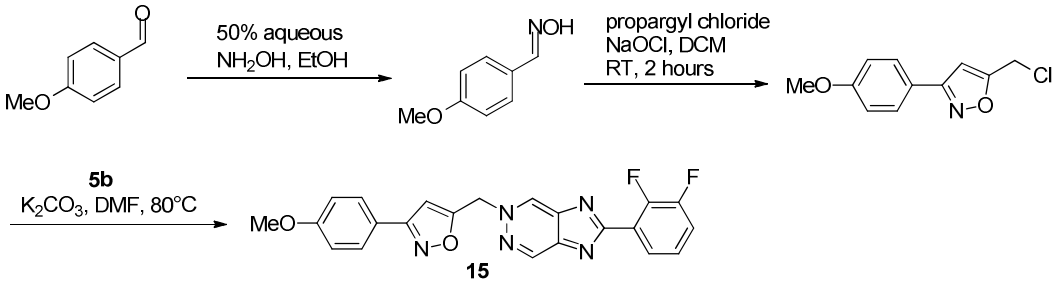


(A) 4-Methylbenzaldehyde oxime. The title compound was prepared from 4-methylbenzaldehyde according to the general procedure for the synthesis of 3-aryl-5-(chloromethyl)isoxazole intermediates (**3**, step A), and was used in the next step without purification.

(B) 5-(Chloromethyl)-3-(p-tolyl)isoxazole. The title compound was prepared from 4-methylbenzaldehyde oxime according to the general procedure for the synthesis of 3-aryl-5-(chloromethyl)isoxazole intermediates (**3**, step B), and was used in the next step without purification.

*(C) 5-((2-(2,3-Difluorophenyl)-5H-imidazo[4,5-d]pyridazin-5-yl)methyl)-3-(p-tolyl)isoxazole (**14**).* The title compound was prepared from 5-(chloromethyl)-3-(p-tolyl)isoxazole and 2-(2,3-difluorophenyl)-1H-imidazo[4,5-d]pyridazine according to procedure described herein for the synthesis of **29**, step C. The crude product was purified by RP-HPLC (C18, MeCN/water with 0.1% TFA) followed by treatment with 2N aqueous HCl to afford **14** as the hydrochloride salt. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.53 (s, 1 H), 9.75 (s, 1 H), 8.14 – 8.18 (m, 1 H), 7.28 – 7.70 (m, 3 H), 7.42 – 7.49 (m, 1 H), 7.29 (d, *J* = 7.8 Hz, 2 H), 7.19 (s, 1 H), 6.33 (s, 2 H), 2.32 (s, 3 H). HRMS *m/z* calcd for $C_{22}H_{15}F_2N_5O$ (M+1): 404.1317; found: 404.1318.

5-((2-(2,3-Difluorophenyl)-5H-imidazo[4,5-d]pyridazin-5-yl)methyl)-3-(4-methoxyphenyl)isoxazole hydrochloride (15).

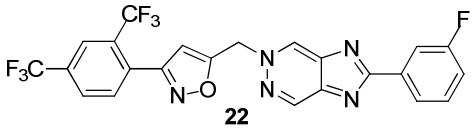


(A) 4-Methoxybenzaldehyde oxime. The title compound was prepared from 4-methoxybenzaldehyde according to the general procedure for the synthesis of 3-aryl-5-(chloromethyl)isoxazole intermediates (**3**, step A), and was used in the next step without purification.

(B) 5-(Chloromethyl)-3-(4-methoxyphenyl)isoxazole. The title compound was prepared from 4-methoxybenzaldehyde oxime according to the general procedure for the synthesis of 3-aryl-5-(chloromethyl)isoxazole intermediates (**3**, step B), and was used in the next step without purification.

*(C) 5-((2-(2,3-Difluorophenyl)-5H-imidazo[4,5-d]pyridazin-5-yl)methyl)-3-(4-methoxyphenyl)isoxazole (**15**).* Compound **15** was prepared from 5-(chloromethyl)-3-(4-methoxyphenyl)isoxazole and **5b** according to procedure described herein for the synthesis of **29**, step C. The crude product was purified by RP-HPLC (C18, MeCN/water with 0.1% TFA) followed by treatment with 2N aqueous HCl to afford **15** as the hydrochloride salt. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.48 (s, 1 H), 9.74 (s, 1 H), 8.13 – 8.22 (m, 1 H), 7.66 – 7.84 (m, 3 H), 7.41 – 7.52 (m, 1 H), 7.18 (s, 1 H), 7.00 – 7.09 (m, 2 H), 6.31 (s, 2 H), 3.80 (s, 3 H). HRMS *m/z* calcd for $C_{22}H_{15}F_2N_5O_2$ (M+1): 420.1267; found: 420.1266.

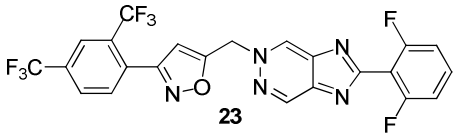
3-(2,4-bis(trifluoromethyl)phenyl)-5-((2-(3-fluorophenyl)-5H-imidazo[4,5-d]pyridazin-5-yl)methyl)isoxazole hydrochloride (22).



22

Compound **22** was prepared via Suzuki coupling of **21** with 3-fluorophenylboronic acid as described herein for the synthesis **24**, step C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.50 (s, 1 H), 9.76 (s, 1 H), 8.20 – 8.29 (m, 5 H), 7.90 (d, *J* = 7.6 Hz, 1 H), 7.67 – 7.70 (m, 1 H), 7.05 (t, *J* = 7.6 Hz, 1 H), 6.39 (s, 2 H). ES-LCMS *m/z*: 508 (M+1).

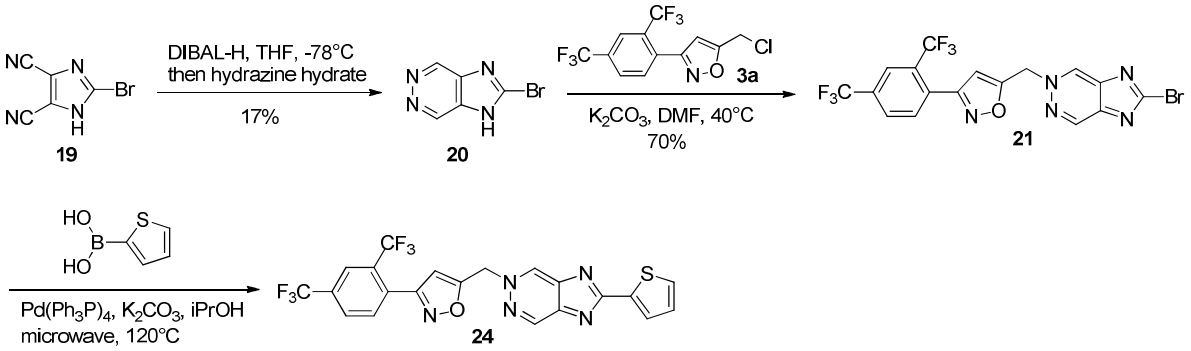
3-(2,4-Bis(trifluoromethyl)phenyl)-5-((2-(2,6-difluorophenyl)-5H-imidazo[4,5-d]pyridazin-5-yl)methyl)isoxazole (23).



23

Compound **23** was prepared via Suzuki coupling of **21** with 2,6-difluorophenylboronic acid as described herein for the synthesis of **24**, step C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.42 (s, 1 H), 9.72 (s, 1 H), 8.25 (m, 3 H), 7.92 (d, *J* = 7.7 Hz 1 H), 7.33 – 7.38 (m, 2 H), 7.08 (s, 1 H), 6.35 (s, 2 H). ES-LCMS *m/z*: 526 (M+1).

3-(2,4-Bis(trifluoromethyl)phenyl)-5-((2-(thiophen-2-yl)-5H-imidazo[4,5-d]pyridazin-5-yl)methyl)isoxazole hydrochloride (24).

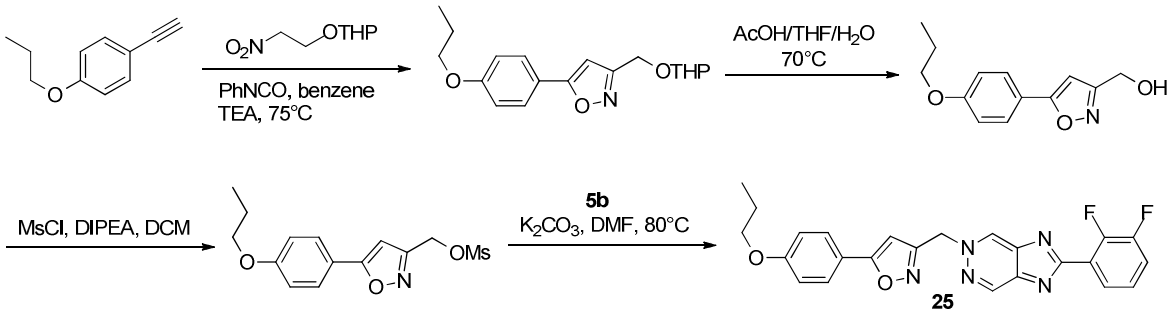


*(A) 2-Bromo-1H-imidazo[4,5-d]pyridazine (**20**).* A solution of 2-bromo-1H-imidazole-4,5-dicarbonitrile (**19**) (2.00 g, 10.2 mmol, see *Heterocycles*, 29, 1325, **1989**) in THF (100 mL) was cooled to -78 °C and treated with 1M DIBAL-H/THF (50 mL, 50.0 mmol) over 10 minutes. The mixture was stirred at -78 °C for 15 minutes and then quenched by addition of 10% aqueous potassium sodium tartrate (80 mL). The solution was then treated with hydrazine hydrate (1.53 g, 30.6 mmol). The mixture was warmed to RT and stirred for 1 hour. The reaction mixture was then cooled to 0°C overnight and filtered to remove solids. The filter cake was washed with MeOH (2x100 mL) and the filtrate concentrated to dryness at reduced pressure. The crude product was subjected to flash chromatography [silica gel, 0-60% MeOH (containing 10% NH_4OH)/DCM] to give **20** (0.350 g, 17%). ES-LCMS *m/z*: 199+201 (M+1).

*(B) 3-(2,4-Bis(trifluoromethyl)phenyl)-5-((2-bromo-5H-imidazo[4,5-d]pyridazin-5-yl)methyl)isoxazole (**21**).* A mixture of **20** (0.350 g, 1.76 mmol), **3a** (0.600 g, 1.82 mmol) and K_2CO_3 (0.500 g, 3.62 mmol) in DMF (5 mL) was heated to 40 °C with stirring. After 1 hour the mixture was cooled to RT and poured into cold water (30 mL). The resulting solid was collected by filtration and dried *in vacuo* to afford **21** (0.590 g, 68%) as a yellow solid.

*(C) 3-(2,4-Bis(trifluoromethyl)phenyl)-5-((2-(thiophen-2-yl)-5H-imidazo[4,5-d]pyridazin-5-yl)methyl)isoxazole hydrochloride (**24**).* A mixture of **21** (0.100 g, 0.203 mmol), thiophen-2-ylboronic acid (33.8 mg, 0.264 mmol), 2M aqueous K_2CO_3 (0.31 mL, 0.61 mmol), and $Pd(Ph_3P)_4$ (11.7 mg, 10.2 µmol) in isopropanol (2 mL) was subjected to microwave heating at 120 °C for 20 minutes. The reaction mixture was filtered to remove solids and the filtrate concentrated to dryness at reduced pressure. The crude residue was subjected to RP-HPLC purification (C18, MeCN/water with 0.1% TFA) followed by treatment with 1N aqueous HCl to afford **24** as the hydrochloride salt. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.34 (s, 1 H), 9.65 (s, 1 H), 8.17 – 8.25 (m, 2 H), 7.97 (dd, *J* = 4.8 Hz, 1.0 Hz, 1 H), 7.90 (d, *J* = 8.4 Hz, 1 H), 7.65 – 7.48 (m, 2H), 7.33 (dd, *J* = 5.1 Hz, 3.8 Hz, 1 H), 7.07 (s, 1 H), 6.35 (s, 2 H). HRMS *m/z* calcd for $C_{21}H_{11}F_6N_5OS$ (M+1): 496.0661; found: 496.0665.

3-((2-(2,3-Difluorophenyl)-5H-imidazo[4,5-d]pyridazin-5-yl)methyl)-5-(4-propoxyphenyl)isoxazole hydrochloride (25).



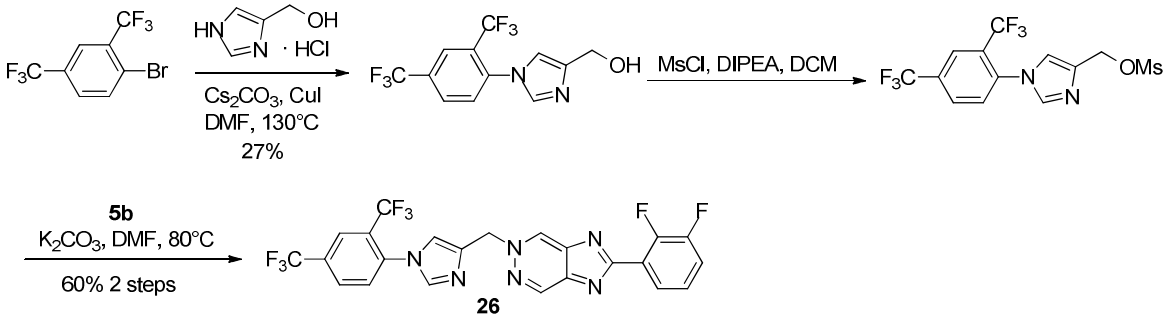
(A) 5-(4-Propoxyphenyl)-3-(((tetrahydro-2H-pyran-2-yl)oxy)methyl)isoxazole. A mixture of phenyl isocyanate (1.13 g, 4.99 mmol), 2-(2-nitroethoxy)tetrahydro-2H-pyran (0.875g, 4.99 mmol) and 1-ethynyl-4-propoxybenzene (0.800 g, 4.99 mmol) was dissolved in benzene (20 mL) and treated with TEA (1.53 mL, 11.0 mmol). The solution was heated to 75 °C in a screw-cap vial overnight. It was then cooled, the solids removed by filtration, and the filtrate concentrated at reduced pressure. The crude product was purified by flash chromatography (silica gel, EtOAc/hexanes) to afford the title compound (0.70 g, 44%).

(B) (5-(4-Propoxyphenyl)isoxazol-3-yl)methanol. A solution of 5-(4-propoxyphenyl)-3-(((tetrahydro-2H-pyran-2-yl)oxy)methyl)isoxazole (0.698 g, 2.20 mmol) in 4:2:1 AcOH/THF/water (10 mL) was stirred at RT overnight and then heated to 75 °C for an additional 5 hours. The solution was cooled to RT and concentrated to dryness at reduced pressure. The residue was dissolved in MeOH and the solution concentrated at reduced pressure to afford the title compound which was used in the next step without purification.

(C) (1-(2,4-Bis(trifluoromethyl)phenyl)-1H-imidazol-4-yl)methyl methanesulfonate. The title compound was prepared from (5-(4-propoxyphenyl)isoxazol-3-yl)methanol as described herein for the synthesis of compound **29**, step B. The crude product was used in the next step without purification.

*(D) 3-((2-(2,3-Difluorophenyl)-5H-imidazo[4,5-d]pyridazin-5-yl)methyl)-5-(4-propoxyphenyl)isoxazole (**25**).* Compound **25** was prepared from (1-(2,4-bis(trifluoromethyl)phenyl)-1H-imidazol-4-yl)methyl methanesulfonate and **5b** as described herein for the synthesis of **29**, step C. The crude product was purified by RP-HPLC purification (C18, MeCN/water with 0.1% TFA) followed by treatment with 1N aqueous HCl to afford **25** as the hydrochloride salt. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.34 (s, 1 H), 9.60 (s, 1 H), 8.08 – 8.16 (m, 1 H), 7.75 (m, 4 H), 7.02 (m, 3H), 6.13 (s, 2 H), 3.96 (t, J = 7.4 Hz, 2 H), 1.70 (m, 2 H), 0.97 (t, J = 7.3 Hz, 3 H). HRMS *m/z* calcd for $C_{24}H_{19}F_2N_5O_2$ (M+1): 448.1580; found: 448.1582.

5-((1-(2,4-Bis(trifluoromethyl)phenyl)-1H-imidazol-4-yl)methyl)-2-(2,3-difluorophenyl)-5H-imidazo[4,5-d]pyridazine hydrochloride (26).

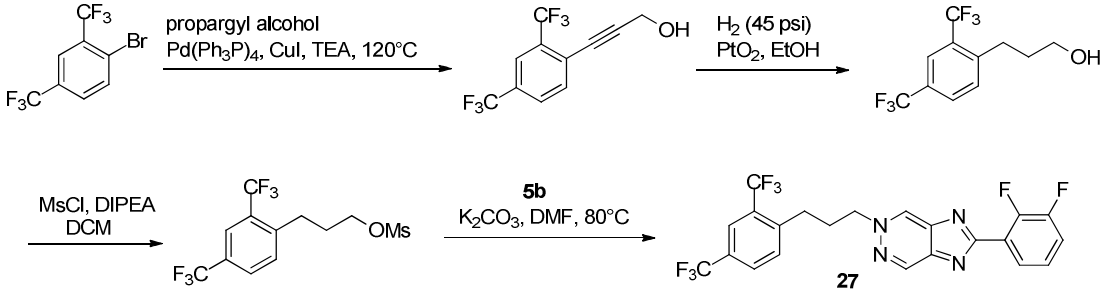


(A) (1-(2,4-Bis(trifluoromethyl)phenyl)-1H-imidazol-4-yl)methanol. A mixture of 1-bromo-2,4-bis(trifluoromethyl)benzene (0.293 g, 1.00 mmol), (1H-imidazol-4-yl)methanol hydrochloride (0.188 g, 1.40 mmol), Cs_2CO_3 (0.980 g, 3.00 mmol), and CuI (38.2 mg, 0.200 mmol) in DMF (3 mL) under argon was heated at 130 °C with stirring. After 3 hours the mixture was cooled to RT and diluted with $CHCl_3$. The solids were removed by filtration through celite and the filtrate concentrated to dryness at reduced pressure. The residue was subjected to flash chromatography [silica gel, 5-30% MeOH (containing 5% NH_4OH)/DCM] to afford the title compound (84 mg, 27%).

(B) (1-(2,4-Bis(trifluoromethyl)phenyl)-1H-imidazol-4-yl)methyl methanesulfonate. The title compound was prepared from (1-(2,4-bis(trifluoromethyl)phenyl)-1H-imidazol-4-yl)methanol as described herein for the synthesis of compound **29**, step B. The crude product was used in the next step without purification.

*(C) 5-((1-(2,4-Bis(trifluoromethyl)phenyl)-1H-imidazol-4-yl)methyl)-2-(2,3-difluorophenyl)-5H-imidazo[4,5-d]pyridazine hydrochloride (**26**).* Compound **26** was prepared from (1-(2,4-bis(trifluoromethyl)phenyl)-1H-imidazol-4-yl)methyl methanesulfonate and **5b** as described herein for the synthesis of **29**, step C. The crude product was purified by RP-HPLC purification (C18, MeCN/water with 0.1% TFA) followed by treatment with 1N aqueous HCl to afford **26** as the hydrochloride salt in 60% yield over 2 steps. ^{1}H NMR (300 MHz, d_4-MeOH): δ 10.51 (s, 1 H), 9.77 (s, 1 H), 9.35 (s, 1 H), 8.21 – 8.36 (m, 4 H), 8.06 (d, J = 7.8 Hz, 1 H), 7.63 – 7.74 (m, 1 H), 7.46 – 7.55 (m, 1 H), 6.36 (s, 2 H). HRMS *m/z* calcd for $C_{23}H_{12}F_8N_6$ (M+1): 525.1069; found: 525.1067.

5-(3-(2,4-Bis(trifluoromethyl)phenyl)propyl)-2-(2,3-difluorophenyl)-5H-imidazo[4,5-d]pyridazine (27).



(A) 3-(2,4-Bis(trifluoromethyl)phenyl)prop-2-yn-1-ol. A vial was charged with 1-bromo-2,4-bis(trifluoromethyl)benzene (0.40 mL, 2.40 mmol), propargyl alcohol (0.400 mL, 6.80 mmol), $Pd(Ph_3P)_4$ (0.115 g,

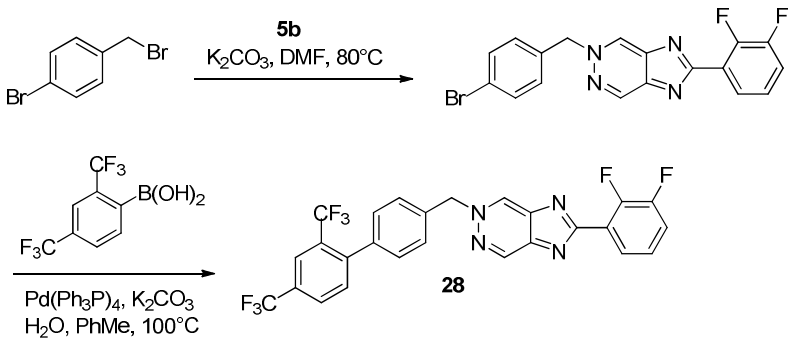
0.100 mmol), CuI (40.0 mg, 0.210 mmol) and TEA (3.00 mL) under argon and the mixture subjected to microwave heating at 120 °C for 10 minutes. The reaction mixture was diluted with EtOAc. The solution was washed with aqueous ammonium chloride (3x), water, brine, dried over Na_2SO_4, and concentrated in the presence of celite. The crude product was purified by flash chromatography (silica gel, 15-60% EtOAc/hexanes) to give the title compound (0.181 g, 28%) as a solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.91 (s, 1 H), 7.70 – 7.78 (m, 2 H), 4.56 (s, 2 H), 1.69 (s, 1 H).

(B) 3-(2,4-Bis(trifluoromethyl)phenyl)propan-1-ol. 3-(2,4-Bis(trifluoromethyl)phenyl)prop-2-yn-1-ol (0.300 g, 1.12 mmol) was dissolved in EtOH (40 mL) and the solution sparged with argon. PtO_2 (50.0 mg, 0.220 mmol) was added and the mixture was shaken for 3 hours under 45 psi of hydrogen. The catalyst was removed by filtration through celite, and the solvents removed at reduced pressure to afford the title compound. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.88 (s, 1 H), 7.74 (d, *J* = 7.5 Hz, 1 H), 7.53 (d, *J* = 7.5 Hz, 1 H), 3.56 (t, *J* = 7.2 Hz, 2 H), 2.95 (t, *J* = 7.1 Hz, 2 H), 2.14 (br s, 1 H), 1.86 – 1.95 (m, 2 H).

(C) 3-(2,4-Bis(trifluoromethyl)phenyl)propyl methanesulfonate. The title compound was prepared from 3-(2,4-bis(trifluoromethyl)phenyl)propan-1-ol as described herein for the synthesis of compound **29**, step B. The crude product was used in the next step without purification.

*(D) 5-(3-(2,4-Bis(trifluoromethyl)phenyl)propyl)-2-(2,3-difluorophenyl)-5H-imidazo[4,5-d]pyridazine (**27**).* Compound **27** was prepared from 3-(2,4-bis(trifluoromethyl)phenyl)propyl methanesulfonate and **5b** as described herein for the synthesis of **29**, step C. The crude product was purified by flash chromatography (silica gel, 0-50% MeOH/DCM) to give **27** as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.93 (s, 1 H), 9.44 (s, 1 H), 8.13 – 8.17 (m, 1 H), 8.00 (d, *J* = 8.4 Hz, 1 H), 7.92 (s, 1 H), 7.78 (d, *J* = 8.4 Hz, 1 H), 7.48 – 7.57 (m, 1 H), 7.29 – 7.36 (m, 1 H), 4.75 (t, *J* = 6.9 Hz, 2 H), 2.82 – 2.93 (m, 2 H), 2.28 – 2.39 (m, 2 H). HRMS *m/z* calcd for $C_{22}H_{14}F_8N_4$ (M+1): 487.1164; found: 487.1166.

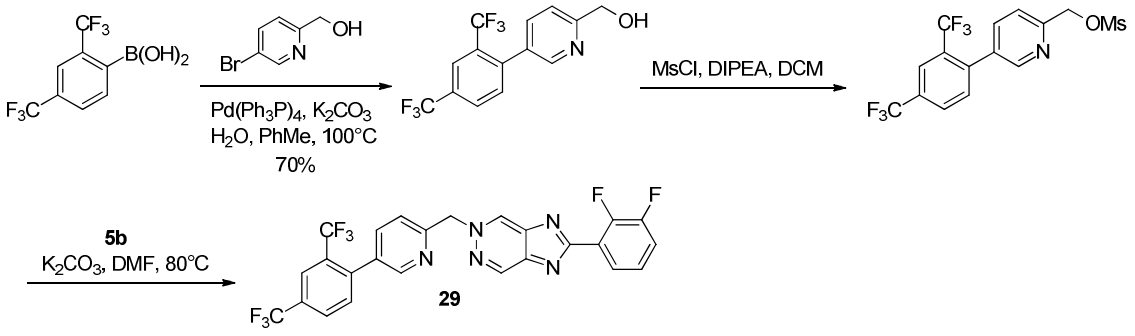
5-((2',4'-Bis(trifluoromethyl)-[1,1'-biphenyl]-4-yl)methyl)-2-(2,3-difluorophenyl)-5H-imidazo[4,5-d]pyridazine (28).



(A) 5-(4-Bromobenzyl)-2-(2,3-difluorophenyl)-5H-imidazo[4,5-d]pyridazine. A mixture of 1-bromo-4-(bromomethyl)benzene (62.0 mg, 0.248 mmol), **5b** (57.6 mg, 0.248 mmol), and K_2CO_3 (68.6 mg, 0.496 mmol) in DMF was stirred at 80 °C for 1 hour and then cooled to RT. The mixture was partitioned between DCM and water and the phases separated. The aqueous phase was extracted with one additional portion of DCM. The combined DCM solutions were washed with saturated brine, dried over Na_2SO_4, and concentrated to dryness at reduced pressure to give the crude title compound which was used in the next step without purification.

*(B) 5-((2',4'-Bis(trifluoromethyl)-[1,1'-biphenyl]-4-yl)methyl)-2-(2,3-difluorophenyl)-5H-imidazo[4,5-d]pyridazine (**28**).* Compound **28** was prepared by Suzuki coupling of 5-(4-bromobenzyl)-2-(2,3-difluorophenyl)-5H-imidazo[4,5-d]pyridazine with (2,4-bis(trifluoromethyl)phenyl)boronic acid as described herein for the synthesis of compound **29**, step A. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.52 (s, 1 H), 9.72 (s, 1 H), 8.15 (m, 3 H), 7.38 – 7.70 (m, 7 H), 6.01 (s, 2 H). HRMS *m/z* calcd for $C_{26}H_{14}F_8N_4$ (M+1): 535.1165; found: 535.1163.

5-((5-(2,4-Bis(trifluoromethyl)phenyl)pyridin-2-yl)methyl)-2-(2,3-difluorophenyl)-5H-imidazo[4,5-d]pyridazine hydrochloride (29).



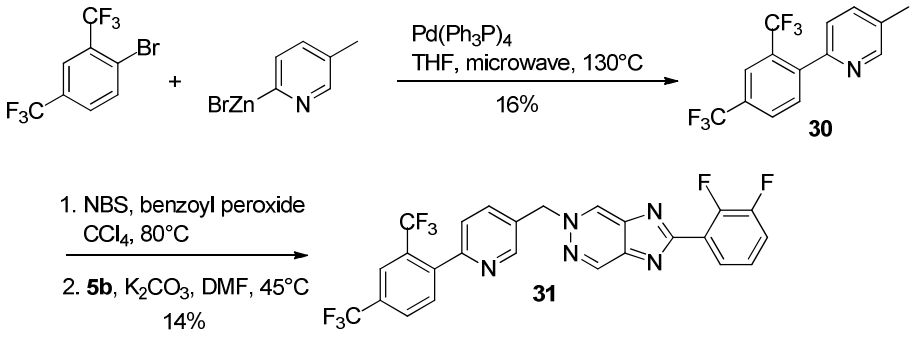
(A) (5-(2,4-Bis(trifluoromethyl)phenyl)pyridin-2-yl)methanol. A mixture of (2,4-bis(trifluoromethyl)phenyl)boronic acid (0.387 g, 1.50 mmol), (5-bromopyridin-2-yl)methanol (0.188 g, 1.00 mmol), $Pd(Ph_3P)_4$ (57.8 mg, 0.0500 mmol), and 2M aqueous Na_2CO_3 (1.00 mL, 2.00 mmol) in toluene (4 mL) was sparged with nitrogen for several minutes and heated to 100 °C with stirring. After 1 hour the mixture was cooled to RT, partitioned between water and EtOAc, and the phases separated. The EtOAc solution was washed with saturated brine, dried over Na_2SO_4, and concentrated to dryness at reduced pressure. The crude material was subjected to flash chromatography (silica gel, 20-100% EtOAc/hexanes) to afford the title compound (0.225 g, 70%).

(B) (5-(2,4-Bis(trifluoromethyl)phenyl)pyridin-2-yl)methyl methanesulfonate. A solution of (5-(2,4-bis(trifluoromethyl)phenyl)pyridin-2-yl)methanol (0.180 g, 0.560 mmol) in DCM (5 mL) was treated with DIPEA (200 µL, 1.15 mmol) followed by MsCl (65 µL, 0.834 mmol). After stirring at RT for 1 hour the solution was partitioned between water and EtOAc and the phases separated. The EtOAc solution was washed with saturated

brine (1x), dried over sodium sulfate, and concentrated to dryness at reduced pressure to give the title compound which was used in the next step without purification.

*(C) 5-((5-(2,4-Bis(trifluoromethyl)phenyl)pyridin-2-yl)methyl)-2-(2,3-difluorophenyl)-5H-imidazo[4,5-d]pyridazine hydrochloride (**29**).* A stirred mixture of (5-(2,4-bis(trifluoromethyl)phenyl)pyridin-2-yl)methyl methanesulfonate (60.0 mg, 0.150 mmol), **5b** (45.4 mg, 0.195 mmol), and K_2CO_3 (60.2 mg, 0.436 mmol) in DMF (4 mL) was heated to 80 °C. After 1 hour the reaction mixture was cooled to RT, filtered to remove solids and the filtrate subjected to RP-HPLC purification (C18, MeCN/water with 0.1% TFA) followed by treatment with 1N aqueous HCl to afford **29** as the hydrochloride salt. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.40 (s, 1 H), 9.68 (s, 1 H), 8.47 (d, *J* = 2.1 Hz, 1 H), 8.10 – 8.18 (m, 3 H), 7.92 (dd, *J* = 8.1 Hz, 2.0 Hz, 1 H), 7.64 – 7.75 (m, 3 H), 7.39 – 7.50 (m, 1 H), 6.23 (s, 2 H). HRMS *m/z* calcd for $C_{25}H_{13}F_8N_5$ (M+1): 536.1116; found: 536.1118.

5-((6-(2,4-Bis(trifluoromethyl)phenyl)pyridin-3-yl)methyl)-2-(2,3-difluorophenyl)-5H-imidazo[4,5-d]pyridazine (31).

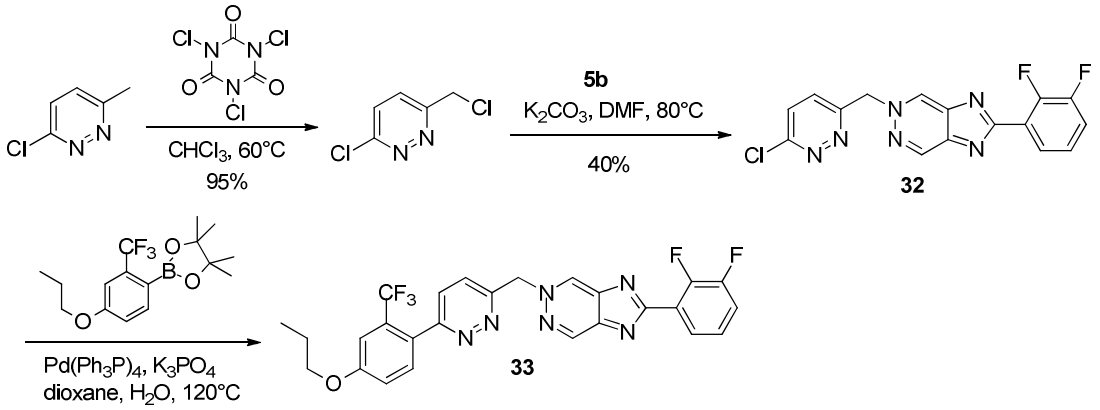


*(A) 2-(2,4-Bis(trifluoromethyl)phenyl)-5-methylpyridine (**30**).* A mixture of 1-bromo-2,4-bis(trifluoromethyl)benzene (1.47 g, 5.00 mmol), $Pd(Ph_3P)_4$ (150 mg, 0.130 mmol), and 0.5 M (5-methylpyridin-2-yl)zinc bromide/THF (10.0 mL, 5.00 mmol) was subjected to microwave heating at 130 °C for 10 minutes. The reaction mixture was partitioned between EtOAc and water and the phases separated. The EtOAc solution was washed with saturated brine, dried over Na_2SO_4, and concentrated to dryness in the presence of celite. Flash chromatography (silica gel, 5-35% EtOAc/hexanes) gave **30** (0.250 g, 16%) as a white solid.

*(B) 5-((6-(2,4-Bis(trifluoromethyl)phenyl)pyridin-3-yl)methyl)-2-(2,3-difluorophenyl)-5H-imidazo[4,5-d]pyridazine (**31**).* A solution of 2-(2,4-bis(trifluoromethyl)phenyl)-5-methylpyridine (0.375 g, 1.23 mmol), NBS (0.284 g, 1.60 mmol), and benzoyl peroxide (50.6 mg, 0.209 mmol) in CCl_4 (12 mL) was stirred at 80 °C for 2 hours and then cooled to RT. The solids were removed by filtration and the filtrate concentrated to dryness at reduced pressure. The residue was dissolved in DMF and the solution treated with **5b** (0.277 g, 1.19 mmol) followed by K_2CO_3 (0.263 g, 1.90 mmol). The mixture was stirred at 45°C for 20 minutes and then cooled to RT. The solids were removed by filtration and the filtrate concentrated in the presence of celite. The crude product was purified by flash

chromatography (silica gel, 0-20% MeOH/DCM) to give **31** (91.0 mg, 14%) as a tan solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.25 (s, 1 H), 9.54 (s, 1 H), 8.85 (s, 1 H), 8.11 – 8.20 (m, 3 H), 8.03 (d, *J* = 10.2 Hz, 1 H), 7.78 (d, *J* = 8.4 Hz, 1 H), 7.52 – 7.68 (m, 2 H), 7.34 (m, 1 H), 5.98 (s, 2 H). HRMS *m/z* calcd for $C_{25}H_{13}F_8N_5$ (M+1): 536.1116; found: 536.1120.

2-(2,3-Difluorophenyl)-5-((6-(4-propoxy-2-(trifluoromethyl)phenyl)pyridazin-3-yl)methyl)-5H-imidazo[4,5-d]pyridazine hydrochloride (33).



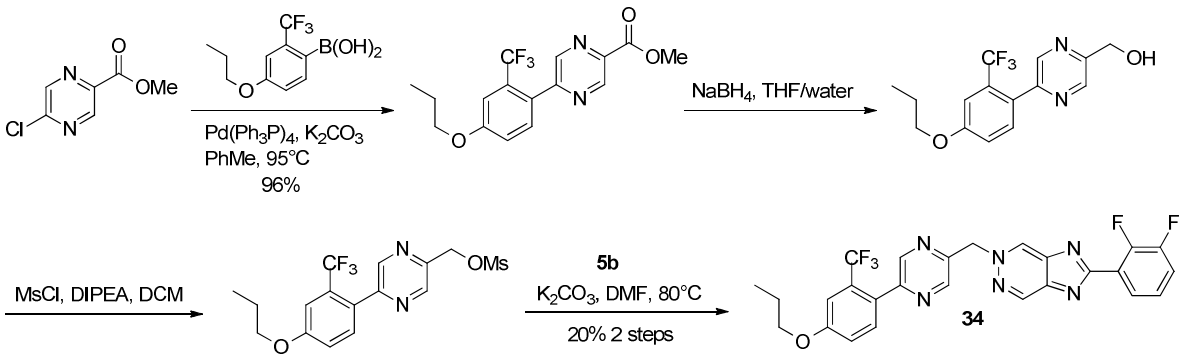
(A) 3-Chloro-6-(chloromethyl)pyridazine. To a stirred solution of 3-chloro-6-methylpyridazine (25.0 g, 194 mmol) in $CHCl_3$ (850 mL) at 60 °C was added trichloroisocyanuric acid (20.9 g, 78.0 mmol). After stirring at 60 °C for 15 hours, an additional charge of trichloroisocyanuric acid (3.00 g, 11.2 mmol) was added and the solution was stirred for an additional hour. The mixture was cooled in an ice bath, solids removed by filtration through celite, and the filtrate concentrated to dryness at reduced pressure to give the title compound (30.0 g, 95%) as a yellow oil which solidified on storage in the freezer.

*(B) 5-((6-Chloropyridazin-3-yl)methyl)-2-(2,3-difluorophenyl)-5H-imidazo[4,5-d]pyridazine (**32**).* To a solution of 3-chloro-6-(chloromethyl)pyridazine (0.140 g, 0.858 mmol) in DMF (1 mL) was added K_2CO_3 (0.198 g, 1.43 mmol) followed by **5b** (0.166 g, 0.715 mmol) and the mixture was heated to 80 °C for 5 minutes. The mixture was cooled to RT and partitioned between EtOAc and water and the phases separated. The aqueous solution was extracted with EtOAc (2x). The combined EtOAc solutions were washed with brine, dried over Na_2SO_4, and concentrated to dryness at reduced pressure to give **32** (64.0 mg, 25%) which was used in the next step without purification.

*(C) 2-(2,3-Difluorophenyl)-5-((6-(4-propoxy-2-(trifluoromethyl)phenyl)pyridazin-3-yl)methyl)-5H-imidazo[4,5-d]pyridazine (**33**).* A mixture of 5-((6-chloropyridazin-3-yl)methyl)-2-(2,3-difluorophenyl)-5H-imidazo[4,5-d]pyridazine (56.0 mg, 0.156 mmol), 4-propoxy-2-(trifluoromethyl)phenyl)boronic acid pinacol ester (51.5 mg, 0.156 mmol), 2N aqueous Na_2CO_3 (0.234 mL, 0.468 mmol), and $Pd(Ph_3P)_4$ (4.51 mg, 3.90 µmol) in toluene (5 mL) was sparged with nitrogen for several minutes and then heated to 80 °C for 30 minutes. The mixture was cooled to RT, partitioned between EtOAc and water, and the phases separated. The aqueous phase was extracted

with EtOAc (2x). The combined EtOAc solutions were washed with brine, dried over Na_2SO_4, and concentrated to dryness at reduced pressure. The crude product was purified by RP-HPLC purification (C18, MeCN/water with 0.1% TFA) followed by treatment with 1N aqueous HCl to afford **33** as the hydrochloride salt. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.42 (s, 1 H), 9.67 (s, 1 H), 8.17 (m, 1 H), 7.96 (d, *J* = 8.7 Hz, 1 H), 7.88 (d, *J* = 8.7 Hz, 1 H), 7.34 – 7.69 (m, 5 H), 6.39 (s, 2 H), 4.07 (t, *J* = 7.4 Hz, 2 H), 1.70 – 1.82 (m, 2 H), 0.98 (t, *J* = 7.3 Hz, 3 H). HRMS *m/z* calcd for $C_{26}H_{19}F_5N_6O$ (M+1): 527.1613; found: 527.1618.

2-(2,3-Difluorophenyl)-5-((5-(4-propoxy-2-(trifluoromethyl)phenyl)pyrazin-2-yl)methyl)-5H-imidazo[4,5-d]pyridazine (34).



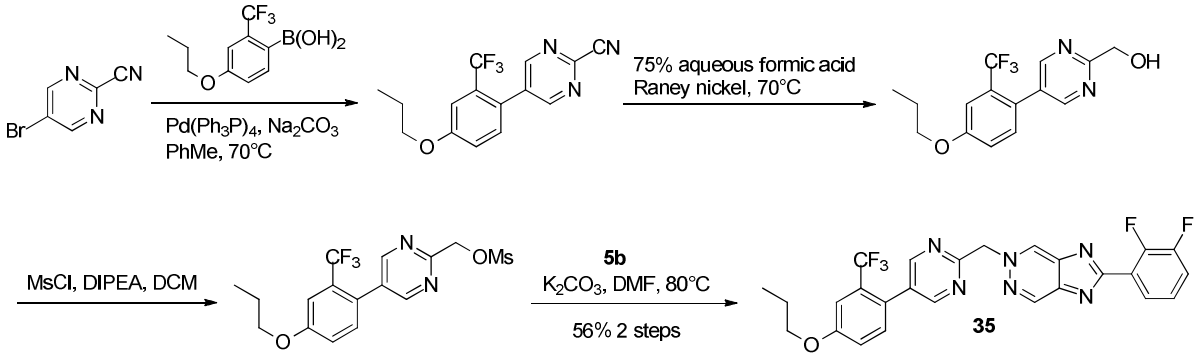
(A) Methyl 5-(4-propoxy-2-(trifluoromethyl)phenyl)pyrazine-2-carboxylate. A mixture of methyl 5-chloropyrazine-2-carboxylate (0.173 g, 1.00 mmol), (4-propoxy-2-(trifluoromethyl)phenyl)boronic acid (0.298 g, 1.20 mmol), $Pd(Ph_3P)_4$ (58.0 mg, 0.0500 mmol) and 2M aqueous K_2CO_3 (0.902 mL, 1.80 mmol) in toluene (1.8 mL) was sparged with argon for several minutes and then stirred at 95 °C. After 1 hour the mixture was cooled to RT, partitioned between EtOAc and water, and the phases separated. The EtOAc solution was washed with brine (1x), dried over Na_2SO_4, and concentrated to dryness at reduced pressure. The crude material was subjected to flash chromatography (silica gel, 20-70% EtOAc/hexanes) to afford the title compound (0.328 g, 96%) as a waxy white solid.

(B) (5-(4-Propoxy-2-(trifluoromethyl)phenyl)pyrazin-2-yl)methanol. To a stirred solution of methyl 5-(4-propoxy-2-(trifluoromethyl)phenyl)pyrazine-2-carboxylate (0.150 g, 0.441 mmol) in 7:2 THF/H_2O (9 mL) was added $NaBH_4$ portionwise (1.50 g, 39.6 mmol). The reaction was briefly warmed, and then stirred without heating for 30 minutes. The solution was partitioned between water and DCM and the phases separated. The DCM solution was dried over Na_2SO_4 and evaporated to dryness in the presence of celite. The crude material was subjected to flash chromatography (silica gel, 30-50% EtOAc/hexanes) to give the title compound (90.0 mg, 65%).

(C) (5-(4-Propoxy-2-(trifluoromethyl)phenyl)pyrazin-2-yl)methyl methanesulfonate. The title compound was prepared from (5-(4-propoxy-2-(trifluoromethyl)phenyl)pyrazin-2-yl)methanol (90 mg, 0.288 mmol) as described herein for the synthesis of compound **29**, step B. The crude product was used in the next step without purification.

*(D) 2-(2,3-Difluorophenyl)-5-((5-(4-propoxy-2-(trifluoromethyl)phenyl)pyrazin-2-yl)methyl)-5H-imidazo[4,5-d]pyridazine (**34**).* Compound **34** was prepared from (5-(4-propoxy-2-(trifluoromethyl)phenyl)pyrazin-2-yl)methyl methanesulfonate and **5b** as described herein for the synthesis of **29**, step C. The crude product was purified by flash chromatography (silica gel, 0-15% MeOH/DCM) to afford **34** (31 mg, 20% for 2 steps). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.54 (s, 1 H), 9.73 (s, 1 H), 8.90 (d, *J* = 1.2 Hz, 1 H), 8.63 (d, *J* = 1.0 Hz, 1 H), 8.10 – 8.14 (m, 1 H), 7.65 – 7.76 (m, 1 H), 7.40 – 7.51 (m, 2 H), 7.27 – 7.31 (m, 2 H), 6.30 (s, 2 H), 4.00 (t, *J* = 6.6 Hz, 2 H), 1.64 – 1.75 (m, 2 H), 0.93 (t, *J* = 7.4 Hz, 3 H). HRMS *m/z* calcd for $C_{26}H_{19}F_5N_6O$ (M+1): 527.1613; found: 527.1615.

2-(2,3-Difluorophenyl)-5-((5-(4-propoxy-2-(trifluoromethyl)phenyl)pyrimidin-2-yl)methyl)-5H-imidazo[4,5-d]pyridazine (35).



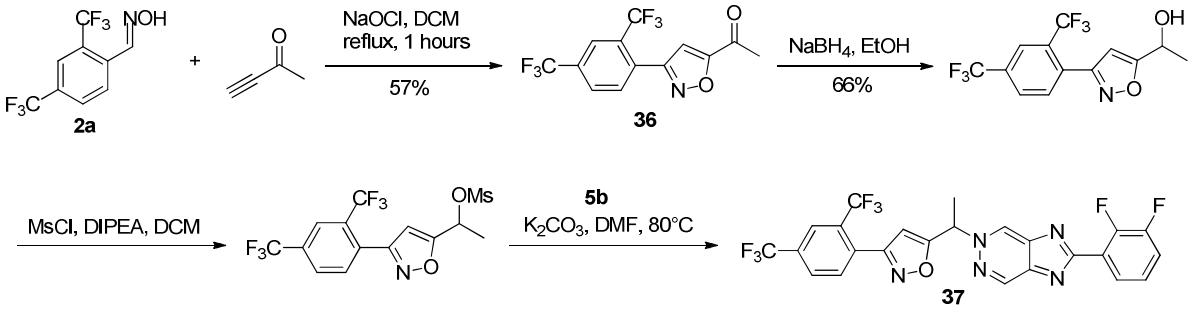
(A) 5-(4-Propoxy-2-(trifluoromethyl)phenyl)pyrimidine-2-carbonitrile. A mixture of 5-bromopyrimidine-2-carbonitrile (0.445 g, 2.42 mmol), (4-propoxy-2-(trifluoromethyl)phenyl)boronic acid (0.900 g, 3.63 mmol), $Pd(Ph_3P)_4$ (0.140 g, 0.121 mmol), and 2M aqueous Na_2CO_3 (1.57 mL, 3.14 mmol) in toluene (12 mL) was briefly sparged with nitrogen and then heated to 70 °C with stirring. After 18 hours the mixture was cooled to RT, partitioned between EtOAc and water, and the phases separated. The EtOAc solution was washed with brine, dried over Na_2SO_4, and concentrated to dryness at reduced pressure. The crude material was subjected to flash chromatography (silica gel, EtOAc/hexanes) to afford the title compound (0.525 g, 71%).

(B) (5-(4-Propoxy-2-(trifluoromethyl)phenyl)pyrimidin-2-yl)methanol. A solution of 5-(4-propoxy-2-(trifluoromethyl)phenyl)pyrimidine-2-carbonitrile (0.500 g, 1.63 mmol) in 75% aqueous formic acid was treated with Raney nickel (approximately 100 mg) and heated to 70 °C. After 18 hours the mixture was cooled to RT, catalyst removed by filtration through celite, and the filtrate concentrated to dryness at reduced pressure. The crude product was subjected to flash chromatography (silica gel, EtOAc/hexanes) to afford the title compound.

(C) (5-(4-Propoxy-2-(trifluoromethyl)phenyl)pyrimidin-2-yl)methyl methanesulfonate. The title compound was prepared from (5-(4-propoxy-2-(trifluoromethyl)phenyl)pyrimidin-2-yl)methanol (40.0 mg, 0.128 mmol) as described herein for the synthesis of compound **29**, step B. The crude product was used in the next step without purification.

*(D) 2-(2,3-Difluorophenyl)-5-((5-(4-propoxy-2-(trifluoromethyl)phenyl)pyrimidin-2-yl)methyl)-5H-imidazo[4,5-d]pyridazine (**35**).* Compound **35** was prepared from (5-(4-propoxy-2-(trifluoromethyl)phenyl)pyrimidin-2-yl)methyl methanesulfonate and **5b** as described herein for the synthesis of **29**, step C. The crude product was purified by flash chromatography (silica gel, MeOH/DCM) to afford **35** (38 mg, 56% for 2 steps). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.39 (s, 1 H), 9.71 (s, 1 H), 8.77 (s, 2 H), 8.13 – 8.17 (m, 1 H), 7.68 – 7.71 (m, 1 H), 7.32 – 7.47 (m, 4 H), 6.25 (s, 2 H), 4.05 (t, *J* = 6.7 Hz, 2 H), 1.72 – 1.77 (m, 2 H), 0.97 (t, *J* = 7.4 Hz, 3 H). HRMS *m/z* calcd for $C_{26}H_{19}F_5N_6O$ (M+1): 527.1613; found: 527.1610.

3-(2,4-Bis(trifluoromethyl)phenyl)-5-(1-(2-(2,3-difluorophenyl)-5H-imidazo[4,5-d]pyridazin-5-yl)ethyl)isoxazole hydrochloride (37).



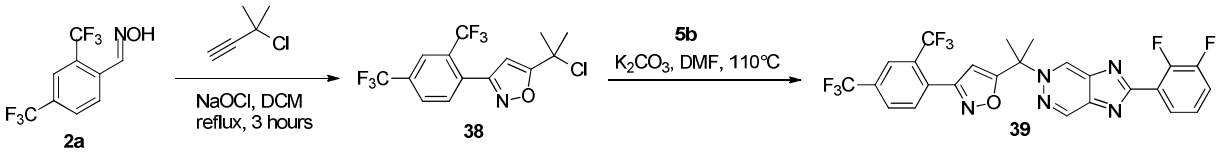
*(A) 1-(3-(2,4-Bis(trifluoromethyl)phenyl)isoxazol-5-yl)ethanone (**36**).* To a stirred solution of **2a** (1.03 g, 4.00 mmol) and but-3-yn-2-one (0.272 g, 4.00 mmol) in DCM at 0 °C was added 10% aqueous NaOCl (6.30 mL, 8.40 mmol). The solution was stirred at 0 °C for 10 minutes and then heated to reflux. After 1 hour the mixture was cooled to RT and partitioned between water and DCM and the phases separated. The DCM solution was washed with saturated aqueous brine (1x), dried over Na_2SO_4, and concentrated to dryness at reduced pressure to give **36** (0.74 g, 57%) which was used in the next step without purification.

(B) 1-(3-(2,4-Bis(trifluoromethyl)phenyl)isoxazol-5-yl)ethanol. A solution of **36** (0.660 g, 2.04 mmol) in EtOH (40 mL) was treated with $NaBH_4$ (0.300 g, 7.93 mmol) and the resulting solution stirred at RT. After 20 minutes the solution was diluted with water and extracted with EtOAc (3x). The combined EtOAc extracts were concentrated to dryness and the residue purified by flash chromatography (silica gel, 0-40% EtOAc/hexanes) to afford the title compound (0.44 g, 66%).

(C) 1-(3-(2,4-Bis(trifluoromethyl)phenyl)isoxazol-5-yl)ethyl methanesulfonate. A solution of 1-(3-(2,4-bis(trifluoromethyl)phenyl)isoxazol-5-yl)ethanol (0.200 g, 0.615 mmol) in DCM (5 mL) was treated with DIPEA (200 µL, 1.15 mmol) followed by MsCl (65 µL, 0.834 mmol). After stirring at RT for 5 minutes the solution was partitioned between water and EtOAc and the phases separated. The EtOAc solution was washed with saturated brine (1x), dried over sodium sulfate, and concentrated to dryness at reduced pressure to give the title compound which was used in the next step without purification.

*(D) 3-(2,4-Bis(trifluoromethyl)phenyl)-5-(1-(2-(2,3-difluorophenyl)-5H-imidazo[4,5-d]pyridazin-5-yl)ethyl)isoxazole (**37**).* A stirred mixture of 1-(3-(2,4-bis(trifluoromethyl)phenyl)isoxazol-5-yl)ethyl methanesulfonate (0.100 g, 0.248 mmol), **5b** (48.0 mg, 0.207 mmol) and K_2CO_3 (69.0 mg, 0.496 mmol) in DMF was subjected to microwave heating at 80 °C for 10 minutes. The reaction mixture was filtered to remove solids and the filtrate subjected to RP-HPLC purification (C18, MeCN/water with 0.1% TFA) followed by treatment with 1N aqueous HCl to afford **37** as the hydrochloride salt. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.33 (s, 1 H), 9.65 (s, 1 H), 8.11 – 8.26 (m, 3 H), 7.92 (d, *J* = 7.8 Hz, 1 H), 7.59 – 7.21 (m, 1 H), 7.37 – 7.45 (m, 1 H), 7.01 (s, 1 H), 6.64 (q, *J* = 6.9 Hz, 1 H), 2.10 (d, *J* = 6.9 Hz, 3 H). HRMS *m/z* calcd for $C_{24}H_{13}F_8N_5O$ (M+1): 540.1065; found: 540.1068.

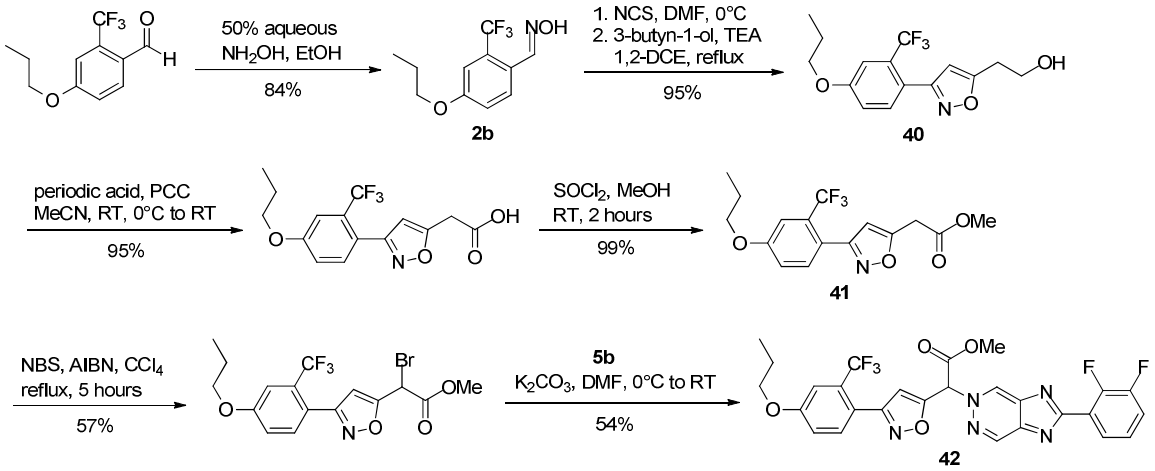
3-(2,4-Bis(trifluoromethyl)phenyl)-5-(2-(2-(2,3-difluorophenyl)-5H-imidazo[4,5-d]pyridazin-5-yl)propan-2-yl)isoxazole hydrochloride (39).



*(A) 3-(2,4-Bis(trifluoromethyl)phenyl)-5-(2-chloropropan-2-yl)isoxazole (**38**).* A suspension of **2a** (1.00 g, 3.89 mmol) and 3-chloro-3-methylbut-1-yne (0.798 g, 7.78 mmol) in DCM (5 mL) at 0 °C was treated with 10% aqueous NaOCl (4.00 mL, 5.38 mmol) by dropwise addition. After stirring at 0 °C for 15 minutes, the solution was heated to reflux. After 3 hours the reaction mixture was cooled to RT, partitioned between DCM and water, and the phases separated. The DCM solution was washed with saturated brine, dried over sodium sulfate and concentrated to dryness at reduced pressure to give crude **38** which was used in the next step without purification.

*(B) 3-(2,4-Bis(trifluoromethyl)phenyl)-5-(2-(2-(2,3-difluorophenyl)-5H-imidazo[4,5-d]pyridazin-5-yl)propan-2-yl)isoxazole (**39**).* A stirred mixture of **38** (0.179 g, 0.500 mmol), **5b** (0.116 g, 0.500 mmol), and K_2CO_3 (0.338 g, 2.45 mmol) in DMF (5 mL) was heated to 80 °C for 18 hours at which point LCMS indicated very little conversion of the starting materials to the desired product. The mixture was then heated to 100 °C for 3 hours and then briefly at 110 °C. The reaction mixture was cooled to RT, filtered to remove solids and the filtrate subjected to RP-HPLC purification (C18, MeCN/water with 0.1% TFA) followed by treatment with 1N aqueous HCl to afford **39** as the hydrochloride salt. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.44 (s, 1 H), 9.69 (s, 1 H), 8.21 – 8.27 (m, 2 H), 8.09 – 8.18 (m, 1 H), 7.91 – 7.99 (m, 1 H), 7.65 – 7.26 (m, 1 H), 7.40 – 7.49 (m, 1 H), 7.12 (s, 1 H), 2.30 (s, 6 H). HRMS *m/z* calcd for $C_{25}H_{15}F_8N_5O$ (M+1): 554.1222; found: 554.1226.

Methyl 2-(2-(2,3-difluorophenyl)-5H-imidazo[4,5-d]pyridazin-5-yl)-2-(3-(4-propoxy-2-(trifluoromethyl)phenyl)isoxazol-5-yl)acetate (42).



*(A) 4-Propoxy-2-(trifluoromethyl)benzaldehyde oxime (**2b**).* A solution of 4-(propyloxy)-2-(trifluoromethyl)benzaldehyde (16.3 g, 70.2 mmol) in EtOH (70 mL) was treated with 50% aqueous hydroxylamine (10.0 mL, 140 mmol) and the resulting solution was stirred at RT. After 2 hours TLC (silica gel, 8:2 hexane/EtOAc) indicated complete conversion of the aldehyde starting material to a new, lower R_f spot. The solution was concentrated by rotary evaporation to a volume of approximately 25 mL at which point a white solid had precipitated. The suspension was diluted with 60 mL of water and stirred for 45 minutes. The solid was collected by vacuum filtration on a medium frit. The filter cake was washed twice with water, suction dried for 20 minutes and then dried in vacuo overnight to afford 16.9 g of a very pale yellow crystalline solid. The crude material was recrystallized from hexane/EtOAc to afford **2b** (14.5 g, 84%) as a white crystalline solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.58 (s, 1 H), 8.19 – 8.23 (m, 1 H), 7.93 (d, *J* = 8.6 Hz, 1 H), 7.21 – 7.30 (m, 2 H), 4.03 (t, *J* = 6.6 Hz, 2H), 1.68 – 1.80 (m, 2H), 0.98 (t, *J* = 7.4 Hz, 3H). ES-LCMS *m/z*: 248 (M+1).

*(B) 2-(3-(4-Propoxy-2-(trifluoromethyl)phenyl)isoxazol-5-yl)ethanol (**40**).* A stirred solution of **2b** (3.00 g, 12.1 mmol) in DMF (25 mL) was cooled to 0 °C and treated with NCS (1.70 g, 12.7 mmol) by slow addition over 1 minute. After 1 hour the solution was diluted with EtOAc, washed with water (2x), brine (1x), dried over Na_2SO_4 and concentrated to dryness at reduced pressure. The residue was dissolved in 1,2-DCE (35 mL) and the solution treated with 3-butyn-1-ol (1.30 g, 18.2 mmol) followed by TEA (2.60 mL, 18.2 mmol). A solid immediately precipitated (TEA-HCl salt). The suspension was heated to reflux with stirring at which point the solid had gone into solution. After 30 minutes the solution was cooled to RT and stirred overnight. The solution was diluted 2-fold with DCM, washed with water (3x), brine (1x), dried over Na_2SO_4 and concentrated to dryness at reduced pressure to afford **40** (4.02 g, 95%) as a viscous, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, *J* = 8.6 Hz, 1 H), 7.27 (d, *J* = 2.3 Hz, 1 H), 7.07 (dd, *J* = 8.5 Hz, 2.5 Hz, 1 H), 6.27 (s, 1 H), 3.94 – 4.02 (m, 4 H), 3.06 (t, *J* = 6.2 Hz, 2 H), 1.78 – 1.88 (m, 2 H), 1.76 (br s, 1H) 1.04 (t, *J* = 7.4 Hz, 3 H). ES-LCMS *m/z*: 316 (M+1).

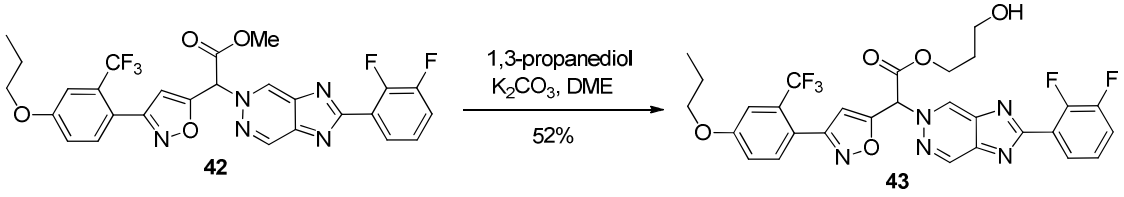
(C) 2-(3-(4-Propoxy-2-(trifluoromethyl)phenyl)isoxazol-5-yl)acetic acid. Periodic acid (4.58 g, 20.1 mmol) was added to anhydrous MeCN (70 mL) and the mixture stirred at RT for 15 minutes. The solid reagent slowly dissolved to afford a clear solution. A solution of **40** (2.88 g, 9.13 mmol) in MeCN (15 mL) was added and the resulting solution was cooled in an ice water bath. The solution was then treated with PCC (40 mg, 0.18 mmol). A light yellow precipitate was rapidly produced. The ice bath was removed and the reaction mixture was allowed to warm to RT with stirring. After 3 hours LCMS indicated complete conversion to the desired product. The mixture was subjected to rotary evaporation to a volume of approximately 15 mL and was then diluted with 100 mL of 9:1 chloroform/iPrOH. The rapidly stirred suspension was treated with 10% aqueous sodium bisulfate (100 mL) and the mixture stirred vigorously for a short time. The mixture was transferred to a separatory funnel and the phases separated. The organic solution was washed with water (3x), brine (1x), dried over sodium sulfate and concentrated to dryness at reduced pressure to afford the title compound (2.85 g, 95%) as a light tan solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.90 (br s, 1 H), 7.58 (d, *J* = 8.4 Hz, 1 H), 7.31 – 7.39 (m, 2 H), 6.57 (s, 1 H), 4.08 (t, *J* = 6.6 Hz, 2 H), 3.98 (s, 2 H), 1.71 – 1.82 (m, 2 H), 1.00 (t, *J* = 7.4 Hz, 3 H). ES-LCMS *m/z*: 330 (M+1).

*(D) Methyl 2-(3-(4-propoxy-2-(trifluoromethyl)phenyl)isoxazol-5-yl)acetate (**41**).* Thionyl chloride (4 mL) was slowly added to a stirred 20 mL portion of MeOH. After 10 minutes a solution of 2-(3-(4-propoxy-2-(trifluoromethyl)phenyl)isoxazol-5-yl)acetic acid (0.530 g, 1.61 mmol) in MeOH (6 mL) was added and the resulting solution was stirred at RT. After 2 hours the solution was concentrated to dryness at reduced pressure and the residue dissolved in EtOAc. The solution was washed with saturated aqueous sodium bicarbonate (2x), brine (1x), dried over Na_2SO_4 and concentrated to dryness at reduced pressure to afford **41** (0.55 g, 99%) as a light yellow oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.53 (d, *J* = 8.4 Hz, 1 H), 7.27 – 7.34 (m, 2 H), 6.55 (s, 1 H), 4.07 (s, 2 H), 4.03 (t, *J* = 6.44 Hz, 2 H), 3.64 (s, 3 H), 1.67 – 1.78 (m, 2 H), 1.00 (t, *J* = 7.4 Hz, 3 H). ES-LCMS *m/z*: 344 (M+1).

(E) Methyl 2-bromo-2-(3-(4-propoxy-2-(trifluoromethyl)phenyl)isoxazol-5-yl)acetate. A mixture of methyl 2-(3-(4-propoxy-2-(trifluoromethyl)phenyl)isoxazol-5-yl)acetate (0.383 g, 1.12 mmol), NBS (0.238 g, 1.34 mmol) and AIBN (10 mg , 0.061 mmol) in CCl_4 (20 mL) was heated to reflux with stirring under a nitrogen atmosphere. After 2 hours TLC (silica gel, 1:1 hexane/EtOAc) indicated approximately 50% conversion of the starting material to a slightly higher R_f component. The mixture was treated with an additional portion of NBS (0.200 g, 1.12 mmol) followed by AIBN (10 mg, 0.061 mmol) and stirring at reflux continued. After 3 more hours of refluxing the reaction mixture was cooled to RT and stirred overnight. The mixture was diluted with DCM and the solids removed by filtration through a medium fritted funnel. The filtrate was concentrated to dryness at reduced pressure and the residue subjected to flash chromatography (silica gel, 0-60% EtOAc/hexanes) to afford the title compound (0.268 g, 57%) as an oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.63 (d, *J* = 8.4 Hz, 1 H), 7.33 – 7.42 (m, 2 H), 6.90 (s, 1 H), 6.44 (s, 1 H), 4.09 (t, *J* = 6.45 Hz, 2 H), 3.81 (s, 3 H), 1.73 – 1.82 (m, 2 H), 1.00 (t, *J* = 7.4 Hz, 3 H).

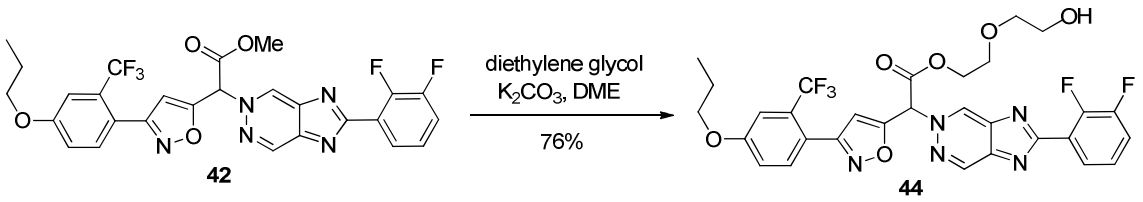
*(F) Methyl 2-(2-(2,3-difluorophenyl)-5H-imidazo[4,5-d]pyridazin-5-yl)-2-(3-(4-propoxy-2-(trifluoromethyl)phenyl)isoxazol-5-yl)acetate (**42**).* A mixture of **5b** (0.313 g, 1.35 mmol) in DMF (10 mL) was briefly warmed to dissolve the starting material. The resulting solution was treated with K_2CO_3 (0.622 g, 4.50 mmol) and cooled in an ice water bath. A solution of methyl 2-bromo-2-(3-(4-propoxy-2-(trifluoromethyl)phenyl)isoxazol-5-yl)acetate (0.380 g, 0.900 mmol) in DMF (4 mL) was added dropwise over 3 minutes. The mixture was allowed to warm to RT. After 1.5 hours the mixture was partitioned between 10% aqueous NaCl and EtOAc and the phases separated. The aqueous phase was back extracted with EtOAc (3x). The combined EtOAc solutions were washed with 10% aqueous NaCl (2x), saturated aqueous brine (1x), dried over Na_2SO_4 and concentrated to dryness at reduced pressure. The crude product was subjected to flash chromatography (silica gel, 0-10% MeOH/DCM) followed by reverse phase HPLC purification (C18, 10-100% MeCN/water with 0.1% TFA). Fractions containing pure product were combined and concentrated to dryness at reduced pressure. The residue was dissolved in EtOAc. The solution was washed with saturated aqueous sodium bicarbonate (1x), brine (1x), dried over sodium sulfate and concentrated to dryness at reduced pressure to afford **42** (0.280 g, 54%) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.40 (s, 1 H), 9.29 (s, 1 H), 8.15 – 8.23 (m, 1 H), 7.58 (d, *J* = 8.6 Hz, 1 H), 7.17 – 7.34 (m, 3 H), 7.12 (dd, *J* = 8.6 Hz, 2.4 Hz, 1 H), 6.88 (s, 1 H), 6.85 (s, 1 H), 4.00 (t, *J* = 6.5 Hz, 2 H), 3.94 (s, 3 H), 1.78 – 1.91 (m, 2 H), 1.05 (t, *J* = 7.4 Hz, 3 H). HRMS *m/z* calcd for $C_{27}H_{20}F_5N_5O_4$ (M+1): 574.1508; found: 574.1504.

3-Hydroxypropyl 2-(2-(2,3-difluorophenyl)-5H-imidazo[4,5-d]pyridazin-5-yl)-2-(3-(4-propoxy-2-(trifluoromethyl)phenyl)isoxazol-5-yl)acetate (43).



A solution of compound **42** (60.0 mg, 0.105 mmol) in 1:1 DME/1,3-propanediol (5 mL) was treated with K_2CO_3 (20.0 mg, 0.145 mmol) and stirred at RT. After 4 hours the solution was treated with glacial acetic acid (0.50 mL) and diluted with EtOAc. The resulting solution was washed with water (2x), brine (1x), dried over sodium sulfate, and concentrated to dryness at reduced pressure. The crude product was subjected to RP-HPLC purification (C18, 10-90% MeCN/H_2O with 0.1% TFA) to afford **43** (34 mg, 52%) as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.44 (s, 1 H), 9.28 (s, 1 H), 8.15 – 8.22 (m, 1 H), 7.57 (d, *J* = 8.6 Hz, 1 H), 7.18 – 7.34 (m, 3 H), 7.12 (dd, *J* = 8.6 Hz, 2.4 Hz, 1 H), 6.91 (s, 1 H), 6.84 (s, 1 H), 4.43 – 4.58 (m, 2 H), 4.00 (t, *J* = 6.6 Hz, 2 H), 3.67 (t, *J* = 5.9 Hz, 2 H), 1.79 – 2.01 (m, 5 H), 1.06 (t, *J* = 7.4 Hz, 3 H). ES-LCMS *m/z*: 618 (M+1).

2-(2-Hydroxyethoxy)ethyl 2-(2-(2,3-difluorophenyl)-5H-imidazo[4,5-d]pyridazin-5-yl)-2-(3-(4-propoxy-2-(trifluoromethyl)phenyl)isoxazol-5-yl)acetate (44).



To a stirred solution of compound **42** (2.00 g, 3.49 mmol) in 1:1 diethylene glycol/DME (25 mL) was added K_2CO_3 (1.46 g, 10.5 mmol) and the resulting mixture stirred at RT. After 18 hours the mixture was treated with glacial acetic acid (3 mL) and diluted with EtOAc. The resulting mixture was washed with water (4x), brine (1x), dried over Na_2SO_4, and concentrated to dryness at reduced pressure. The crude material was subjected to flash chromatography (silica gel, 50-100% EtOAc/hexanes) followed by recrystallization from EtOAc/hexanes to afford compound **44** (1.71 g, 76%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.10 (s, 1 H), 9.55 (s, 1 H), 8.15 – 8.22 (m, 1 H), 7.72 (s, 1 H), 7.54 – 7.68 (m, 2 H), 7.33 – 7.42 (m, 3 H), 7.13 (s, 1 H), 4.55 (t, *J* = 5.2 Hz, 1 H), 4.33 – 4.49 (m, 2 H), 4.09 (t, *J* = 6.6 Hz, 2 H), 3.62 (t, *J* = 4.5 Hz, 2 H), 3.28 – 3.43 (m, 4 H), 1.71 – 1.84 (m, 2 H), 1.00 (t, *J* = 7.4 Hz, 3 H). HRMS *m/z* calcd for $C_{30}H_{27}F_5N_5O_6$ (M+1): 648.1882; found: 648.1888.

2. InVivo/In Vitro DMPK Profiling

All studies were conducted after review by the Institutional Animal Care and Use Committee at GSK (or at the institution where the work was performed, if not at GSK) and in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals

2a. Pharmacokinetics

Male CD rats (Charles River Labs, Raleigh, NC) were dosed intravenously at 1 mg/kg (n=2) and orally at doses of 5 mg/kg (n=2 or n=3) and for some test compounds at higher doses as indicated in the text, for pharmacokinetic evaluations. All prodrugs were administered as equivalents of the parent compound. Male beagle dogs were dosed intravenously at 1 mg/kg and orally at 5 mg/kg for pharmacokinetic evaluations, with n=3 animals for each route of administration. Animals were serially sampled for all studies. Samples were extracted by protein precipitation and centrifugation and analyzed by LC/MS/MS. If ester prodrugs were administered in pharmacokinetic studies, all steps of sample preparation were conducted on ice in order to prevent conversion of the ester to parent compound, which occurred at room temperature in blood and plasma.

2b. Hepatocyte Stability

Test compounds (0.5 µM) were incubated with pooled cryopreserved hepatocytes from mouse, rat, dog, monkey or human donors for 120 minutes. At time points after initiation of the incubation, 100 µL of incubation was transferred to a 96-well plate containing 200 µL of acetonitrile/0.1% formic acid to quench the reaction. The samples were extracted by protein precipitation and centrifugation prior to LC/MS/MS analysis. The metabolic stability was expressed as the percentage of the test compound remaining as calculated by the peak area ratio of the test compound remaining after incubation timepoint (t_x) compared to the time zero (t_0) incubation. The half-life ($t_{1/2}$) in hepatocytes was calculated using the following equation:

$T_{1/2} = -\ln(2)/k,$

Where k is the turnover rate constant of the ln % remaining vs. time regression. The intrinsic clearance (Cl'int) was calculated using correction factors for the number of cells in the incubation, the number of cells per gram of liver tissue, and the grams of liver tissue per kilogram body weight for each respective species. The predicted clearance was calculated by normalizing for liver blood flow using the well-stirred model.

2c. Microsomal Stability

Microsomes were purchased from XenoTech (Lenexa, Kansas). The stock protein content of all microsomal preparations was 20 mg/mL. Microsomes were diluted to a concentration of 0.89 mg/mL with Tris-HCl buffer, pH 7.4. Incubations were carried out in 96-well polypropylene plates on a shaker maintained at 37 °C. A mixture of

0.5 μM of the test compound and 0.5 mg/mL microsomes were preincubated for 5 minutes with shaking at 37°C and the reaction was initiated by adding NaDPH and $MgCl_2$ to a final concentration of 2 mM and 5 mM, respectively. 100-μL aliquots were collected at 0, 5, 10, 15, 20, 25, and 30 min. Each aliquot was added to 200 μL of ice-cold acetonitrile:methanol (80:20, v/v). Precipitated protein was pelleted by centrifugation and the resultant supernatant was transferred to a new 96-well polypropylene plate for LC/MS/MS analysis. The metabolic viability of the microsomes preparations was confirmed using quality control (QC) standards (Verapamil, Haloperidol, and Amitriptyline). The clearance (ml/min/kg) classification as: High (>70% hepatic blood flow); Moderate (30~70% hepatic blood flow; Low (<30% hepatic blood flow).

2d. Plasma Protein Binding

Plasma protein binding was measured using the Rapid Equilibrium Dialysis (RED) device and inserts, obtained from Pierce. The RED inserts, with plasma and buffer compartments, were added to the 48 well Teflon RED plate. A 10 μl aliquot of test compound (1 mM stock in DMSO) was added to plasma to yield a final concentration of 5 μM. Triplicate aliquots (300 μl) of plasma containing test compounds at a concentration of 5 μM were pipetted to plasma side (red color) of the insert and 500μl of 0.1 M PBS (phosphate buffered saline) pH 7.4 was placed into the receiver side (white color) of the insert. The plate was covered with sealing tape and incubated in a 37 °C orbital shaker water bath at approximately 100 rpm for 4 hours. Following incubation, 100 μl of the plasma dialysate was added to 100 μl of blank PBS and 100 μl of PBS dialysate was added to 100 μl blank plasma from the corresponding species. Triplicate aliquots (100 μL) of test compounds at a concentration of 5 μM that were not dialyzed from each species were added to blank PBS as a measure of recovery. Aliquots of the samples were extracted by protein precipitation and centrifugation prior to analysis by LC/MS/MS.

2e. Stability of Ester 42 and compound 16 in buffer and biological media

Stability of compounds **16** and **42** were measured in vitro over time in blood and in buffer at pH 2 and pH 7. Duplicate incubations of each compound were prepared at a concentration of 1 μM (parent equivalent) in a deep-well 96-well plate on an orbital shaker, with samples collected at time zero and through 60 minutes. Samples were extracted by protein precipitation and analyzed by LC/MS/MS for the ester **42** and compound **16**.

2f. 7-Day toxicity studies (Compound 44)

DOG. The objective of this study was to determine the toxicity and toxicokinetics of compound **44** in a 7-day, oral, dose range-finding study in beagle dogs. Compound **44** was wet bead milled and formulated as a suspension in 1% hydroxypropyl methylcellulose (Pharmacoat™ 603)/0.2% sodium dodecyl sulfate in reverse osmosis-treated water and administered to dogs at a dose volume of 10 mL/kg.

Compound **44** was given to dogs (1/sex/group) at doses of 10, 60, or 600 mg/kg/day once daily for 7 days by oral gavage. This study was conducted at GlaxoSmithKline, Research Triangle Park, NC, USA. The following endpoints/parameters were evaluated: clinical observations, body weights, hematology and clinical chemistry results, and macroscopic and microscopic observations. Toxicokinetic evaluation was performed on samples collected on days 1 and 7.

RAT: The objective of this study was to determine the toxicity and toxicokinetics of compound **44** in a 7-day, oral, repeat-dose study in rats. Compound **44** was formulated as a suspension in 1% hydroxypropyl methylcellulose (Pharmacoat™ 603)/0.2% sodium dodecyl sulfate and administered to rats at a dose volume of 10 mL/kg. Compound **44** was given to male rats (4/group) at doses of 0 (vehicle), 10, 100 or 1000 mg/kg/day once daily for 7 days by oral gavage. Three male animals were added at each test article dose level for toxicokinetic evaluation. Single dose-escalation pharmacokinetic studies with compound **44** showed that 10, 100, and 1000 mg/kg/day produced approximate exposures of 12, 60, and 320 μg.h/mL of the parent compound **16**, respectively.

This study was conducted at GlaxoSmithKline, Research Triangle Park, NC, USA. The following endpoints/parameters were evaluated for toxicology animals: clinical observations, body weights, hematology and clinical chemistry results, liver weights, macroscopic and microscopic observations, and hepatic gene expression analysis. Toxicokinetic evaluation was performed on samples collected on days 1 and 7.

3. Solubility Determinations- CLND

An aliquot of 5 μl of a 10 mM stock solution of compound in DMSO was diluted to 100 μl with a 10 mM phosphate buffered saline, pH 7.4, equilibrated for 1 h at RT, and filtered through Millipore MultiscreenHTS-PCF filter plates (MSSL BPC). The eluent was quantified by suitably calibrated flow injection ChemiLuminescence Nitrogen Detection (CLND). Hill, A.; Young, R.; *Drug Discovery Today*, **2010**, *15*, 648 - 655.

4. HCV Replicon Assays

4a. Stable HCV replicon assays for compounds 1, 6 and 7

Huh-luc/neo-ET replicon cells were removed from flasks with a 0.05% trypsin/EDTA solution and resuspended in medium supplemented with 10% FBS, penicillin-streptomycin, non-essential amino acids, glutamine and geneticin. The cell density was adjusted to 7.9×10^4 cells/mL, and 95 μl of cell suspension were placed in columns 2-12 of 96-well plates using a MultiDrop liquid dispenser (Thermo LabSystems), for a density of 7,500 cells/well. 95 μl of

medium was dispensed to column 1 for the background controls. Two sets of plates were prepared: white opaque plates for assay of luciferase activity and clear plates for assay of cytotoxicity. The cell plates were incubated overnight at 37 °C, 5% CO_2. Compound plates were prepared with 10-point, 2-fold serial dilutions of the test compounds in duplicate, with four compounds assayed per 96-well plate. 10 µL from the compound dilution plates were transferred to the cell culture plates using a multi-channel pipettor or a RapidPlate 96/384 Workstation(Caliper Lifesciences). The cell plates were incubated for 48 hr at 37 °C, 5% CO_2. To determine luciferase activity, 100 µL of Steady-Glo reagent (Promega) were added to the culture medium and the plates were shaken on a plate shaker. The plates were incubated for 15 to 30 min at room temperature and the luminescence was read on an Analyst HT luminometer (Molecular Devices) with a one second integration time. To determine cytotoxicity, 10 µL of WST-detection reagent (Roche) were added to culture medium. Plates were incubated at 37 °C, 5% CO_2, for approximately 60 min. Absorbance at 440 nm was measured using the SpectraMax Plus microplate spectrophotometer (Molecular Devices). Data analysis for the luciferase and cytotoxicity assays was performed using Graphpad Prism v.4.0 (Graphpad Software, Inc.) in conjunction with DS Accord for EXCEL 6.0 (Accelrys, Microsoft Corp.). The percent inhibition and percent cytotoxicity values were plotted against compound concentration, and the data were fit to a constrained four parameter sigmoidal fit, equivalent to the "four parameter logistic equation." The curve fit equation employed was: Y = Bottom + (Top -Bottom)/(1+((10^($logEC_{50}$ - X)^Hillslope)), where Bottom is the minimum Y value, Top is the maximum Y value, and Hillslope is the slope of the linear portion of the semi-log curve. Top and Bottom were constrained to values of 100% and 0%, respectively. The minimum n (number of independent measurements) for each compound was 2. The average pEC_{50} SD in the gt1a data was less than 0.21 and in the gt1b less than 0.25

4b. Other stable and transient HCV replicon assays

Experimental details for all other stable and transient replicon assays can be found at *http://dx.doi.org/10.1021/jm400317w*.

4c. Replicon data with 95% confidence intervals

	Gt1a					Gt1b					CC50				
Compound	**pXC50**	**EC50 (nM)**	**95% CI low**	**95% CI high**	**N**	**pXC50**	**EC50 (nM)**	**95% CI low**	**95% CI high**	**N**	**pXC50**	**CC50 (uM)**	**95% CI low**	**95% CI high**	**N**
1	7.47	34	29	39	82	8.34	5	4	5	91	<4.3	>50	NA	NA	18
6	NA	NA	NA	NA	NA	6.77	170	NA	NA	2	4.52	31	NA	NA	2
7	6.91	122	85	177	11	7.79	16	13	20	26	5.00	10	NA	NA	2
8	7.88	13	7	25	8	8.67	2	1	4	10	<4.3	>50	NA	NA	4
9	NA	NA	NA	NA	NA	6.50	313	NA	NA	2	4.30	50	NA	NA	1
10	5.86	1385	NA	NA	2	6.66	221	25	1981	3	4.30	50	NA	NA	1
11	4.30	50000	NA	NA	1	4.30	50000	NA	NA	1	4.30	50	NA	NA	1
12	7.19	65	NA	NA	2	7.63	24	15	38	6	<4.3	>50	NA	NA	2
13	7.09	81	NA	NA	2	8.00	10	5	22	5	4.30	50	NA	NA	1
14	6.98	106	NA	NA	2	7.63	23	NA	NA	2	4.30	50	NA	NA	1
15	6.48	328	NA	NA	1	7.03	93	NA	NA	1	4.30	50	NA	NA	1
16	7.66	22	21	23	181	8.47	3	3	4	190	4.48	33	15	72	3
18	5.43	3737	1728	8079	4	6.95	113	89	143	12	4.51	31	19	50	5
22	NA	NA	NA	NA	NA	4.30	50000	NA	NA	1	4.30	50	NA	NA	1
23	NA	NA	NA	NA	NA	5.31	4860	416	56790	3	NA	NA	NA	NA	0
24	NA	NA	NA	NA	NA	5.76	1750	NA	NA	1	4.30	50	NA	NA	1
25	6.96	110	NA	NA	2	7.41	39	NA	NA	2	4.53	30	NA	NA	1
26	4.30	50000	NA	NA	1	5.13	7470	NA	NA	1	4.30	50	NA	NA	1
27	4.79	16180	NA	NA	1	4.28	52520	NA	NA	1	4.30	50	NA	NA	1
28	6.93	118	64	220	5	7.76	18	14	23	8	4.59	26	6	108	3
29	6.51	307	126	749	5	7.44	36	20	66	6	4.48	33	NA	NA	1
31	6.81	156	104	235	6	7.79	16	10	27	7	4.52	30	19	49	3
33	7.74	18	1	360	3	8.12	7	1	42	3	4.81	16	NA	NA	1
34	7.22	60	29	122	3	7.50	32	20	50	3	4.30	50	NA	NA	1
35	6.52	300	NA	NA	2	6.63	236	NA	NA	2	4.64	23	NA	NA	1
37	6.04	909	NA	NA	2	7.11	77	46	129	5	4.60	25	NA	NA	1
39	5.26	5440	NA	NA	1	5.82	1520	NA	NA	1	4.30	50	NA	NA	1
42	7.67	21	10	46	4	8.50	3	2	4	4	<4.3	>50	NA	NA	4
43	7.63	23.71	NA	NA	2	8.23	5.89	NA	NA	2	<4.3	>50	NA	NA	8
44	7.96	11.08	6.47	18.9	8	8.47	3.37	2.15	5.31	12	<=4.4	>=39	NA	NA	8

5. hERG Determination

Compounds **1**, **8, 16** and were tested in IonWorks Quattro Instrument. Continuous and frozen CHO-hERGV2-S1 cells from GRITS 32575batch of BioCat97761 were thawed and plated into T-225 flasks at a density of 6-7 million cells per flask (frozen vial, split @2:3). These cells were grown at 30 °C. Growth time in total is for 72 hrs in DMEM/F12 +10% FBS before running in the assay. Compounds were assayed in IonWorks PPC mode, frozen cell culture technology, compounds handled through compound management's automated system and dispensed into 384 well plastic plates, and Pluronic acid F-127 in the assay buffer. 500 ul of 10% Pluronic solution in H_2O was added to 500 ml PBS (Final conc= 0.001%). Tail currents (resulting from the voltage step from +40 mV to -50 mV in the PPC protocol) from pre and post- compound scans were exported to .iha files for analysis in XC50 excel templates. Max responses and pIC50 values were compared across experimental groups.

Compound **7** was tested in PatchXPress system. HEK293 hERG Warf cells produced in Craig January's lab at University of Wisconsin were used. Cells were cultured in Eagle's Minimum Essential Medium (EMEM), 2mM glutamine, 1% Na-pyruvate, 1% penicillin/streptomycin and 10% FBS, 1% non-essential amino acids, and 400µg/ml geneticin (except for harvesting). Cells were grown and maintained at 37 °C in a humidified environment containing 5% CO_2 in air. Cells with 50-60% confluency were detached from the T75 culture flask for passage and harvesting using TripLe. After media aspiration 9 ml Ca2+/Mg2+ free DPBS and 5 ml 1:5 diluted triple were added for 30-40s until cells detached. The flask was tilted few times, the suspension triturated and 10 ml medium were added. The suspension was centrifuged for 1.5 minutes at 1100 r.p.m. The pellet was resuspended in 200 µl, 5 ml medium were added and placed in the 37 °C incubator for ten minutes. The suspension was centrifuged at 1100 r.p.m. for 1.5 min. The pellet was resuspended in 200 µl external solution and transferred to the instrument. The intracellular solution contained the following (in mM): K aspartate 130, KCl 10, HEPES 10, EGTA 5, MgATP 5, $CaCl_2$ 0.1, $MgCl_2$ 1, pH to 7.2 with KOH. The extracellular solution contained the following (in mM): NaCl 140, KCl 4, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, glucose 10, pH adjusted to 7.4 with NaOH. Standard whole-cell configuration was achieved through an automated protocol on PatchXpress™. Pulse protocol: holding potential: -80mV, step to -50 mV for 500ms then to 20 mV for 5 s then to -50 mV for 5 s. The peak current is measured at the deactivation phase (returning to -50mV) and subtracted from the resting current at -50 mV before activation. Inhibition is measured as the reduction in peak amplitude.

Compound **31** was tested at Cerep (Redmond Washington) by automated Patch-clamp System.

6. pKa Calculation

The pKa acidity constants were calculated with Jaguar, a quantum chemistry program (see citation below). Briefly, Jaguar computes the energies of the protonated and unprotonated forms of the compound in aqueous solution,

and then uses an empirical relationship to convert the energy difference into a predicted pKa value. Jaguar starts by optimizing the geometries, and then calculates the energies of the protonated and unprotonated compounds in vacuum using density functional theory. The effects of solvation are then modelled by using self-consistent reaction field methods to solve the Poisson-Boltzmann equation for the protonated and unprotonated compounds. The Jaguar methodology includes empirical energy-to-pKa conversion formulas for each of several different ionizable groups, which are in each case adjusted to fit experimental pKa values. For the compounds of Table 1, Jaguar selected the "heterocyclic nitrogen" conversion formula. To save computer time and minimize complexity, the Jaguar calculations were run on the model compounds shown below, rather than the full compounds shown in Table 1; these model compounds include the B, C and D rings, but exclude the di-CF3-phenyl A ring. For each model compound, this is the protonation site with the lowest energy in quantum calculations. Likewise, the energies of the protonated and unprotonated forms were calculated using the lowest energy conformations obtained from prior quantum mechanical conformational search calculations. An earlier attempt to calculate the pKa values with a purely empirical method gave anomalous results, raising questions as to whether the method included reference compounds suitable for the imidazopyridine and imidazopyridazine ring systems. While the Jaguar quantum mechanical method uses empirical data to parameterize the energy-to-pKa conversion formulas, it does not require a close match to any reference compound. Because of these concerns, the Jaguar quantum chemistry method was used instead of any empirical method for the pKa acidity constants reported here.

Jaguar version 8.0, Schrodinger LLC, New York, NY; Jasna J. Klicic, Richard A. Friesner, Shi-Yi Liu and Wayne C. Guida, "Accurate Prediction of Acidity Constants in Aqueous Solution via Density Functional Theory and Self-Consistent Reaction Field Methods," Journal of Physical Chemistry A, 2002, 106, 1327-1335; Art D. Bochevarov, Edward Harder, Thomas F. Hughes, Jeremy R. Greenwood, Dale A. Braden, Dean M. Philipp, David Rinaldo, Mathew D. Halls, Jing Zhang and Richard A. Friesner, "Jaguar: A High-Performance Quantum Chemistry Software Program with Strengths in Life and Materials Sciences," International Journal of Quantum Chemistry, 2013, 113, 2110-2142.