Supporting Information

Identification of Potent and Selective RIPK2 Inhibitors for the Treatment of Inflammatory Diseases

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Contents include synthetic schemes and procedures for the preparation of **4**, **5a-p**, **6a-6h**, **7a-g** and **8**, Kinome profile data for compound **8**, experimental details for *in vitro* assays and *in vivo* animal models, X-ray crystallography data of compound **1** and **7f**, and docking study with compound **4** in co-crystal structure of RIPK2 with compound **1**.

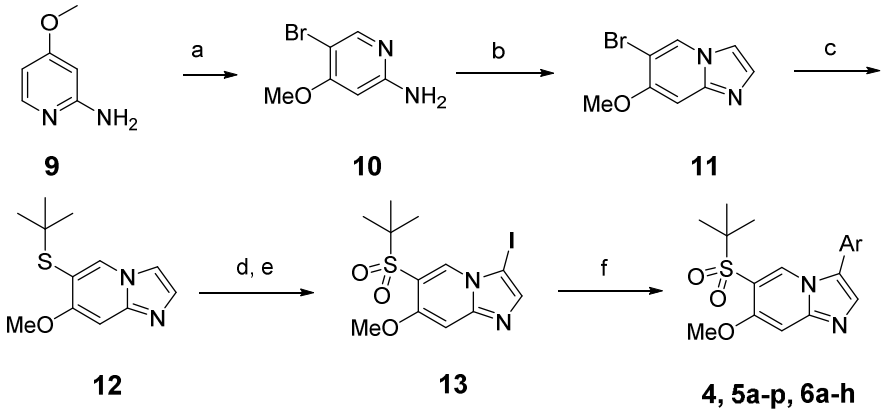
CHEMISTRY

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Removal of solvent under reduced pressure or concentration refers to distillation using Büchi rotary evaporator attached to a vacuum pump. Products obtained as solids or high boiling oils were dried under vacuum (1 mmHg). All microwave experiments were performed in a self-tuning single mode Biotage Initiator Microwave Synthesizer or a CEM Discover microwave reactor.

Sample purification by flash column chromatography was conducted on a Teledy Isco purification system using pre-packed commercially available silica gel columns. Reverse-phase LCMS (RP-LCMS) purification was conducted on a Waters autopurification system consisting of a 2767 autosampler/ fraction collector, a 2525 binary gradient module, a 2487 UV detector, and a ZQ mass spectrometer. Compounds were purified using flow rate of 100 mL/min with an Atlantis® prep T3 OBD™ 10µm 19x50 mm column. A 3.0 min linear gradient from 10% solvent A (acetonitrile with 0.035% trifluoroacetic acid) in solvent B (water with 0.05% trifluoroacetic acid) to 30−90% solvent A was used, followed by a 1.0 min hold at 90% solvent A. Elemental analyses were carried out by Midwest Microlabs LLC, Indianapolis, IN. NMR spectra were recorded on Bruker XWINNMR (400 MHz) spectrometer. Chemical shifts are listed in ppm correlated to the solvent used as an internal standard. All final compounds were analyzed with a Waters ZQ 2000 LC/MS system. All compounds were determined to be > 95% pure by LC/MS analysis, and further confirmed by NMR analysis.

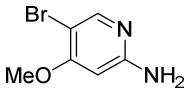
Synthetic methods for 4, 5a-p, 6a-6h:

Scheme SI-1[a]



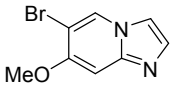
[a]Reagents and conditions: (a) Br_2, AcOH, rt., 74%; (b) 2-chloroacetaldehyde, $NaHCO_3$, EtOH, 80˚C, 65%; (c) *t*-butylthiol, $Pd_2(dba)_3$, xantphos, K_2CO_3, dioxane, 120˚C, 99%; (d) oxone, MeOH/water (3:1); (e) NIS, MeOH/water (3:1), 95%; (f) aryl boronic acid or aryl pinacolboronate, $PdCl_2(dppf)$, Na_2CO_3, dioxane/EtOH (1:1), microwave, 140 ˚C, 20 min, 5~79%.

5-Bromo-4-methoxypyridin-2-amine **(10)**



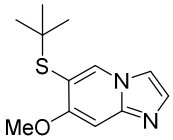
Br_2 (351mg, 2.2 mmol) was slowly added into a solution of 4-methoxypyridin-2-amine **9** (248 mg, 2.0 mmol) in AcOH (5 mL) at room temperature. The mixture solidified after 5min. More AcOH (3mL) was added to the mixture and stirred at room temperature for 1hr. Solid was collected by filtration and washed by AcOH (1mL). It was air dried overnight to provide 5-bromo-4-methoxypyridin-2-amine (391mg, 74%) as AcOH salt. Exact mass calculated for $C_6H_7BrN_2O$: 202.0; found: MS *m/z* 203.0 (M+1).

6-Bromo-7-methoxyimidazo[1,2-*a*]pyridine **(11)**



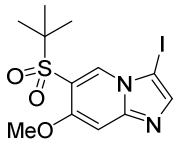
To a solution of a 5-bromo-4-methoxypyridin-2-amine (2.00g, 9.85mmol) in EtOH (30mL) was added $NaHCO_3$ (2.48g, 29.8mmol) followed by 2-chloroacetaldehyde (2.11g, 14.8mmol). The mixture was heated at 80°C for 16hr, reaction mixture turned dark in color. It was cooled to room temperature, solid was filtered. The filtrate was mixed with 10g of silica gel and concentrated. The resulted mixture was loaded on cartridge and purified by flash column chromatography (0~8%MeOH/DCM) to provide ~1.46g (65.3%) of 6-bromo-7-methoxyimidazo[1,2-*a*]pyridine. Exact mass calculated for $C_8H_7BrN_2O$: 226.0; found: MS *m/z* 227.1 (M+1).

6-(*tert*-Butylthio)-7-methoxyimidazo[1,2-*a*]pyridine **(12)**



A mixture of 6-bromo-7-methoxyimidazo[1,2-*a*]pyridine (1.46g, 6.43mmol), Pd_2dba_3 (59mg, 0.064mmol), xantphos (56mg, 0.096mmol) and K_2CO_3 (1.066g, 7.72mmol) in dioxane (15mL) was degassed by applying vacuum and N_2 alternatively for three times, and 2-methylpropane-2-thiol (0.87g, 1.087mL, 9.65mmol) was added. The mixture was heated to 120°C for 16hr. After cooling down to room temperature, the mixture was treated with sat. NH_4Cl (40 mL) and extracted with EtOAc (5×20 mL). Organic layers were combined and concentrated. Crude material was purified by flash column chromatography (0~8%MeOH/DCM) to provide 6-(*tert*-butylthio)-7-methoxyimidazo[1,2-*a*]pyridine (1.5 g, 99%) as brownish yellow solid. Exact mass calculated for $C_{12}H_{16}N_2OS$: 236.1; found: MS *m/z* 237.1 (M+1).

6-(*tert*-Butylsulfonyl)-3-iodo-7-methoxyimidazo[1,2-*a*]pyridine **(13)**



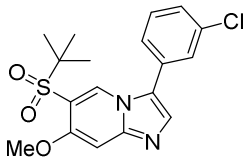
To 6-(*tert*-butylthio)-7-methoxyimidazo[1,2-*a*]pyridine (236 mg, 1.0mmol) in MeOH/H_2O (3 mL/1 mL) was added oxone (800 mg, 1.3 mmol). The mixture was stirred at room temperature for 36 hr. for completion of oxidation. To this reaction mixture, water (3 mL) was added to clear up the suspension. NIS (270 mg, 1.2 mmol) was added, the mixture was stirred at room temperature for 30min., and then was treated with sat. $NaHCO_3$ (5 mL) and extracted with DCM (3×10 mL). The combined organic phases were concentrated *in vacuo* to give the crude product, which was purified by flash column chromatography on silica gel (0~6%MeOH/DCM/NH_3) to provide 6-(*tert*-butylsulfonyl)-3-iodo-7-methoxyimidazo[1,2-*a*]pyridine (~370 mg, 95%) as white solid. Exact mass calculated for $C_{12}H_{15}IN_2O_3S$: 394.0; found: MS *m/z* 395.0 (M+1).

General procedure for Suzuki coupling

To a microwave reaction vessel was added 3-iodo-7-alkoxyimidazo[1,2-*a*]pyridine (0.1 mmol), phenylboronic acid or phenyl pinacolboronate (0.13 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ adduct (0.005 mmol), Na_2CO_3 (2M aqueous solution, 0.3mmol) and EtOH/dioxane (1:1, 1 mL). The reaction tube was degassed by applying vacuum and N_2 alternatively for three times, sealed under N_2 and heated in microwave reactor to 140°C for 20min. Reaction mixture was then treated with sat. NH_4Cl (5 mL), extracted with EtOAc (3×5mL). Organic layers were combined, concentrated and purified by silica gel flash column chromatography or RP-LCMS to provide desired products.

Compounds **4**, **5a-p**, **6a-h** were prepared using the general procedure for Suzuki coupling:

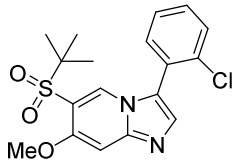
6-(*tert*-Butylsulfonyl)-3-(3-chlorophenyl)-7-methoxyimidazo[1,2-*a*]pyridine (**4**)



6-(*tert*-Butylsulfonyl)-3-(3-chlorophenyl)-7-methoxyimidazo[1,2-*a*]pyridine (**4**) was synthesized using general Suzuki coupling condition from 6-(*tert*-butylsulfonyl)-3-iodo-7-methoxyimidazo[1,2-a]pyridine (**13**) and 3-chlorophenyl boronic acid and purified by RP-LCMS. Yield, 32%. ^{1}H NMR

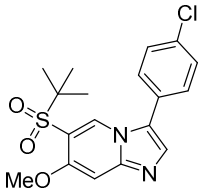
(400MHz, 300K, MeOH-d_4): δ 8.86 (s, 1H), 8.17 (s, 1H), 7.77 (s, 1H), 7.68-7.65 (m, 3H), 7.52 (s, 1H), 4.18 (s, 3H), 1.42 (s, 9H). Exact mass calculated for $C_{18}H_{19}ClN_2O_3S$: 378.1; found: MS *m/z* 379.1 (M+1).

6-(*tert*-Butylsulfonyl)-3-(2-chlorophenyl)-7-methoxyimidazo[1,2-*a*]pyridine (**5a)**:



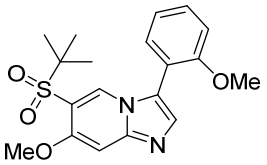
Compound **5a** was synthesized from 6-(*tert*-butylsulfonyl)-3-iodo-7-methoxyimidazo[1,2-*a*]pyridine (**13**) and 2-chlorophenyl boronic acid and purified by RP-LCMS. Yield, 31%. ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 8.44 (s, 1H), 8.17 (s, 1H), 7.79-7.60 (m, 4H), 7.55 (s, 1H), 4.18 (s, 3H), 1.40 (s, 9H). Exact mass calculated for $C_{18}H_{19}ClN_2O_3S$: 378.1; found: MS *m/z* 379.1 (M+1).

6-(*tert*-Butylsulfonyl)-3-(4-chlorophenyl)-7-methoxyimidazo[1,2-*a*]pyridine (**5b**)



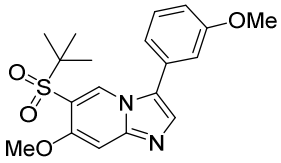
Compound **5b** was synthesized from 6-(*tert*-butylsulfonyl)-3-iodo-7-methoxyimidazo[1,2-*a*]pyridine (**13**) and 4-chlorophenyl boronic acid and purified by RP-LCMS. Yield, 31%. ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 8.84 (s, 1H), 8.15 (s, 1H), 7.71 (s, 4H), 7.53 (s, 1H), 4.18 (s, 3H), 1.42 (s, 9H). Exact mass calculated for $C_{18}H_{19}ClN_2O_3S$: 378.1; found: MS *m/z* 379.1 (M+1).

6-(*tert*-Butylsulfonyl)-7-methoxy-3-(2-methoxyphenyl)imidazo[1,2-*a*]pyridine **(5c**)



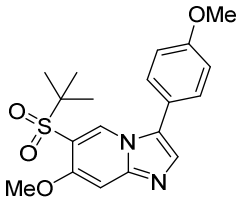
Compound **5c** was synthesized from 6-(*tert*-butylsulfonyl)-3-iodo-7-methoxyimidazo[1,2-*a*]pyridine (**13**) and 2-methoxyphenyl boronic acid and purified by RP-LCMS. Yield, 31%. ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 8.47 (s, 1H), 8.04 (s, 1H), 7.69 (ddd, *J* = 8.4, 7.5, 1.7 Hz, 1H), 7.55 (dd, *J* = 7.6, 1.7 Hz, 1H), 7.50 (s, 1H), 7.32 (dd, *J* = 8.7, 0.8 Hz, 1H), 7.22 (td, *J* = 7.5, 1.0 Hz, 1H), 4.17 (s, 3H), 3.85 (s, 3H), 1.41 (s, 9H). Exact mass calculated for $C_{19}H_{22}N_2O_4S$: 374.1; found: MS *m/z* 375.1 (M + 1).

6-(*tert*-Butylsulfonyl)-7-methoxy-3-(3-methoxyphenyl)imidazo[1,2-*a*]pyridine (**5d**)



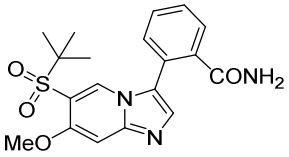
Compound **5d** was synthesized from 6-(*tert*-butylsulfonyl)-3-iodo-7-methoxyimidazo[1,2-*a*]pyridine (**13**) and 3-methoxyphenyl boronic acid and purified by RP-LCMS. Yield, 31%. ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 8.93 (s, 1H), 8.11 (s, 1H), 7.59 (t, J = 8.2 Hz, 1H), 7.50 (s, 1H), 7.33- 7.14 (m, 3H), 4.17 (s, 3H), 3.89 (s, 3H), 1.41 (s, 9H). Exact mass calculated for $C_{19}H_{22}N_2O_4S$: 374.1; found: MS *m/z* 375.2 (M + 1).

6-(*tert*-Butylsulfonyl)-7-methoxy-3-(4-methoxyphenyl)imidazo[1,2-*a*]pyridine (**5e)**



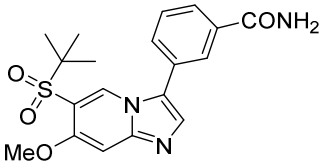
Compound **5e** was synthesized from 6-(*tert*-butylsulfonyl)-3-iodo-7-methoxyimidazo[1,2-*a*]pyridine (**13**) and 4-methoxyphenyl boronic acid and purified by RP-LCMS. Yield, 31%. ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 8.82 (s, 1H), 8.01 (s, 1H), 7.68-7.54 (m, 2H), 7.49 (s, 1H), 7.31- 7.16 (m, 2H), 4.16 (s, 3H), 3.92 (s, 3H), 1.40 (s, 9H). Exact mass calculated for $C_{19}H_{22}N_2O_4S$: 374.1; found: MS *m/z* 375.2 (M + 1).

2-(6-(*tert*-Butylsulfonyl)-7-methoxyimidazo[1,2-*a*]pyridin-3-yl)benzamide (**5f)**



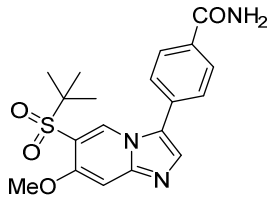
Compound **5f** was synthesized from 6-(*tert*-butylsulfonyl)-3-iodo-7-methoxyimidazo[1,2-*a*]pyridine (**13**) and 2-aminocarbonylphenyl boronic acid and purified by RP-LCMS. Yield, 5%. ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 8.38 (s, 1H), 7.98 (q, J = 3.4, 3.0 Hz, 2H), 7.83-7.76 (m, 2H), 7.69-7.63 (m 1H), 7.48 (s, 1H), 4.15 (s, 3H), 1.38 (s, 9H). Exact mass calculated for $C_{19}H_{21}N_3O_4S$: 387.1; found: MS *m/z* 388.2 (M + 1).

3-(6-(*tert*-Butylsulfonyl)-7-methoxyimidazo[1,2-*a*]pyridin-3-yl)benzamide (**5g**)



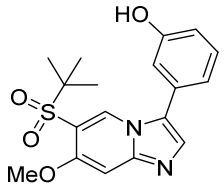
Compound **5g** was synthesized from 6-(*tert*-butylsulfonyl)-3-iodo-7-methoxyimidazo[1,2-*a*]pyridine (**13**) and 3-aminocarbonylphenyl boronic acid and purified by RP-LCMS. Yield, 25%. ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 8.87 (s, 1H), 8.19 (d, J = 7.9 Hz, 2H), 8.13 (d, J = 6.7 Hz, 1H), 7.89 (d, J = 7.8 Hz, 1H), 7.79 (t, J = 7.8 Hz, 1H), 7.54 (s, 1H), 4.18 (s, 3H), 1.42 (s, 9H). Exact mass calculated for $C_{19}H_{21}N_3O_4S$: 387.1; found: MS *m/z* 388.2 (M + 1).

4-(6-(*tert*-Butylsulfonyl)-7-methoxyimidazo[1,2-*a*]pyridin-3-yl)benzamide (**5h**)



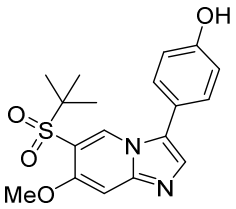
Compound **5h** was synthesized from 6-(*tert*-butylsulfonyl)-3-iodo-7-methoxyimidazo[1,2-*a*]pyridine (**13**) and 4-aminocarbonylphenyl boronic acid and purified by RP-LCMS. Yield, 30%. ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 8.92 (s, 1H), 8.21 (s, 1H), 8.16 (d, J = 8.5 Hz, 2H), 7.82 (d, J = 8.5 Hz, 2H), 7.53 (s, 1H), 4.18 (s, 3H), 1.41 (s, 9H). Exact mass calculated for $C_{19}H_{21}N_3O_4S$: 387.1; found: MS *m/z* 388.2 (M + 1).

3-(6-(*tert*-Butylsulfonyl)-7-methoxyimidazo[1,2-*a*]pyridin-3-yl)phenol (**5i**)



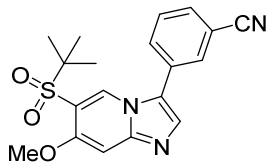
Compound **5i** was synthesized from 6-(*tert*-butylsulfonyl)-3-iodo-7-methoxyimidazo[1,2-*a*]pyridine (**13**) and 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol and purified by RP-LCMS. Yield, 33%. ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 8.93 (s, 1H), 8.05 (s, 1H), 7.47 (s, 2H), 7.15-7.08 (m, 1H), 7.01 (m, 2H), 4.16 (s, 3H), 1.41 (s, 9H). Exact mass calculated for $C_{18}H_{20}N_2O_4S$: 360.1; found: MS *m/z* 361.2 (M + 1).

4-(6-(*tert*-Butylsulfonyl)-7-methoxyimidazo[1,2-*a*]pyridin-3-yl)phenol (**5j**)



Compound **5j** was synthesized from 6-(*tert*-butylsulfonyl)-3-iodo-7-methoxyimidazo[1,2-*a*]pyridine (**13**) and 4-hydroxyphenyl boronic acid and purified by RP-LCMS. Yield, 33%. ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 8.82 (s, 1H), 7.98 (s, 1H), 7.52-7.46 (m, 3H), 7.09-7.02 (m, 2H), 4.16 (s, 3H), 1.40 (s, 9H). Exact mass calculated for $C_{18}H_{20}N_2O_4S$: 360.1; found: MS *m/z* 361.1 (M + 1).

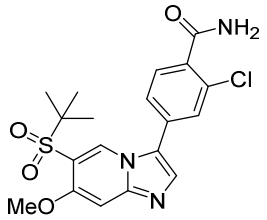
3-(6-(*tert*-Butylsulfonyl)-7-methoxyimidazo[1,2-*a*]pyridin-3-yl)benzonitrile (**5k**)



Compound **5k** was synthesized from 6-(*tert*-butylsulfonyl)-3-iodo-7-methoxyimidazo[1,2-*a*]pyridine (**13**) and 3-cyanophenyl boronic acid and purified by RP-LCMS. Yield, 79%. ^{1}H NMR (400MHz, 300K,

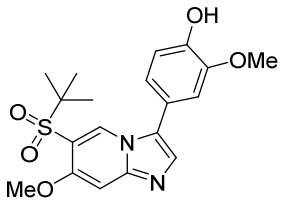
MeOH-d_4): δ 8.84 (s, 1H), 8.23 (s, 1H), 8.14 (s, 1H), 8.02 (dd, *J* = 8.0, 1.6 Hz, 2H), 7.87 (t, *J* = 7.6 Hz, 1H), 7.54 (s, 1H), 4.18 (s, 3H), 1.42 (s, 9H). Exact mass calculated for $C_{19}H_{19}N_3O_3S$: 369.1; found: MS *m/z* 370.2 (M + 1).

4-(6-(*tert*-Butylsulfonyl)-7-methoxyimidazo[1,2-*a*]pyridin-3-yl)-2-chlorobenzamide (**5l**)



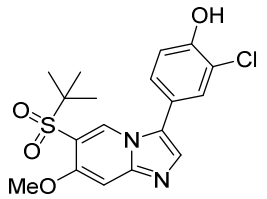
Compound **5l** was synthesized from 6-(*tert*-butylsulfonyl)-3-iodo-7-methoxyimidazo[1,2-*a*]pyridine (**13**) and 2-chloro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzamide and purified by RP-LCMS. Yield, 27%. ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 8.85 (s, 1H), 8.23 (s, 1H), 7.87 (d, *J* = 1.6 Hz, 1H), 7.80 (d, *J* = 8.0 Hz, 1H), 7.72 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.53 (s, 1H), 4.18 (s, 3H), 1.42 (s, 9H). Exact mass calculated for $C_{19}H_{20}ClN_3O_4S$: 421.1; found: MS *m/z* 422.1 (M + 1).

4-(6-(*tert*-Butylsulfonyl)-7-methoxyimidazo[1,2-*a*]pyridin-3-yl)-2-methoxyphenol (**5m**)



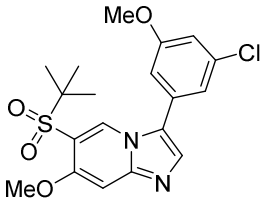
Compound **5m** was synthesized from 6-(*tert*-butylsulfonyl)-3-iodo-7-methoxyimidazo[1,2-*a*]pyridine (**13**) and 2-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol and purified by RP-LCMS. Yield, 30%. **5m**: ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 8.92 (s, 1H), 8.01 (s, 1H), 7.49 (s, 1H), 7.23 (d, *J* = 1.9 Hz, 1H), 7.13 (dd, *J* = 8.1, 2.0 Hz, 1H), 7.05 (d, *J* = 8.1 Hz, 1H), 4.16 (s, 3H), 3.92 (s, 3H), 1.41 (s, 9H). Exact mass calculated for $C_{19}H_{22}N_2O_5S$: 390.1; found: MS *m/z* 391.2 (M + 1).

4-(6-(*tert*-Butylsulfonyl)-7-methoxyimidazo[1,2-*a*]pyridin-3-yl)-2-chlorophenol (**5n**)



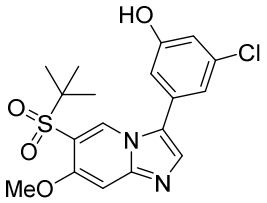
Compound **5n** was synthesized from 6-(*tert*-butylsulfonyl)-3-iodo-7-methoxyimidazo[1,2-*a*]pyridine (**13**) and 3-chloro-4-hydroxyphenyl boronic acid and purified by RP-LCMS. Yield, 30%. ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 8.80 (s, 1H), 8.03 (s, 1H), 7.67 (d, *J* = 2.1 Hz, 1H), 7.49 (s, 1H), 7.49-7.41 (m, 1H), 7.17 (d, *J* = 8.4 Hz, 1H), 4.16 (s, 3H), 1.41 (s, 9H). Exact mass calculated for $C_{18}H_{19}ClN_2O_4S$: 394.1; found: MS *m/z* 395.2 (M + 1).

6-(*tert*-Butylsulfonyl)-3-(3-chloro-5-methoxyphenyl)-7-methoxyimidazo[1,2-*a*]pyridine (**5o**)



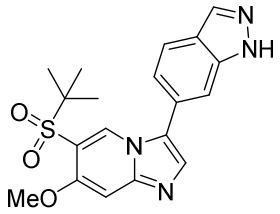
Compound **5o** was synthesized from 6-(*tert*-butylsulfonyl)-3-iodo-7-methoxyimidazo[1,2-*a*]pyridine (**13**) and 3-chloro-5-methoxyphenyl boronic acid and purified by RP-LCMS. Yield, 29%. ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 8.91 (s, 1H), 8.16 (s, 1H), 7.51 (s, 1H), 7.31 (t, J = 1.6 Hz, 1H), 7.26 (t, J = 2.1Hz, 1H), 7.22 (dd, J = 2.4, 1.4 Hz, 1H), 4.17 (s, 3H), 3.90 (s, 3H), 1.42 (s, 9H). Exact mass calculated for $C_{19}H_{21}ClN_2O_4S$: 408.1; found: MS *m/z* 409.1 (M + 1).

3-(6-(*tert*-Butylsulfonyl)-7-methoxyimidazo[1,2-*a*]pyridin-3-yl)-5-chlorophenol (**5p**)



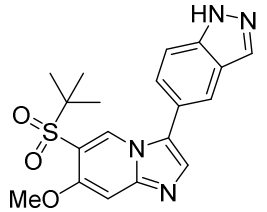
Compound **5p** was synthesized from 6-(*tert*-butylsulfonyl)-3-iodo-7-methoxyimidazo[1,2-*a*]pyridine (**13**) and 3-chloro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol and purified by RP-LCMS. Yield, 52%. ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 8.89 (s, 1H), 8.11 (s, 1H), 7.52 (s, 1H), 7.17 (t, J = 1.6Hz, 1H), 7.06 (t, J = 2.0 Hz, 1H), 7.03 (t, J = 1.6 Hz, 1H), 4.17 (s, 3H), 1.42 (s, 9H). Exact mass calculated for $C_{18}H_{19}ClN_2O_4S$: 394.1; found: MS *m/z* 395.1 (M + 1).

6-(6-(*tert*-Butylsulfonyl)-7-methoxyimidazo[1,2-*a*]pyridin-3-yl)-1H-indazole (**6a**)



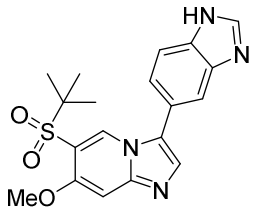
Compound **6a** was synthesized from 6-(*tert*-butylsulfonyl)-3-iodo-7-methoxyimidazo[1,2-*a*]pyridine (**13**) and (1H-indazol-6-yl)boronic acid and purified by RP-LCMS. Yield, 31%. ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 8.96 (s, 1H), 8.23 (d, J = 1.0 Hz, 1H), 8.19 (s, 1H), 8.09 (dd, J = 8.3, 0.9 Hz, 1H), 7.92 (q, J = 1.1 Hz, 1H), 7.54 (s, 1H), 7.42 (dd, J = 8.4, 1.4 Hz, 1H), 4.18 (s, 3H), 1.41 (s, 9H). Exact mass calculated for $C_{19}H_{20}N_4O_3S$: 384.1; found: MS *m/z* 385.2 (M + 1).

5-(6-(*tert*-Butylsulfonyl)-7-methoxyimidazo[1,2-*a*]pyridin-3-yl)-1H-indazole (**6b**)



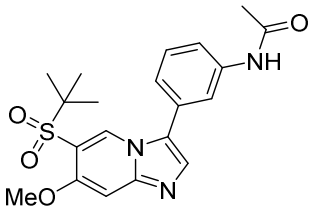
Compound **6b** was synthesized from 6-(*tert*-butylsulfonyl)-3-iodo-7-methoxyimidazo[1,2-*a*]pyridine (**13**) and 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indazole and purified by RP-LCMS. Yield, 39%. ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 8.82 (s, 1H), 8.18 (s, 1H), 8.03 (s, 1H), 7.77 (d, J = 8.8 Hz, 1H), 7.68 (s, 1H), 7.59 (dd, J = 8.8, 1.6 Hz, 1H), 7.15 (s, 1H), 4.02 (s, 3H), 1.38 (s, 9H). Exact mass calculated for $C_{19}H_{20}N_4O_3S$: 384.1; found: MS *m/z* 385.2 (M + 1).

5-(6-(*tert*-Butylsulfonyl)-7-methoxyimidazo[1,2-*a*]pyridin-3-yl)-1H-benzo[d]imidazole (**6c**)



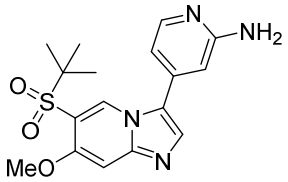
Compound **6c** was synthesized from 6-(*tert*-butylsulfonyl)-3-iodo-7-methoxyimidazo[1,2-*a*]pyridine (**13**) and 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-benzo[d]imidazole and purified by RP-LCMS. Yield, 15%. ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 8.87 (s, 1H), 8.31 (s, 1H), 7.84 (b, 2H), 7.68 (s, 1H), 7.50 (dd, J = 8.4, 1.6 Hz, 1H), 7.14 (s, 1H), 4.01 (s, 3H), 1.38 (s, 9H). Exact mass calculated for $C_{19}H_{20}N_4O_3S$: 384.1; found: MS *m/z* 385.2 (M + 1).

N-(3-(6-(*tert*-Butylsulfonyl)-7-methoxyimidazo[1,2-*a*]pyridin-3-yl)phenyl)acetamide (**6d**)



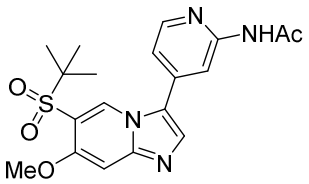
Compound **6d** was synthesized from 6-(*tert*-butylsulfonyl)-3-iodo-7-methoxyimidazo[1,2-*a*]pyridine (**13**) and 3-acetamidophenyl boronic acid and purified by RP-LCMS. Yield, 43%. ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 8.94 (s, 1H), 8.22 (s, 1H), 8.11 (s, 1H), 7.57-7.61 (m, 2H), 7.50 (s, 1H), 7.40 (m, 1H), 4.17 (s, 3H), 2.17 (s, 3H), 1.42 (s, 9H). Exact mass calculated for $C_{20}H_{23}N_3O_4S$: 401.1; found: MS *m/z* 402.2 (M + 1).

4-(6-(*tert*-Butylsulfonyl)-7-methoxyimidazo[1,2-*a*]pyridin-3-yl)pyridin-2-amine (**6e**)



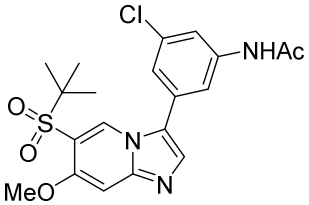
Compound **6e** was synthesized from 6-(*tert*-butylsulfonyl)-3-iodo-7-methoxyimidazo[1,2-*a*]pyridine (**13**) and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-amine and purified by RP-LCMS. Yield, 47%. ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 9.07 (s, 1H), 8.51 (s, 1H), 8.09 (d, J = 6.8 Hz, 1H), 7.59 (s, 1H), 7.34 (s, 1H), 7.19 (dd, J = 6.8, 0.8 Hz, 1H), 4.19 (s, 3H), 1.45 (s, 9H). Exact mass calculated for $C_{17}H_{20}N_4O_3S$: 360.1; found: MS *m/z* 361.1 (M + 1).

N-(4-(6-(*tert*-Butylsulfonyl)-7-methoxyimidazo[1,2-*a*]pyridin-3-yl)pyridin-2-yl)acetamide (**6f**)



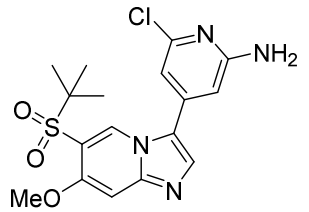
Compound **6f** was synthesized from 6-(*tert*-butylsulfonyl)-3-iodo-7-methoxyimidazo[1,2-*a*]pyridine (**13**) and N-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl)acetamide and purified by RP-LCMS. Yield, 31%. ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 9.07 (s, 1H), 8.43 (d, J = 4.4 Hz, 2H), 7.91 (s, 1H), 7.34 (dd, J = 5.6, 1.6 Hz, 1H), 7.19 (s, 1H), 4.03 (s, 3H), 2.21 (s, 3H), 1.44 (s, 9H). Exact mass calculated for $C_{19}H_{22}N_4O_4S$: 402.1.1; found: MS *m/z* 403.2 (M + 1).

N-(3-(6-(*tert*-butylsulfonyl)-7-methoxyimidazo[1,2-*a*]pyridin-3-yl)-5-chlorophenyl)acetamide (**6g**)



Compound **6g** was synthesized from 6-(*tert*-butylsulfonyl)-3-iodo-7-methoxyimidazo[1,2-*a*]pyridine (**13**) and N-(3-chloro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acetamide and purified by RP-LCMS. Yield, 41%. ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 8.92 (s, 1H), 8.14 (s, 1H), 8.08 (t, J = 1.6 Hz, 1H), 7.71 (t, J = 2.0 Hz, 1H), 7.48 (s, 1H), 7.43 (t, J = 1.6 Hz, 1H), 4.16 (s, 3H), 2.17 (s, 3H), 1.43 (s, 9H). Exact mass calculated for $C_{20}H_{22}ClN_3O_4S$: 435.1; found: MS *m/z* 436.1 (M + 1).

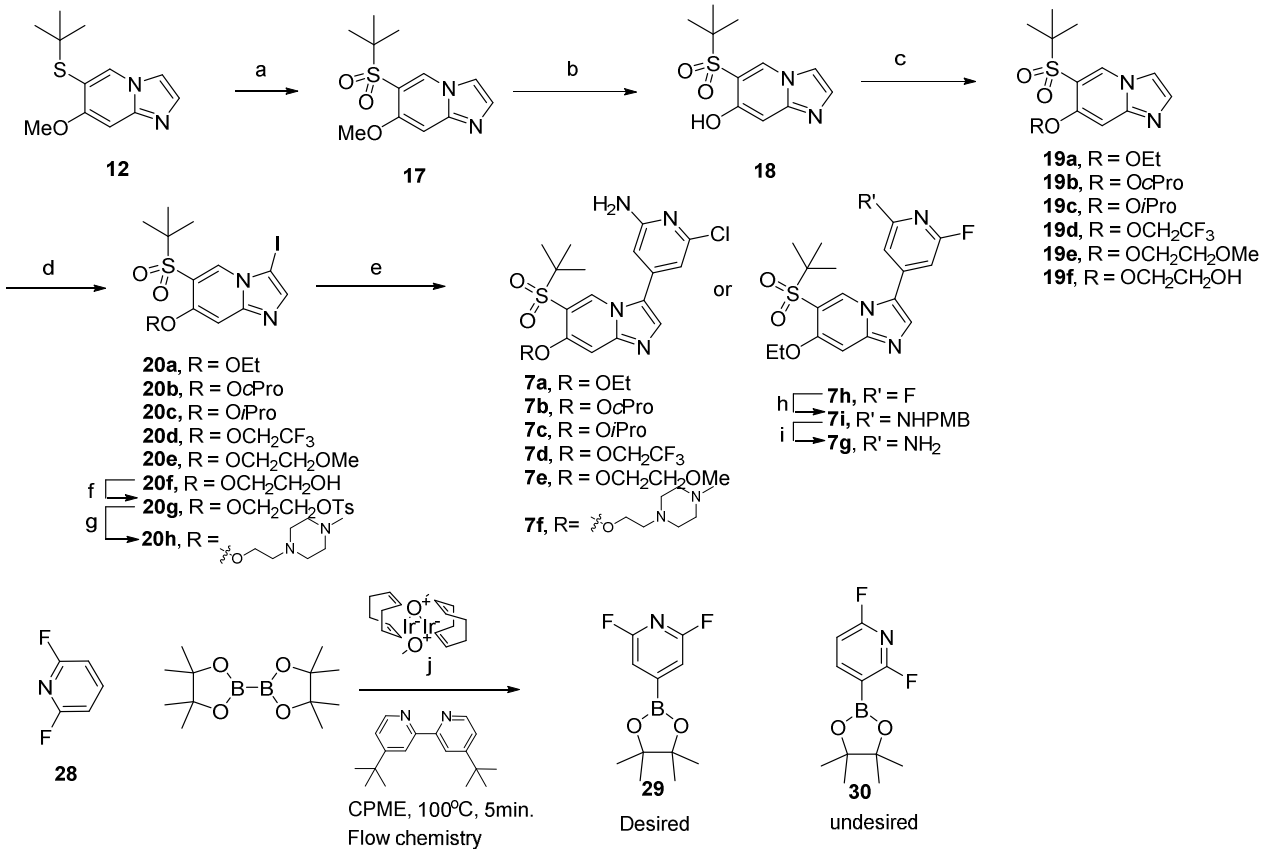
4-(6-(*tert*-Butylsulfonyl)-7-methoxyimidazo[1,2-*a*]pyridin-3-yl)-6-chloropyridin-2-amine (**6h**)



Compound **6h** was synthesized from 6-(*tert*-butylsulfonyl)-3-iodo-7-methoxyimidazo[1,2-*a*]pyridine (**13**) and 6-chloro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-amine and purified by RP-LCMS. Yield, 34%. ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 8.98 (s, 1H), 8.26 (s, 1H), 7.53 (s, 1H), 6.87 (s, 1H), 6.73 (s, 1H), 4.18 (s, 3H), 1.43 (s, 9H). Exact mass calculated for $C_{17}H_{19}ClN_4O_3S$: 394.1; found: MS *m/z* 395.1 (M + 1).

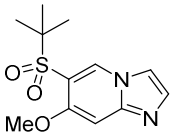
Synthetic methods for 7a-g.

Scheme SI-2[a]



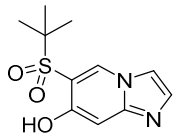
[a]Reagents and conditions: (a) oxone, MeOH/water, 87%; (b) sodium propane-2-thiolate, DMF, 150˚C, 87%; (c) K_2CO_3, DMF, RX; (d) NIS, MeOH/water (3:1), 95%~99%; (e) 6-chloro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-amine or **29**, $PdCl_2$(dppf), Na_2CO_3, dioxane/EtOH (1:1), microwave, 140 ˚C, 20 min, 9%~76% (f) TsCl, TEA, DCM, 96%; (g) N-methylpiperazine, THF; (h) *p*-methoxybenzylamine, DIEA, IPA, microwave, 140˚C, 25min, 75%; (i) TFA, DCM, 32%; (j) (1,5-Cyclooctadi-ene) (methoxy)iridium(I) dimer, 4,4'-di-*tert*-butyl-2,2'-bipyridine, CPME, microwave, 100˚C, 5min, 50.8%.

6-(*tert*-Butylsulfonyl)-7-methoxyimidazo[1,2-*a*]pyridine (**17**)



To 6-(*tert*-butylthio)-7-methoxyimidazo[1,2-*a*]pyridine (**12**, 236 mg, 1.0 mmol) in MeOH/H_2O (3 mL/1 mL) was added oxone (800 mg, 1.3 mmol). The mixture was stirred at room temperature for 36 hr. for completion of oxidation. The mixture was then treated with sat. $NaHCO_3$ till pH~9 and extracted with EtOAc (5×10 mL). Organic layer was concentrated and purified by flash column chromatography (0~10%MeOH/DCM) to provide 6-(*tert*-butylsulfonyl)-7-methoxyimidazo[1,2-*a*]pyridine (236 mg, 0.87 mmol, 87 % yield) as white powder. Exact mass calculated for $C_{12}H_{16}N_2O_3S$: 268.1; found: MS *m/z* 269.1 (M + 1).

6-(*tert*-Butylsulfonyl)imidazo[1,2-*a*]pyridin-7-ol (**18**)



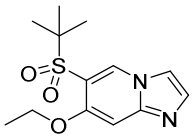
To 6-(*tert*-butylsulfonyl)-7-methoxyimidazo[1,2-*a*]pyridine (**17**, 6.9 g, 25.7 mmol) in DMF (40 mL) was added sodium propane-2-thiolate (5.05 g, 51.4 mmol). The mixture was heated to 150°C for 1hr., LCMS indicated completion of reaction. The mixture was concentrated. The resulted residue was suspended in water (50 mL) and cooled to 0°C. HCl (1N, ~45 mL) was added to neutralize the mixture to pH 4~5. Solid was collected by filtration and washed by water (5 mL) and ACN (5 mL) to provide 4.36 g 6-(*tert*-butylsulfonyl)imidazo[1,2-*a*]pyridin-7-ol. Filtrate was concentrated and purified by flash column chromatography (dry loaded by MeOH, 0~10%MeOH/DCM) provide more of product 6-(*tert*-butylsulfonyl)-imidazo[1,2-*a*]pyridin-7-ol (1.31 g). Combining the products provided a total yield of 6-(*tert*-butylsulfonyl)imidazo[1,2-*a*]pyridin-7-ol (5.67 g, 22.30 mmol, 87 % yield) as light yellow solid. Exact mass calculated for $C_{11}H_{14}N_2O_3S$: 254.1; found: MS *m/z* 255.1 (M + 1).

General procedure for the alkylation of 6-(*tert*-butylsulfonyl)imidazo[1,2-*a*]pyridin-7-ol (18):

To 6-(*tert*-Butylsulfonyl)imidazo[1,2-*a*]pyridin-7-ol (**18**) (1.02 g, 4.00 mmol) in DMF (10 mL) was added Cs_2CO_3 (1.96 g, 6.00 mmol). The mixture was stirred at room temperature for 5min. before the addition of RX (5.2 mmol). The mixture was stirred at designated temperature until completion analyzed by LCMS. Reaction mixture was filtered. The filtrate was concentrated and purified by silica gel flash column chromatography or RP-LCMS to provide the desired 6-(*tert*-butylsulfonyl)-7-alkoxyimidazo[1,2-*a*]pyridines.

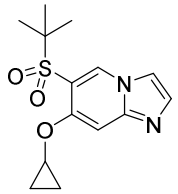
Compounds **19a-f** were prepared by the general procude for the alkylation of **18**:

6-(*tert*-Butylsulfonyl)-7-ethoxyimidazo[1,2-*a*]pyridine (**19a**)



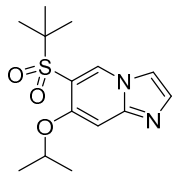
Compound **19a** was prepared using 6-(*tert*-butylsulfonyl)imidazo[1,2-*a*]pyridin-7-ol (**18**) and iodo-ethane at room temperature for 6hr and purified by silica gel flash column chromatography (0~80% (25% EtOH/EtOAc)/hexane). Yield, 53%. Exact mass calculated for $C_{13}H_{18}N_2O_3S$: 282.1; found: MS *m/z* 283.1 (M + 1).

6-(*tert*-Butylsulfonyl)-7-cyclopropoxyimidazo[1,2-*a*]pyridine (**19b**)



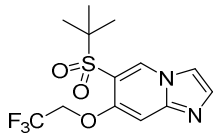
Compound **19b** was prepared using 6-(*tert*-butylsulfonyl)imidazo[1,2-*a*]pyridin-7-ol (**18**) and bromocyclopropane at 150°C for 16hr and purified by RP-LCMS. Yield, 11%. Exact mass calculated for $C_{14}H_{18}N_2O_3S$: 294.1; found: MS *m/z* 295.2 (M + 1).

6-(*tert*-Butylsulfonyl)-7-isopropoxyimidazo[1,2-*a*]pyridine (**19c**)



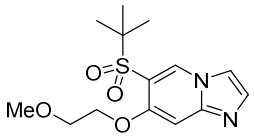
Compound **19c** was prepared using 6-(*tert*-butylsulfonyl)imidazo[1,2-*a*]pyridin-7-ol (**18**) and 2-iodopropane at room temperature for 16hr and purified by silica gel flash column chromatography (0~80%(25%EtOH/EtOAc)/hexane). Yield, 45%. Exact mass calculated for $C_{14}H_{20}N_2O_3S$: 296.1; found: MS *m/z* 297.2 (M + 1).

6-(*tert*-Butylsulfonyl)-7-cyclopropoxyimidazo[1,2-*a*]pyridine (**19d**)



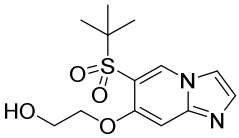
Compound **19d** was prepared using 6-(*tert*-butylsulfonyl)imidazo[1,2-*a*]pyridin-7-ol (**18**) and 2,2,2-trifluoroethyl trifluoromethanesulfonate at room temperature for 16hr and purified by silica gel flash column chromatography (0~80%(25%EtOH/EtOAc)/hexane). Yield, 68%. Exact mass calculated for $C_{13}H_{15}F_3N_2O_3S$: 336.1; found: MS *m/z* 337.2 (M + 1).

6-(*tert*-Butylsulfonyl)-7-(2-methoxyethoxy)imidazo[1,2-*a*]pyridine (**19e**)



Compound **19e** was prepared using 6-(*tert*-butylsulfonyl)imidazo[1,2-*a*]pyridin-7-ol (**18**) and 1-bromo-2-methoxyethane at room temperature for 16hr and purified by silica gel flash column chromatography (0~80% (25%EtOH/EtOAc)/hexane). Yield, 81%. Exact mass calculated for $C_{14}H_{20}N_2O_4S$: 312.1; found: MS *m/z* 313.2 (M + 1).

2-((6-(*tert*-Butylsulfonyl)imidazo[1,2-*a*]pyridin-7-yl)oxy)ethan-1-ol (**19f**)



Compound **19f** was prepared using 6-(*tert*-butylsulfonyl)imidazo[1,2-*a*]pyridin-7-ol (**18**) and 2-bromoethanol at 60°C for 16hr and purified by silica gel flash column chromatography

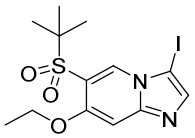
(0~80%(25%EtOH/EtOAc)/hexane). Yield, 43%. Exact mass calculated for $C_{13}H_{18}N_2O_4S$: 298.1; found: MS *m/z* 299.2 (M + 1).

General procedure for the iodonation of 6-(*tert*-butylsulfonyl)-7-alkoxyimidazo[1,2-*a*]pyridines

To imidazo[1,2-*a*]pyridine (1mmol) in MeOH (6 mL) was added NIS (1.2 mmol) at room temperature. The mixture was stirred at room temperature for 1hr. until LCMS indicated completion of the reaction. It was treated with sat. $NaHCO_3$(10 mL) and extracted with EtOAc (3×15 mL). The organic layers were combined and concentrated for purification by silica gel flash column chromatography (0~80% (25%EtOH/EtOAc)/hexane) to provide the desired 3-iodoimidazo[1,2-*a*]pyridines.

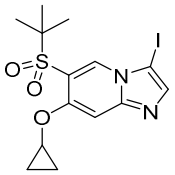
Compounds **20a-20f** were prepared using the general procedure for iodination:

6-(*tert*-Butylsulfonyl)-7-ethoxy-3-iodoimidazo[1,2-*a*]pyridine (**20a**)



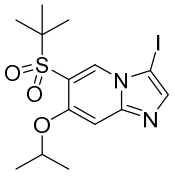
Compound **20a** was prepared from 6-(*tert*-butylsulfonyl)-7-ethoxyimidazo[1,2-*a*]pyridine (**19a**) and purified by silica gel flash column chromatography (0~80% (25%EtOH/EtOAc)/hexane). Yield, 98%. Exact mass calculated for $C_{13}H_{17}IN_2O_3S$: 408.0; found: MS *m/z* 409.0 (M + 1).

6-(*tert*-Butylsulfonyl)-7-cyclopropoxy-3-iodoimidazo[1,2-*a*]pyridine (**20b**)



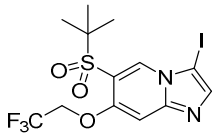
Compound **20b** was prepared from 6-(*tert*-butylsulfonyl)-7- cyclopropoxy imidazo[1,2-*a*]pyridine (**19b**) and purified by silica gel flash column chromatography (0~80% (25%EtOH/EtOAc)/hexane). Yield, 99%. Exact mass calculated for $C_{14}H_{17}IN_2O_3S$: 420.0; found: MS *m/z* 421.0 (M + 1).

6-(*tert*-Butylsulfonyl)-3-iodo-7-isopropoxyimidazo[1,2-*a*]pyridine (**20c**)



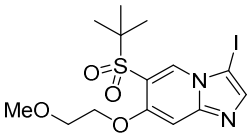
Compound **20c** was prepared from 6-(*tert*-butylsulfonyl)-7- isopropoxy imidazo[1,2-*a*]pyridine (**19c**) and purified by silica gel flash column chromatography (0~80% (25%EtOH/EtOAc)/hexane). Yield, 99%. Exact mass calculated for $C_{14}H_{19}IN_2O_3S$: 422.0; found: MS *m/z* 423.0 (M + 1).

6-(*tert*-Butylsulfonyl)-3-iodo-7-(2,2,2-trifluoroethoxy)imidazo[1,2-*a*]pyridine (**20d**)



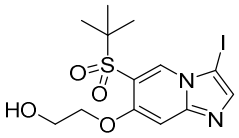
Compound **20d** was prepared from 6-(*tert*-butylsulfonyl)-7-(2,2,2-trifluoroethoxy)imidazo[1,2-*a*]pyridine (**19d**) and purified by silica gel flash column chromatography (0~80%(25%EtOH/EtOAc)/ hexane). Yield, 99%. Exact mass calculated for $C_{13}H_{14}F_3IN_2O_3S$: 462.0; found: MS *m/z* 463.0 (M + 1).

6-(*tert*-Butylsulfonyl)-3-iodo-7-(2-methoxyethoxy)imidazo[1,2-*a*]pyridine (**20e**)



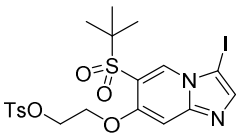
Compound **20e** was prepared from 6-(*tert*-butylsulfonyl)-7-(2-methoxyethoxy)imidazo[1,2-*a*]pyridine (**19e**) and purified by silica gel flash column chromatography (0~80% (25%EtOH/EtOAc)/hexane). Yield, 99%. Exact mass calculated for $C_{14}H_{19}IN_2O_4S$: 438.0; found: MS *m/z* 439.0 (M + 1).

2-((6-(*tert*-Butylsulfonyl)-3-iodoimidazo[1,2-*a*]pyridin-7-yl)oxy)ethan-1-ol (**20f**)



Compound **20f** was prepared from 2-((6-(*tert*-butylsulfonyl)imidazo[1,2-*a*]pyridin-7-yl)oxy)ethan-1-ol (**19f**) using general procedure for iodination and purified by silica gel flash column chromatography (0~80% (25%EtOH/EtOAc)/hexane). Yield, 95%. Exact mass calculated for $C_{13}H_{17}IN_2O_4S$: 424.0; found: MS *m/z* 425.0 (M + 1).

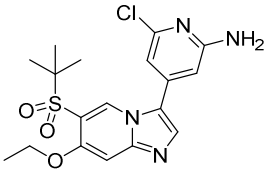
2-((6-(*tert*-Butylsulfonyl)-3-iodoimidazo[1,2-*a*]pyridin-7-yl)oxy)ethyl 4-methylbenzenesulfonate (**20g**)



2-((6-(*tert*-Butylsulfonyl)-3-iodoimidazo[1,2-*a*]pyridin-7-yl)oxy)ethan-1-ol (**20f**, 200 mg, 0.471 mmol) in DCM (5mL) was added TsCl (270 mg, 1.414 mmol) followed by triethylamine (0.197 mL, 1.414 mmol), mixture was stirred at room temperature for 48hr. Mixture was treated with $NaHCO_3$ (sat. 5mL) and extracted with DCM (3×10 mL). Organic layeres were combined, concentrated and purified by silica gel flash column chromatography(0~5%MeOH/DCM) to provide 2-((6-(*tert*-butylsulfonyl)-3-iodoimidazo[1,2-*a*]pyridin-7-yl)oxy)ethyl 4-methylbenzenesulfonate **20g** (263 mg, 0.455 mmol, 96 % yield). Exact mass calculated for $C_{20}H_{23}IN_2O_6S_2$: 578.0; found: MS *m/z* 579.1 (M + 1).

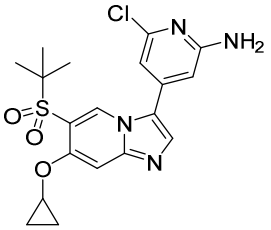
Compounds **7a-e** were prepared using the general procedure for Suzuki coupling:

4-(6-(*tert*-Butylsulfonyl)-7-ethoxyimidazo[1,2-*a*]pyridin-3-yl)-6-chloropyridin-2-amine (**7a**)



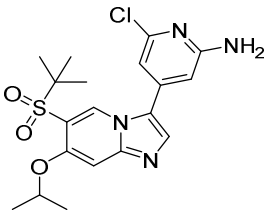
Compound **7a** was synthesized from 6-(*tert*-butylsulfonyl)-7-ethoxyimidazo[1,2-*a*]pyridine (**20a**) and 6-chloro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-amine and purified by RP-LC/MS. Yield, 30%. ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 8.98 (s, 1H), 8.26 (s, 1H), 7.50 (s, 1H), 6.89 (s, 1H), 6.75 (s, 1H), 4.44 (q, J = 4.4 Hz, 2H), 1.57 (t, J = 4.4 Hz, 3H), 1.45 (s, 9H). Exact mass calculated for $C_{18}H_{21}ClN_4O_3S$: 408.1; found: MS *m/z* 409.1 (M + 1).

4-(6-(*tert*-Butylsulfonyl)-7-cyclopropoxyimidazo[1,2-*a*]pyridin-3-yl)-6-chloropyridin-2-amine (**7b**)



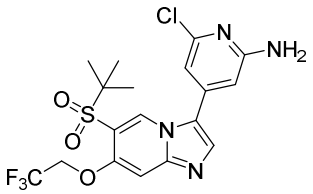
Compound **7b** was synthesized from 6-(*tert*-butylsulfonyl)-7-cyclopropoxy-3-iodoimidazo[1,2-*a*]pyridine (**20b**) and 6-chloro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-amine and purified by RP-LC/MS. Yield, 9%. ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 8.98 (s, 1H), 8.27 (s, 1H), 7.80 (s, 1H), 6.85 (d, J = 1.2 Hz, 1H), 6.71 (d, J = 1.2 Hz, 1H), 4.25 (m, 1H), 1.41 (s, 9H), 1.06 (m, 2H), 0.98 (m, 2H). Exact mass calculated for $C_{19}H_{21}ClN_4O_3S$: 420.1 (M + 1); found: MS *m/z* 421.1 (M + 1).

4-(6-(*tert*-Butylsulfonyl)-7-isopropoxyimidazo[1,2-*a*]pyridin-3-yl)-6-chloropyridin-2-amine (**7c**)



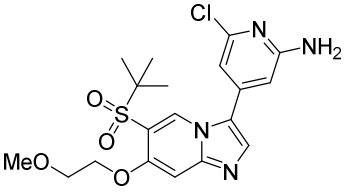
Compound **7c** was synthesized from 6-(*tert*-butylsulfonyl)-3-iodo-7-isopropoxyimidazo[1,2-*a*]pyridine (**20c**) and 6-chloro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-amine and purified by RP-LC/MS. Yield, 42%. ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 9.00 (s, 1H), 8.31 (s, 1H), 7.57 (s, 1H), 6.98 (s, 1H), 6.88 (s, 1H), 5.09 (m, 1H), 1.51 (d, J = 5.6 Hz, 6H), 1.45 (s, 9H). Exact mass calculated for $C_{19}H_{23}ClN_4O_3S$: 422.1; found: MS *m/z* 423.1 (M + 1).

4-(6-(*tert*-Butylsulfonyl)-7-(2,2,2-trifluoroethoxy)imidazo[1,2-*a*]pyridin-3-yl)-6-chloropyridin-2-amine (**7d**)



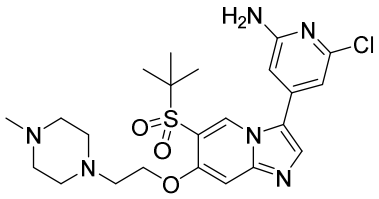
Compound **7d** was synthesized from 6-(*tert*-butylsulfonyl)-3-iodo-7-(2,2,2-trifluoroethoxy)imidazo-[1,2-*a*]pyridine (**20d**) and 6-chloro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-amine and purified by RP-LC/MS. Yield, 51%. ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 9.03 (s, 1H), 8.33 (s, 1H), 7.65 (s, 1H), 6.87 (d, J = 1.2 Hz, 1H), 6.72 (d, J = 1.2 Hz, 1H), 5.02 (m, 2H), 1.45 (s, 9H). Exact mass calculated for $C_{18}H_{18}F_3ClN_4O_3S$: 462.1; found: MS *m/z* 463.1 (M + 1).

4-(6-(*tert*-Butylsulfonyl)-7-(2-methoxyethoxy)imidazo[1,2-*a*]pyridin-3-yl)-6-chloropyridin-2-amine (**7e**)



Compound **7e** was synthesized from 6-(*tert*-butylsulfonyl)-3-iodo-7-(2-methoxyethoxy)imidazo[1,2-*a*]pyridine (**20e**) and 6-chloro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-amine and purified by RP-LC/MS. Yield, 53%. ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 8.99 (s, 1H), 8.26 (s, 1H), 7.55 (s, 1H), 6.88 (d, J = 1.2 Hz, 1H), 6.74 (d, J = 1.2 Hz, 1H), 4.51 (m, 2H), 3.87 (m, 2H), 3.43 (s, 3H), 1.45 (s, 9H). Exact mass calculated for $C_{19}H_{23}ClN_4O_4S$: 438.1; found: MS *m/z* 439.1 (M + 1).

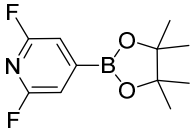
4-(6-(*tert*-Butylsulfonyl)-7-(2-(4-methylpiperazin-1-yl)ethoxy)imidazo[1,2-*a*]pyridin-3-yl)-6-chloropyridin-2-amine (**7f**)



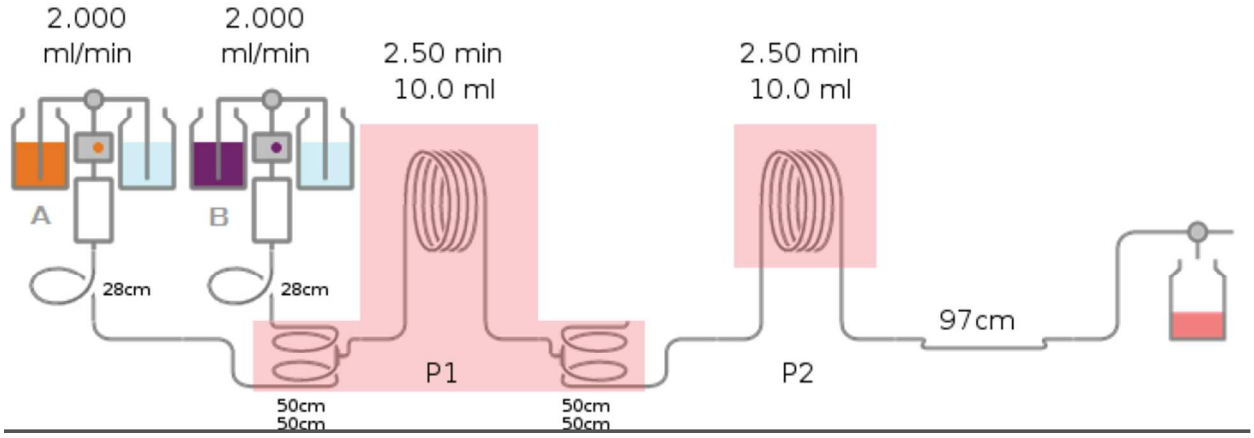
To 2-((6-(*tert*-butylsulfonyl)-3-iodoimidazo[1,2-*a*]pyridin-7-yl)oxy)ethyl 4-methylbenzenesulfonate (**20g**, 20 mg, 0.035 mmol) in THF (0.5 mL) was added 1-methylpiperazine (11 mg, 0.104 mmol). The mixture was stirred at 60°C for 16hr. with completed conversion to intermediate 6-(*tert*-butylsulfonyl)-3-iodo-7-(2-(4-methylpiperazin-1-yl)ethoxy)imidazo[1,2-*a*]pyridine (**20h)** by LCMS. The mixture was then cooled to room temperature, added 6-chloro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-amine (10.56 mg, 0.041 mmol), $PdCl_2$(dppf)·CH_2Cl_2 adduct (0.003 mmol), Na_2CO_3 (2M aqueous solution, 0.05 mL, 0.1 mmol) and ethanol (0.5 mL). The reaction tube was degassed by applying vacuum and N_2 alternatively for three times, sealed under N_2 and heated in microwave reactor to 140°C for 20min. The reaction mixture was filtered and directly purified by RP-LCMS to provide 4-(6-(*tert*-butylsulfonyl)-7-(2-(4-methylpiperazin-1-yl)ethoxy)imidazo[1,2-*a*]pyridin-3-yl)-6-chloropyridin-2-amine (**7f**, 9 mg, 0.015 mmol, 44.2 % yield). ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 9.01 (s, 1H), 8.37 (s, 1H), 7.69 (s, 1H),

6.96 (s, 1H), 6.84 (d, J = 0.8 Hz, 1H), 4.95 (m, 2H), 4.40-3.60 (m, 10H), 3.01 (s, 3H), 1.47 (s, 9H). Exact mass calculated for $C_{23}H_{31}ClN_6O_3S$: 506.2; found: MS m/z 507.2 (M + 1).

2,6-Difluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (**29**)

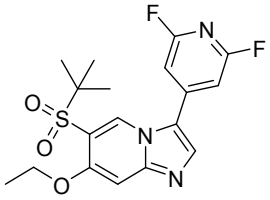


The reaction was performed in flow with the following setup:



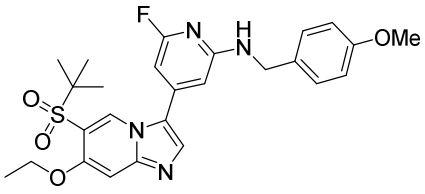
4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (55.2 g, 217 mmol) was dissolved in cyclopentyl methyl ether (CPME, 250 mL) and 2,6-difluoropyridine (**28**, 19.72 mL, 217 mmol) was added. In a separate container, 4,4'-di-*tert*-butyl-2,2'-bipyridine (0.700 g, 2.61 mmol), (1,5-cyclooctadiene)(methoxy)iridium(I) dimer (1.728 g, 2.61 mmol) and cyclopentyl methyl ether (250 mL) were combined and the mixture was sonicated. To dissolve the solids (catalyst mixture), tetrahydrofuran (10 mL) was added. The mixture was sonicated until a homogenous mixture was obtained (dark brown) and then stirred continuously with a stir bar while the mixture was flowing through the system. Additional cyclopentyl methyl ether was added to the flask containing 2,6-difluoropyridine until equal volumes were obtained between the two flasks. The reaction was performed at 2 mL/min in each reagent at 100°C. The solvent was removed (aspirator) and the crude material was purified by flash column chromatography (silica gel, loaded neat with hexane rinse, 0-10% EtOAc/hexanes) to provide 2,6-difluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (**29**, 26.6g, 50.8%) as colorless needlelike crystals. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.23 (s, 2H), 1.32 (s, 12H). 2,6-difluoro-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (**30**) as by-product was eluted afterward. Exact mass calculated for $C_{11}H_{14}BF_2NO_2$: 241.1; Mass of boronic acid was observed. MS m/z 160.1 (M-82+1).

6-(*tert*-Butylsulfonyl)-3-(2,6-difluoropyridin-4-yl)-7-ethoxyimidazo[1,2-*a*]pyridine (**7h**)



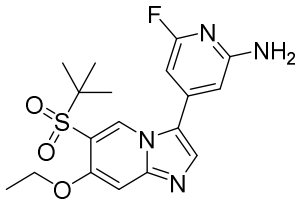
Compound **7h** was synthesized from 6-(*tert*-butylsulfonyl)-7-ethoxy-3-iodoimidazo[1,2-*a*]pyridine (**20a**) and 2,6-difluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (**29**) using general procedure for Suzuki coupling reaction and purified by silica gel flash column chromatography (0~80% (25%EtOH/EtOAc)/hexane). Yield, 76%. Exact mass calculated for $C_{18}H_{19}F_2N_3O_3S$: 395.1; found: MS *m/z* 396.1 (M + 1).

4-(6-(*tert*-Butylsulfonyl)-7-ethoxyimidazo[1,2-*a*]pyridin-3-yl)-6-fluoro-N-(4-methoxybenzyl)pyridin-2-amine (**7i**)



6-(*tert*-Butylsulfonyl)-3-(2,6-difluoropyridin-4-yl)-7-ethoxyimidazo[1,2-*a*]pyridine (**7h**, 60 mg, 0.152 mmol), (4-methoxyphenyl)methanamine (0.040 mL, 0.30 mmol) and DIEA (0.040 mL, 0.228 mmol) in IPA (1 mL) was heated at 150°C in microwave reactor for 30 min. The mixture was concentrated and purified by silica gel flash column chromatography (0~80%(25%EtOH/EtOAc)/hexane) to provide 4-(6-(*tert*-butylsulfonyl)-7-ethoxyimidazo[1,2-*a*]pyridin-3-yl)-6-fluoro-N-(4-methoxybenzyl)pyridin-2-amine (**7i**, 59 mg, 0.115 mmol, 75 % yield) as white solid. Exact mass calculated for $C_{26}H_{29}FN_4O_4S$: 512.2; found: MS *m/z* 513.2 (M + 1).

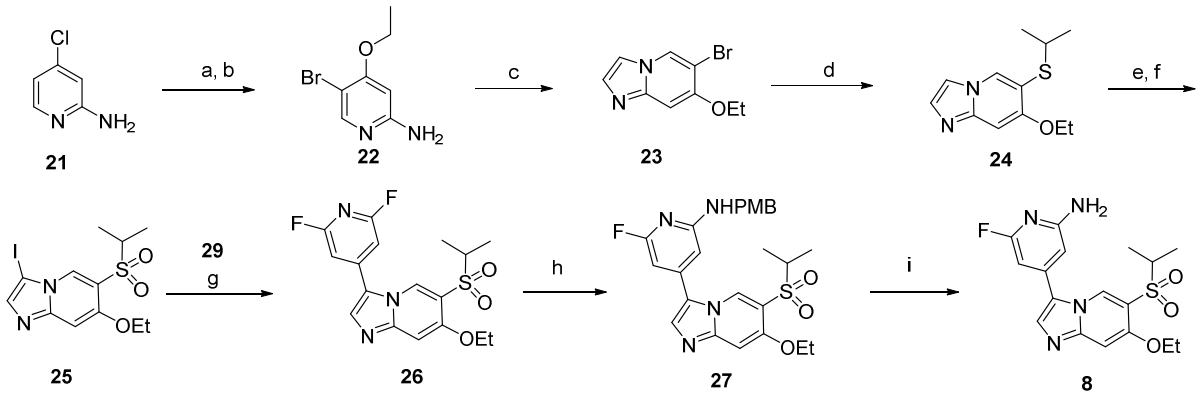
4-(6-(*tert*-Butylsulfonyl)-7-ethoxyimidazo[1,2-*a*]pyridin-3-yl)-6-fluoropyridin-2-amine (**7g**)



4-(6-(*tert*-Butylsulfonyl)-7-ethoxyimidazo[1,2-*a*]pyridin-3-yl)-6-fluoro-N-(4-methoxybenzyl)pyridin-2-amine (**7i**, 55mg, 0.107mmol) in DCM (2mL) was added TFA (0.4mL) at rt. The mixture was stirred at room temperature for 16hr. and concentrated, treated with sat. $NaHCO_3$ (3mL) and extracted with DCM (3×5 mL). The organic layers were combined, concentrated and purified by silica gel flash column chromatography (0~80%(25%EtOH/EtOAc)/hexane) to provide 4-(6-(*tert*-butylsulfonyl)-7-ethoxyimidazo[1,2-*a*]pyridin-3-yl)-6-fluoropyridin-2-amine (**7g**, 20 mg, 0.048 mmol, 32 % yield). ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 8.97 (s, 1H), 7.82 (s, 1H), 7.12 (s, 1H), 6.59 (t, J = 1.0 Hz, 1H), 6.37 (s, 1H), 4.27 (q, J = 5.6 Hz, 2H), 1.51 (t, J = 5.6 Hz, 3H), 1.43 (s, 9H). Exact mass calculated for $C_{18}H_{21}FN_4O_3S$: 392.1; found: MS *m/z* 393.1 (M + 1).

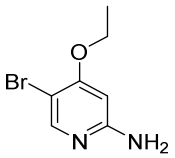
Synthesis of 4-(7-ethoxy-6-(isopropylsulfonyl)imidazo[1,2-*a*]pyridin-3-yl)-6-fluoropyridin-2-amine (8).

Scheme SI-3[a]



[a]Reagents and conditions: (a) NaOEt, EtOH; (b) Br_2, AcOH, rt., 61%; (c) 2-chloroacetaldehyde, $NaHCO_3$, EtOH, 80˚C, 78%; (d) ^{i}ProSH, $Pd_2(dba)_3$, xantphos, K_2CO_3, dioxane, 110˚C, 99%; (e) oxone, MeOH/water (3:1); (f) NIS, MeOH/water (3:1), 72%; (g) 2,6-difluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine, $PdCl_2$(dppf), $NaHCO_3$, dioxane, microwave, 125 ˚C, 25 min, 70.2%; (h) p-Methoxybenzylamine, DIEA, IPA, microwave, 140˚C, 25min, 99%; (i) TFA, DCM, 62%.

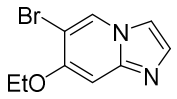
5-Bromo-4-ethoxypyridin-2-amine (**22**)



4-Chloropyridin-2-amine (**21**, 25 g, 194 mmol) and sodium ethoxide (181 ml, 486 mmol, 21% solution in EtOH) were sealed in a 350 mL pressure flask. The content was heated to 145 ˚C for 16 h. The reaction mixture was allowed to cool to room temperature. Water (200 mL) and AcOH (150 mL) were added and the mixture was stirred for 10 min. The solvent was removed (aspirator), EtOAc (200 mL) was added, the mixture was sonicated and then filtered through Celite® to remove NaCl. Additional EtOAc (400 mL total) was used to rinse. Solvent was removed (aspirator) to provide the crude intermediate 4-ethoxypyridin-2-amine. Exact mass calculated for $C_7H_{10}N_2O$: 138.1.0; found: MS *m/z* 139.1 (M + 1).

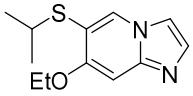
Crude 4-ethoxypyridin-2-amine from above was dissolved in AcOH (200 mL). 25 mL of a solution of bromine in AcOH (194 mmol bromine in 50mL AcOH) was added dropwise. After 10 min, partial conversion was observed to the mono-bromo product by LCMS. Additional bromine from the previously mentioned bromine solution was added in small increments (~5 mL increments) until nearly full conversion was observed by LCMS. Solvent volume was reduced by a half (aspirator) and the solid was filtered and washed with minimal amount of AcOH to give 5-bromo-4-ethoxypyridin-2-amine (25.7 g, 118 mmol, 61 % yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (s, 1H), 7.73 (s, 2H), 6.42 (s, 1H), 4.23 (q, *J* = 7.0 Hz, 2H), 1.41 (t, *J* = 7.0 Hz, 3H). Exact mass calculated for $C_7H_9BrN_2O$: 216.0; found: MS *m/z* 217.0 (M + 1).

6-Bromo-7-ethoxyimidazo[1,2-*a*]pyridine (**23**)



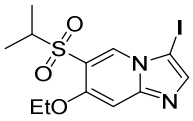
To a solution of 5-bromo-4-ethoxypyridin-2-amine (**22**) (26.4 g, 122 mmol) in ethanol (405 ml) was added $NaHCO_3$ (30.7 g, 365 mmol) followed by 2-chloroacetaldehyde (20.50 ml, 182 mmol, 55% in water). The mixture was heated at 80°C for 16hr, and turned into a dark colored solution. It was then cooled down to room temperature. The volumn of solvent was reduced by half (aspirator) and H_2O (200 mL) was added. The mixture was sonicated until a precipitate formed. The solid was filtered and washed with additional water. The filtrate was concentrated (aspirator), the residue was dissolved in DCM/MeOH (~10% MeOH in DCM, ~200 mL) and the mixture was passed through a silica gel plug (60 grams silica gel). An additional 500 mL of 10% MeOH in DCM was used to rinse the plug. The solvent was fully removed (aspirator) and water (200 mL) was added to precipitate the product. The mixture was filtered and the solid was washed with water (200 mL). The solids were combined to give 6-bromo-7-ethoxyimidazo[1,2-*a*]pyridine (**23**) (22.8 g, 95 mmol, 78 % yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.89 (s, 1H), 7.71 (dd, J = 1.2, 0.7 Hz, 1H), 7.43 (d, J = 1.3 Hz, 1H), 7.07 (s, 1H), 4.16 (q, J = 7.0 Hz, 2H), 1.40 (t, J = 7.0 Hz, 3H). Exact mass calculated for $C_9H_9BrN_2O$: 240.0; found: MS *m/z* 241.1 (M + 1).

7-Ethoxy-6-(isopropylthio)imidazo[1,2-*a*]pyridine (**24**)



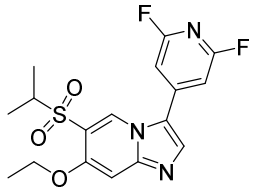
A mixture of 6-bromo-7-ethoxyimidazo[1,2-*a*]pyridine (**23**) (20 g, 83 mmol), $Pd_2(dba)_3 \cdot CH_2Cl_2$ (0.429 g, 0.415 mmol), xantphos (0.480 g, 0.830 mmol) and K_2CO_3 (13.76 g, 100 mmol) in dioxane (250 mL) was flushed with N_2 and propane-2-thiol (8.48 mL, 91 mmol) was added. The reaction mixture was heated to 110˚C for 16hr. After cooling down to room temperature, solid was removed by filtration over Celite® and washed with ACN (200 mL) and DCM (200 mL). The filtrate was concentrated and purified by flash column chromatography (silica gel, loaded neat with DCM rinse, 0-8% MeOH /DCM) to provide 7-ethoxy-6-(isopropylthio)imidazo[1,2-*a*]pyridine (**24**) (19.5g, 99%) as light brownish solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 (s, 1H), 7.75 - 7.69 (m, 1H), 7.39 (d, J = 1.3 Hz, 1H), 6.95 (s, 1H), 4.12 (q, J = 7.0 Hz, 2H), 3.40 (p, J = 6.7 Hz, 1H), 1.39 (t, J = 7.0 Hz, 3H), 1.20 (d, J = 6.7 Hz, 6H). Exact mass calculated for $C_{12}H_{16}N_2OS$: 236.1; found: MS *m/z* 237.1 (M + 1).

7-Ethoxy-3-iodo-6-(isopropylsulfonyl)imidazo[1,2-*a*]pyridine (**25**)



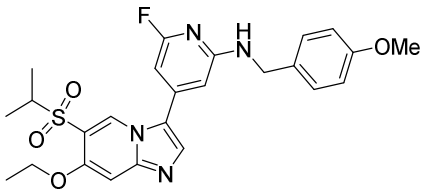
To a mixture of 7-ethoxy-6-(isopropylthio)imidazo[1,2-*a*]pyridine (**24**) (19.5 g, 83 mmol) in MeOH (423 ml) and water (127 ml) was added oxone (76 g, 124 mmol, Aldrich) and the mixture was stirred at room temperature overnight. LC/MS indicated complete formation of intermediate 7-ethoxy-6-(isopropylsulfonyl)imidazo[1,2-*a*]pyridine. To the reaction mixture was then added NIS (18.56 g, 83 mmol) and the mixture was stirred at room temperature for 2hr. To the mixture was added $NaHCO_3$ (saturated, until pH ~8 was obtained for the mixture), then extracted with EtOAc (x3, ~ 1500 mL total volume). The organics were combined and dried (Na_2SO_4). After filtering off the drying agent, the solvent was reduced in volume to about 250 mL, the resulting solid was collected by filtra-tion and washed with EtOAc (100 mL) to provide 7-ethoxy-3-iodo-6-(isopropylsulfonyl)imidaz[1,2-*a*]pyridine (**25**) (23 grams, 72%). Exact mass calculated for $C_{12}H_{15}IN_2O_3S$: 394.0; found: MS *m/z* 395.0 (M + 1)

3-(2,6-Difluoropyridin-4-yl)-7-ethoxy-6-(isopropylsulfonyl)imidazo[1,2-*a*]pyridine (**26**)



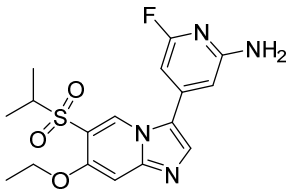
7-Ethoxy-3-iodo-6-(isopropylsulfonyl)imidazo[1,2-*a*]pyridine (**25**) (5.7 g, 14.46 mmol), 2,6-difluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (**29)** (6.97 g, 28.9 mmol), $PdCl_2(dppf).CH_2Cl_2$ adduct (590 mg, 0.723 mmol) and $NaHCO_3$ (43mL, 43 mmol, saturated solution) in dioxane (96mL) was degassed by applying vacuum and N_2 alternatively three times, sealed under N_2, and heated to 125°C for 25 min in a microwave reactor. The mixture was added to a separatory funnel and diluted with EtOAc (100mL) and NH_4Cl (sat. 50mL). The layers were separated, the organics were washed with brine, dried (Na_2SO_4) and filtered. The filtrate was concentrated and purified by flash column chromatography (silica gel, loaded neat with DCM rinse, 0-70% (25% EtOAc/EtOH)/hexane). Impure fractions were purified again with an additional column (silica gel, loaded neat with DCM rinse, 0-8% MeOH/DCM) to give 3-(2,6-difluoropyridin-4-yl)-7-ethoxy-6-(isopropylsulfonyl)imidazo[1,2-*a*]pyridine (**26**) (3.87 g, 10.15 mmol, 70.2 % yield) after combining. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.92 (s, 1H), 8.18 (s, 1H), 7.57 (s, 2H), 7.38 (s, 1H), 4.32 (q, J = 7.0 Hz, 2H), 3.75 (p, J = 6.9 Hz, 1H), 1.42 (t, J = 7.0 Hz, 3H), 1.25 (d, J = 6.9 Hz, 6H). Exact mass calculated for $C_{17}H_{17}F_2N_3O_3S$: 381.1; found: MS *m/z* 382.1 (M + 1).

4-(7-Ethoxy-6-(isopropylsulfonyl)imidazo[1,2-*a*]pyridin-3-yl)-6-fluoro-N-(4-methoxybenzyl)pyridin-2-amine (**27**)



A mixture of 3-(2,6-difluoropyridin-4-yl)-7-ethoxy-6-(isopropylsulfonyl)imidazo[1,2-*a*]pyridine (**26**) (3.87 g, 10.15 mmol), (4-methoxyphenyl)methanamine (2.65 ml, 20.29 mmol) and DIEA (3.54 ml, 20.29 mmol) in IPA (85 ml) was heated at 140°C in a microwave reactor for 25min. The solvent was removed (aspirator) and the crude material was purified by flash column chromatography (silica gel, loaded neat with DCM rinse, 0-70% (3:1 EtOAc:EtOH)/hexane) to give 4-(7-ethoxy-6-(isopropylsulfonyl)imidazo-[1,2-*a*]pyridin-3-yl)-6-fluoro-N-(4-methoxybenzyl)pyridin-2-amine (**27**) (5.02 g, 10.07 mmol, 99 % yield) after combining the pure fractions. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.84 (s, 1H), 7.91 (s, 1H), 7.73 (t, J = 5.7 Hz, 1H), 7.35 – 7.26 (m, 3H), 6.96 – 6.86 (m, 2H), 6.61 (s, 1H), 6.46 (s, 1H), 4.40 (d, J = 5.8 Hz, 2H), 4.29 (q, J = 7.0 Hz, 2H), 3.73 (s, 3H), 1.41 (t, J = 6.9 Hz, 3H), 1.23 (d, J = 6.9 Hz, 6H). Exact mass calculated for $C_{25}H_{27}FN_4O_4S$: 498.2; found: MS *m/z* 499.2 (M + 1).

4-(7-Ethoxy-6-(isopropylsulfonyl)imidazo[1,2-*a*]pyridin-3-yl)-6-fluoropyridin-2-amine (**8**)



To a solution of 4-(7-ethoxy-6-(isopropylsulfonyl)imidazo[1,2-*a*]pyridin-3-yl)-6-fluoro-N-(4-methoxybenzyl)pyridin-2-amine (**27**) (2.36 g, 6.24 mmol, 61.9 % yield) in DCM (50 mL) was added trifluoroacetic acid (15.51 mL, 201 mmol) at room temperature and the mixture was stirred for 3 hr. The mixture was concentrated and residue was partitioned between DCM (200 mL) and sat. $NaHCO_3$ (100mL). The organic layer was collected and the aqueous layer was extracted with DCM (2x100mL). The organics layers were dried (Na_2SO_4), filtered off drying agents and concentrated. The crude material was purified by flash column chromatography (silica gel, dry loaded, 0-6% MeOH/DCM). The solids were suspended in MeOH (30 mL), collected by filtration, and washed with MeOH (3x 10 mL). The solids were air dried on the filter paper and then under high vacuum to give 4-(7-ethoxy-6-(isopropylsulfonyl)imidazo[1,2-*a*]pyridin-3-yl)-6-fluoropyridin-2-amine (**8**) (2.36 g, 6.24 mmol, 61.9 % yield). ^{1}H NMR (400MHz, 300K, MeOH-d_4): δ 8.99 (s, 1H), 7.82 (s, 1H), 7.14 (s, 1H), 6.62 (t, J = 1.0 Hz, 1H), 6.40 (s, 1H), 4.32 (q, J = 5.6 Hz, 2H), 3.83 (m, 1H), 1.53 (t, J = 5.6 Hz, 3H), 1.33 (d, J = 5.6 Hz, 6H). ^{13}C NMR (400 MHz, 300K, DMSO- d_6) δ 162.4, 159.8, 152.6, 148.5, 141.2, 135.4, 129.6, 123.6, 117.7, 101.1, 96.9, 91.8, 65.3, 53.6, 14.8, 14.0. ^{19}F NMR (400 MHz, 300K, DMSO-d_6) δ 69.9. Exact mass calculated for $C_{17}H_{19}FN_4O_3S$: 378.1; found: LC/MS *m/z* 379.1 (M + 1). Anal. Calc. for $C_{17}H_{19}FN_4O_3S$: C, 53.96; H, 5.06; N, 14.81; found: C 54.04; H, 4.97; N, 14.73.

BIOLOGY ASSAYS

ADP transcreener Assay:

The ADP transcreener assay was carried out according to the manufactural specifications (Cis-Bio). Briefly, the assay was carried out in 1536-well white plates in 50 mM HEPES pH7, 10 mM $MgCl_2$, 2 mM $MnCl_2$, 5 mM beta glycerol-2-phosphate, 1mM EGTA, 0.5 mM sodium orthovanadate, 0.01% Tween 20, 2 mM DTT, and 0.01% BSA. 6.25 ng full length RIPK2 in 2.5 µL were incubated with 50 nL compounds for 30 min, followed by the addition of 2.5 µL of 20 µM ATP. The reaction was incubated for 2 hours at room temperature, then 2.5 µl of 1× ADP-d2 followed by 2.5 µL of 1× anti-ADP cryptate was added to each well. Plates were further incubated for 60 minutes and fluorescence was read at Ex 320 nm/(Em 620 nm) (donor) and at Ex 320 nm/(Em 665 nm) (acceptor) on a PHERAstar reader. The fluorescence ratio (acceptor/donor) was calculated and used to determine RIPK2 activity.

Novartis enzymatic kinase profiling panel description:

Compounds were tested against ~50 kinases in this panel. Kinases were expressed as either histidine- or GST-tagged fusion proteins using the baculovirus expression technology. The kinase activities were measured in the LabChip mobility-shift assay (PerkinElmer). The assay was performed at 30 °C for 60 min. The effect of compound on the enzymatic activity was obtained from the linear progress curves in the absence and presence of compound and routinely determined from one reading (end point measurement).

Nanosyn 250 kinases kinome profiling data for compound **8:**

Compound **8** were tested against 250 kinases kinome panel at Nanosyn (http://www.nanosyn.com) using microfluidics capillary electrophoresis technology to measure the change in electrophoretic mobility of the substrate upon phosphorylation. Compound was tested in duplicate mode at 0.5 µM and 5 µM. Data of the two runs were averaged and converted to % inhibition of enzyme activity (Table SI-1)

Table SI-1. Nanosyn 250 kinases kinome profile of compound **8**.

Kinase	Conc. Tested (μM)	Compd 8	Conc. Tested (μM)	Compd 8	Kinase	Conc. Tested (μM)	Compd 8	Conc. Tested (μM)	Compd 8
ABL1	0.5	11	5	60	MAPKAPK-2	0.5	3	5	5
AKT1	0.5	4	5	2	MAPKAPK-3	0.5	2	5	4
AKT2	0.5	3	5	2	MARK1	0.5	-18	5	-14
AKT3	0.5	8	5	8	MARK3	0.5	-1	5	-4
ALK	0.5	14	5	18	MARK4	0.5	-8	5	-6
AMP-A1B1G1	0.5	2	5	4	MEK1	0.5	-9	5	-5
AMP-A2B1G1	0.5	-3	5	0	MEK2	0.5	5	5	7
ARG	0.5	1	5	28	MEK3	0.5	8	5	5
ARK5	0.5	0	5	2	MELK	0.5	0	5	23
AURORA-A	0.5	19	5	27	MER	0.5	-3	5	23
AURORA-B	0.5	11	5	12	MET	0.5	4	5	7
AURORA-C	0.5	-1	5	-2	MKNK1	0.5	11	5	24
AXL	0.5	22	5	33	MNK2	0.5	14	5	21
BLK	0.5	14	5	37	MRCK-ALPHA	0.5	2	5	3
BMX	0.5	3	5	13	MRCK-BETA	0.5	-1	5	1
BRAF	0.5	6	5	16	MSK1	0.5	10	5	12
BRK	0.5	42	5	83	MSK2	0.5	9	5	10
BRSK1	0.5	4	5	17	MSSK1	0.5	-7	5	-5
BRSK2	0.5	-2	5	0	MST1	0.5	21	5	13
BTK	0.5	12	5	18	MST2	0.5	19	5	12
CAMK1A	0.5	7	5	7	MST3	0.5	28	5	6
CAMK1D	0.5	2	5	3	MST4	0.5	20	5	-7
CAMK2A	0.5	0	5	0	MUSK	0.5	10	5	15
CAMK2B	0.5	2	5	3	NDR2	0.5	-4	5	-2
CAMK2D	0.5	-2	5	-1	NDRG1	0.5	6	5	0
CAMK2G	0.5	-15	5	-12	NEK1	0.5	8	5	1
CAMK4	0.5	-2	5	-3	NEK2	0.5	-2	5	5
CDK1	0.5	16	5	7	NEK6	0.5	4	5	4
CDK2	0.5	3	5	6	NEK7	0.5	3	5	4
CDK2-CYCLINE	0.5	7	5	13	NEK9	0.5	3	5	6
CDK3-CYCLINE	0.5	3	5	4	P38-ALPHA	0.5	17	5	20
CDK4-CYCLIND	0.5	5	5	8	P38-BETA	0.5	6	5	7
CDK5	0.5	3	5	4	P38-DELTA	0.5	5	5	6
CDK5-P25	0.5	3	5	6	P38-GAMMA	0.5	4	5	6
CDK6-CYCLIND3	0.5	-4	5	-2	P70S6K1	0.5	5	5	6
CDK9-CYCLINT1	0.5	17	5	29	P70S6K2	0.5	1	5	4
CHEK1	0.5	-2	5	-1	PAK1	0.5	-1	5	-1
CHEK2	0.5	-6	5	-5	PAK2	0.5	-14	5	-14
CK1	0.5	16	5	51	PAK3	0.5	-8	5	-7
CK1-EPSILON	0.5	6	5	30	PAK4	0.5	3	5	4
CK1-GAMMA1	0.5	14	5	46	PAK5	0.5	-5	5	-4
CK1-GAMMA2	0.5	10	5	30	PAK6	0.5	7	5	12
CK1-GAMMA3	0.5	12	5	58	PAR-1B-ALPHA	0.5	1	5	2
CLK1	0.5	-8	5	16	PASK	0.5	-2	5	1
CLK2	0.5	-13	5	12	PDGFR-ALPHA	0.5	51	5	89
CLK3	0.5	-14	5	1	PDGFR-BETA	0.5	76	5	95
CLK4	0.5	44	5	91	PDK1	0.5	-1	5	0
CRAF	0.5	1	5	3	PERK	0.5	-2	5	-4
CSK	0.5	18	5	34	PHK-GAMMA1	0.5	14	5	19
DAPK1	0.5	3	5	2	PHK-GAMMA2	0.5	-10	5	4
DAPK3	0.5	-1	5	1	PI3-KINASE-ALPHA	0.5	-9	5	-9
DCAMKL2	0.5	6	5	7	PI4-K-BETA	0.5	-3	5	27
DDR1	0.5	23	5	65	PIM-1-KINASE	0.5	-2	5	1
DDR2	0.5	9	5	15	PIM2	0.5	2	5	1
DYRK1A	0.5	0	5	6	PIM3	0.5	1	5	2
DYRK1B	0.5	-8	5	-4	PKACB	0.5	-1	5	1
DYRK2	0.5	8	5	22	PKC-ALPHA	0.5	3	5	6
DYRK3	0.5	3	5	9	PKC-BETA1	0.5	1	5	6
DYRK4	0.5	-3	5	-3	PKC-BETA2	0.5	5	5	5
EGFR	0.5	7	5	5	PKC-EPSILON	0.5	14	5	4
EPH-A1	0.5	28	5	68	PKC-ETA	0.5	6	5	11
EPH-A2	0.5	37	5	82	PKC-GAMMA	0.5	4	5	9
EPH-A3	0.5	53	5	86	PKC-IOTA	0.5	9	5	3
EPH-A4	0.5	32	5	74	PKC-THETA	0.5	0	5	7

Kinase	Conc. Tested (μM)	Compd 8	Conc. Tested (μM)	Compd 8	Kinase	Conc. Tested (μM)	Compd 8	Conc. Tested (μM)	Compd 8
EPH-A5	0.5	39	5	80	PKC-ZETA	0.5	11	5	13
EPH-A8	0.5	31	5	72	PKN1	0.5	5	5	50
EPH-B1	0.5	20	5	46	PKN2	0.5	6	5	4
EPH-B2	0.5	64	5	92	PLK1	0.5	10	5	15
EPH-B3	0.5	13	5	17	PLK3	0.5	2	5	6
EPH-B4	0.5	20	5	56	PLK4	0.5	-4	5	2
ERB-B2	0.5	7	5	-1	PRAK	0.5	6	5	5
ERB-B4	0.5	12	5	8	PRKACA	0.5	5	5	5
FAK	0.5	11	5	11	PRKD1	0.5	4	5	7
FER	0.5	5	5	15	PRKD2	0.5	7	5	9
FES	0.5	4	5	8	PRKD3	0.5	2	5	6
FGFR1	0.5	23	5	39	PRKG1	0.5	4	5	6
FGFR2	0.5	13	5	28	PRKG2	0.5	14	5	14
FGFR3	0.5	2	5	9	PRKX	0.5	-1	5	-4
FGFR4	0.5	13	5	16	PTK5	0.5	8	5	22
FGR	0.5	24	5	51	PYK2	0.5	-1	5	6
FLT-1	0.5	16	5	46	RET	0.5	10	5	32
FLT-3	0.5	14	5	40	RIPK2	0.5	95	5	96
FLT-4	0.5	37	5	81	ROCK1	0.5	2	5	3
FMS	0.5	30	5	38	ROCK2	0.5	10	5	-2
FRAP1	0.5	-1	5	-1	RON	0.5	4	5	7
FYN	0.5	27	5	64	ROS	0.5	12	5	29
GRK6	0.5	1	5	3	RSK1	0.5	-4	5	2
GRK7	0.5	13	5	15	RSK2	0.5	1	5	5
GSK-3-ALPHA	0.5	8	5	12	RSK3	0.5	-1	5	3
GSK-3-BETA	0.5	6	5	13	RSK4	0.5	-1	5	1
HASPIN	0.5	13	5	39	SGK1	0.5	10	5	11
HCK	0.5	18	5	53	SGK2	0.5	13	5	13
HIPK1	0.5	3	5	29	SGK3	0.5	5	5	6
HIPK2	0.5	1	5	26	SIK	0.5	14	5	33
HIPK3	0.5	3	5	15	SLK	0.5	0	5	12
IGF1R	0.5	14	5	16	SNF1LK2	0.5	8	5	29
IKK-ALPHA	0.5	14	5	9	SPHK1	0.5	4	5	4
IKK-BETA	0.5	7	5	8	SPHK2	0.5	18	5	18
IKK-EPSILON	0.5	23	5	24	SRC	0.5	19	5	46
INSR	0.5	14	5	17	SRMS	0.5	7	5	6
IRAK1	0.5	4	5	21	SRPK1	0.5	5	5	8
IRAK4	0.5	5	5	13	SRPK2	0.5	-7	5	5
IRR	0.5	3	5	5	STK16	0.5	13	5	20
ITK	0.5	-2	5	31	STK25	0.5	5	5	6
JAK1	0.5	3	5	4	SYK	0.5	12	5	3
JAK2	0.5	17	5	17	TAK1-TAB1	0.5	3	5	4
JAK3	0.5	9	5	4	TAOK2	0.5	-2	5	7
JNK1	0.5	9	5	12	TAOK3	0.5	-4	5	-2
JNK2	0.5	2	5	5	TBK1	0.5	-8	5	-7
JNK3	0.5	12	5	14	TEC	0.5	4	5	5
KDR	0.5	30	5	68	TIE2	0.5	2	5	8
KIT	0.5	80	5	88	TNIK	0.5	6	5	39
LATS1	0.5	-1	5	-2	TNK1	0.5	12	5	12
LATS2	0.5	-23	5	-23	TNK2	0.5	3	5	17
LCK	0.5	14	5	38	TRKA	0.5	4	5	6
LOK	0.5	1	5	8	TRKB	0.5	0	5	5
LRRK2-G2019S	0.5	-6	5	-2	TRKC	0.5	7	5	19
LTK	0.5	0	5	14	TSSK1	0.5	11	5	12
LYNA	0.5	15	5	53	TSSK2	0.5	4	5	3
LYNB	0.5	20	5	53	TTK	0.5	2	5	4
MAP4K2	0.5	6	5	6	TXK	0.5	13	5	44
MAP4K4	0.5	6	5	45	TYK2	0.5	10	5	12
MAP4K5	0.5	14	5	19	TYRO3	0.5	10	5	26
MAPK1	0.5	4	5	4	YES	0.5	16	5	56
MAPK3	0.5	1	5	2	ZAP70	0.5	14	5	15

MDP-stimulated human PBMC IL8 assay:

Peripheral blood mononuclear cells (PBMC) were prepared using Ficoll-Paque PLUS and resuspended in RPMI 1640 medium supplemented with 5% heat inactivated fetal bovine serum and 1 mM HEPES. 40,000 cells were plated in 96-well plates and treated with compounds and MDP (0.3 μg/mL). IL8 concentrations in supernatants were measured 18h later using V-PLEX MSD kit. Data (IC_{50}) are reported as an average of triplicate measurements.

MDP-stimulated mouse BMDM IL6 assay:

Bone marrow cells were isolated from C57BL/6 mice using standard protocol [1] and cultured in petri dishes for 6 days in RPMI 1640-containing 10 % fetal bovine serum, and 50 ng/mL murine M-CSF. 100,000 differentiated macrophage cells were plated in 96 well plates per well in 100 μL of RPMI 1640-containing 10 % fetal bovine serum, and 50 ng/mL murine M-CSF. Next day, compounds were added 30 min prior to addition of MDP and LPS to a final concentration of 5μg/mL MDP and 1 ng/mL LPS. IL6 concentrations in supernatants were measured 18 hours later using mouse IL6 V-plex MSD kit (Meso Scale Discovery). Data (IC_{50}) are reported as an average of triplicate measurements.

MDP-stimulated rat whole blood TNFα assay:

Rat whole blood was collected in heparin tubes, 100 μL of blood was dispensed per well in 96 well plates followed by the addition of 5 μL 1.5 μg/mL MDP diluted in RPMI-1640, 5% HI serum. 5h later, plasma was collected and TNFα levels were measured using V-PLEX Meso Scale Discovery plates. Data (IC_{50}) are reported as an average of triplicate measurements.

MDP-stimulated human whole blood TNFα assay:

Human blood was obtained from The Scripps Research Institute (TSRI). All samples were drawn from normal, healthy volunteers with informed written consents as part of TSRI's Normal Blood Drawing Service approved by the Office of Research Subjects Protection of the Scripps Health Human Subjects Committee.

Human whole blood was collected in heparin tubes. 1 vol. of blood was diluted with 0.6 vol. of serum free RMPI 1640 media, and 100 μL per well were dispensed in 96 well plates. 20 μL of 10× compounds were added per well, and 30 min. later, 20 μL MDP was added to a final concentration of 0.3 μg/mL. Compounds and MDP dilutions were done in RPMI 5% heat inactivated serum, 10 mM HEPES, 1% P/S/G. 20h later, plasma was collected and TNFα concentrations were measured using V-PLEX Meso Scale Discovery plates. Data (IC_{50}) are reported as an average of triplicate measurements.

MDP-stimulated human PBMC pS6 CyTOF assay

CyTOF Methods: CyTOF or mass cytometry, incorporates flow cytometry technology with a time-of-flight inductively coupled plasma mass spectrometry (ICP-MS). It allows for the simultaneous detection and quantification of over 40 parameters from a single cell. It utilizes rare-earth metal conjugated monoclonal antibodies to specific cell surface or intracellular molecules.

Human PBMCs were incubated with compound for 15 minutes prior to stimulations. The cells were then stimulated with MDP or LPS for 30 minutes. The cells were fixed with 1.6% PFA to preserve phosphorylation status on signaling molecules. The cells were then stained with a combination of cell surfaces receptors for specific lineages and intracellular signaling molecules. The samples where then

acquired and analyzed on the CyTOF. CD14+ HLA-DR+ cells where gated on and analyzed for phosphorylation of S6.

viSNE Analysis: Visualization of t-Distributed Stochastic Neighbor Embedding (t-SNE) algorithm (viSNE) allows for visualization of high-dimensional single-cell data by finding the two dimensional representation of single-cell data that best preserves their local and global geometry. The resulting "maps" provide visual representation of the single-cell data that are similar to biaxial plots. The positions of dots (cells) reflect their proximity in high-dimensional space. The user defines the parameters (in this case, cell-surface markers) used to cluster the data. Then we overlay individual parameters to identify "islands" (lineages and subpopulations) such as myeloid cells and pS6.

MDP-stimulated human PBMC pS6 FACS assay

Human PBMC were suspended in RPMI 1640 medium supplemented with 5% heat inactivated fetal bovine serum and 1 mM HEPES and one million cells per point were treated for 15 min with compounds then for 30 min with 0.3 µg/mL MDP. After washing with FACS buffer (PBS, 5% FBS, 1mM EDTA), cells were incubated for 20 min on ice with anti hCD14 Alexa 647 antibody, washed once with FACS buffer then fixed with 1.6% paraformaldehyde for 15 min at 37ºC. Followed fixation, cells were washed once with FACS buffer then permeablized with Perm buffer III for 30 min on ice. Intracellular pS6 staining was performed using monoclonal antibody against pS6 Ser235/236 PE for 30 min on ice and in the dark. Primary antibodies were diluted at the optimal dilutions according to the manufacturer's instructions and appropriate isotype control for CD14 cell surface marker and rabbit (DA1E) mAb IgG XP® Isotype Control (Alexa Fluor® 647 Conjugate) for pS6 were used to facilitate the gating of specific cell populations of interest.

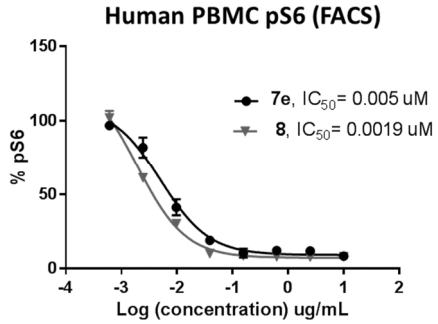


Figure SI-1. Human PBMC FACS experiments. Compounds **7e** and **8** inhibit phosphorylation of S6 in MDP stimulated human PBMC.

MDP-induced rat *ex vivo* experiment

A baseline bleed of 200 µL of whole blood was collected from the saphenous vein of female Wistar rats into heparinized collection tubes. The animals were then dosed orally with Compound **8** at 4 mL/kg (30 mg/kg equivalent)**.** A further 200 µL of blood was drawn into heparinized collection tubes at 0.5, 3, 5, and 24 hours post dosing. 100 µL of blood from different time points were used to test the plasma concentration of compound **8**. 100 µL of blood was dispensed per well in 96 well plates followed by the addition of 5 µL 1.5 µg/mL MDP diluted in RPMI-1640, 5% HI serum. 5h later, plasma was collected and cytokines (IL6 and TNFα) levels were measured using V-PLEX Meso Scale Discovery plates.

MDP-induced rat *in vivo* model

Female Wistar rats were used for the *in vivo* study. Compound **8** was formulated in a vehicle of 0.5% Methyl Cellulose and 0.5% Tween 80 at a concentration of 7.50 mg/mL. It was administered by oral gavage at a dosing volume at 4 mL/kg (30 mg/kg equivalent) followed by challenge of MDP (100 µg, *i.p.*) in 2.5 mL of saline, one hour after dosing with Compound **8**. Blood and gut were collected 4 hours after MDP injection (5 hours after dosing with Compound **8**). Serum Cytokine levels were measured using rat V-PLEX MSD kit (Meso Scale Discovery).

Intestine tissues were lysed in a 50mM Hepes pH 7.5, 150 mM NaCl, 5mM $MgCl_2$, 10% glycerol, 1% triton, and 1mM EDTA buffer in the presence of proteinase inhibitors. Cytokines concentrations were measured using rat V-PLEX MSD kit (Meso Scale Discover)

All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Genomics Institute of the Novartis Research Foundation and strictly followed the NIH guidelines for humane treatment of animals.

PK Studies.

Rodent PK studies were conducted in male CD-1® mice and male Wistar rats. Dog PK study of compound **8** was conducted in male beagle dogs. Plasma concentrations of compounds were determined by liquid chromatography−tandem mass spectrometry (Applied Biosystems, Foster City, CA, USA). The lower limit of detection was 1 ng/mL. Pharmacokinetic parameters were calculated by noncompartmental regression analysis using an in-house fitting program.

Microsomal stability assay.

Compounds (1µM) were incubated in 96 well format with a reaction mixture consisting of animal or human liver microsomal protein (0.5 mg/mL), NADPH (1.0 mM), and $MgCl_2$ (2.0 mM) in 50 mM potassium phosphate buffer (pH 7.4). At various time points (0, 0.5, 5, 15, and 30min), the reactions at 37°C were quenched by the addition of quench solution (80%acetonitrile/10%MeOH/10% MilliQ H_2O with 665 nM propranolol). The reaction mixtures were centrifuged at 4,000*g* for 10 min, and aliquots of the supernatant were analyzed by LC/MS/MS to measure the parent compound concentrations. Liver microsomal extraction ratio (ER), intrinsic clearance (CL_{int}), and in vitro half-life ($t_{½}$) values were calculated[2] and reported.

XRAY CRYSTALLOGRAPHY

Protein Purification and Crystallization

RIP2 kinase residues 2-311 were cloned into a pFastBacHT plasmid (Invitrogen) containing an N-terminal His-tag followed by a TEV cleavage site. Protein expression in SF9 insect cells was performed according to the Invitrogen User Guide.

Protein was purified by Ni-NTA affinity chromatography, followed by removal of the His-tag with TEV protease and subsequent reverse Ni-NTA chromatography. A final purification step involved S200 size-exclusion chromatography using a running buffer of 20 mM Tris pH 8.5, 500 mM NaCl, 10% Glycerol, 1 mM TCEP. The purified protein was concentrated to 20 mg/mL and flash frozen for storage at -80ºC.

For compound co-crystallization experiments, thawed RIP2 protein was diluted with 20 mM Tris pH 8.5, 500 mM NaCl, 10% Glycerol, 1 mM TCEP to ~8-9 mg/ml. Compound was then added to the protein to a final concentration of 0.8-1.0 mM and incubated at 4ºC overnight before crystallization by sitting drop vapor diffusion utilizing the JCSG coarse screens.[3]

Crystals for compounds **1** and **7f** were both cryo-protected by immersing them into reservoir solution containing 25% (v/v) glycerol followed by flash cooling in liquid nitrogen. Data was collected from single crystals at Beamline 5.0.3 of the Advanced Light Source (ALS) at Lawrence Berkeley National Laboratory in Berkeley, CA to 2.9 Å. Data processing and structure determination were done using the software packages PHENIX[4] HKL2000[5], MOLREP[6] and PHASER[7] within the CCP4 program suite. Structure refinement for both structures was carried out using BUSTER,[8] whist COOT[9] was used for iterative cycles of model building.

Crystals of compound **1** in space group $P2_12_12_1$ with 2 molecules per asymmetric unit were grown at 20ºC in 1.0 M lithium chloride, 10% PEG6000, 0.1 M Tris pH 8. Data were processed and refined via the procedures detailed above (Table SI-2). A reduced bias simulated annealing omit map depicts the difference electron density for compound **1** (Figure SI-2 left)

Compoound **7f** Data Analysis and Structure Solution

Crystals of compound **7f** in space group P1 with 16 RIP2 molecules per asymmetric unit grew at 20ºC in 1.6 M ammonium sulfate, 0.1 M citric acid pH 4.0.

Analysis of the diffraction intensities for the complex between RIP2 and compound **7f** indicated an orthorhombic crystal system with systematic absences indicating $P222_1$ as the likely space group. However analysis of the intensities with POINTLESS and phenix.xtriage[4] showed deviations from the expected distribution of intensities (Table SI-3) suggesting that twinning or some non-random arrangement of the molecules in the asymmetric unit was present. These conclusions were strengthened by a number of other observations, ambiguous systematic absences along axes, molecular replacement solutions for the four molecules in the asymmetric unit related by peusdosymmetrical rotations coincident with the point group matrices of the orthorhombic crystal system, and the recalcitrance of the molecular replacement solution to refine to a satisfactory agreement with the observed data. Despite this, at all stages from molecular replacement onwards, clear electron density could be observed for compound **7f** indicating a bound ligand within the ATP binding site of all the kinase domains of the asymmetric unit.

Due to the unequal lengths of the a, b, c axis in orthorhombic crystals hemihedral twinning laws are not possible, however, tetartohedral twinning can exist whereby four twin laws, corresponding to the point group symmetry of the orthorhombic crystal system (h,k,l), (-h, k –l), (h, -k –l), (-h –k –l) can produce the intensity distributions seen.[10] These systems often coincide with pseudosymmetry[11] that coincides with the twin domain. This phenomenon matches where is seen for the Compound **7f** data set and matches well with what is described in detail by Roversi *et al*.[10] For the compound **7f** data this would imply that the crystal system was triclinic (spacegroup P1) with cell dimensions the same as the orthorhombic system but with 16 molecules in the asymmetric unit, arranged as 8 RIP2 dimers, with tetrameric arrangements related by 222 point group symmetry. This arrangement of the molecules enabled the successful refinement of the structure (Table SI-3). REFMAC was able to detect and refine the twinning fractions for these domains and estimated the fractions to be 0.36, 0.23, 0.18 and 0.23 for the twin laws detailed, respectively. However, conventional refinement in P1 with BUSTER[8] was also able to refine the structure (Table SI-3). Lebedev et. al. [12] have noticed these phenomena whilst reviewing the

deposited diffraction intensities in the Protein Data and this has further been expounded with relation to the crystallographic R-factor by Mushodov.[13]

However, in early rounds of refinement, prior to the placement of Compound **7f**, clear difference electron density was available for all 16 molecules in the asymmetric unit., illustrated by the simulated annealing omit map on right side of Figure SI-2 constructed at the end of the refinement process. Because of the relatively low resolution of the data, strict NCS was used throughout the refinement of this structure to enhance the low observations to parameters ratio, as such at the end of the refinement the agreement between the 16 molecules in the asymmetric unit was good, with deviations concentrated in a few higher flexibility loops, illustrated via a plot of root mean square deviation of all atoms per residue (Figure SI-3). Density for molecule **7f** shows unequivocally 2FoFc density for all 16 subunits with deviation between chains confined to the more solvent exposed methyl-pyrazine (Figure SI-2 right).

The coordinates and structure factors have been deposited in Protein Data Bank with accession codes 5W5J (compound **1**) and 5W5O (compound **7f**), respectively.

Table SI-2: RIP2 + compound **1** (5W5J.pdb):

Data Collection		*Refinement and Statistics*	
Resolution, (Å)	46-2.85 (2.95-2.85)*	Rwork, (%)	22.5
Space group	P21 21 21	Rfree, (%)	27.7
a, (Å)	58.2	RMSD from ideal geometry:	
b, (Å)	92.3	Bond lengths, (Å)	0.010
c, (Å)	126.6	Bond angles, (°)	1.16
Molecules per asymmetric unit	2	Numbers of atoms:	
Unique Reflections	16533 (1628)*	Protein (non-hydrogen)	3990
Multiplicity	3.6 (3.6)*	Water oxygen atoms	25
Average I/σ(I)	5.3 (1.63)*	Ligand atoms	48
Rmerge, (%)	10.1 (64.5)*	Ramachandran	
Completeness, (%)	99.8 (100)*	Favored/Allowed/Outliers (%)	94/5.6/0.4
*The values in the parenthesis are those from the highest resolution shell			

Table SI-3: RIP2 + compound **7f** (5W5O.pdb)

Data Collection		*Refinement and Statistics*	
Resolution, (Å)	47-2.9 (2.94-2.9)*	Rwork, (%)	21.9
Space group	P 1	Rfree, (%)	25.0
a (Å); α (º)	80.3; 90.15	RMSD from ideal geometry:	
b (Å); β (º)	107.48; 89.77	Bond lengths, (Å)	0.01
c (Å); γ (º)	210.19; 89.96	Bond angles, (°)	1.12
Molecules per asymmetric unit	16	Numbers of atoms:	
Unique Reflections	139065 (10631)*	Protein (non-hydrogen)	34833
Multiplicity	2.1 (1.8)*	Water oxygen atoms	312
Average I/σ(I)	6.3 (0.5)*	Ligand atoms	544

Rmerge, (%)	11.7 (>100.0)*	Ramachandran	
Completeness, (%)	90.9 (81.3)*	Favored/Allowed/Outliers (%)	
Intensity Statistics	Calculated	Untwinned	Perfect
$\langle I^2 \rangle / \langle I \rangle^2$	1.796	2.0	1.5
$\langle F \rangle^2 / \langle F^2 \rangle$	0.839	0.785	0.885
$\langle \lvert E^2 - 1 \rvert \rangle$	0.639	0.736	0.541
$\langle \lvert L \rvert \rangle$	0.418	0.5	0.375
$\langle L^2 \rangle$	0.244	0.333	0.2
*The values in the parenthesis are those from the highest resolution shell			

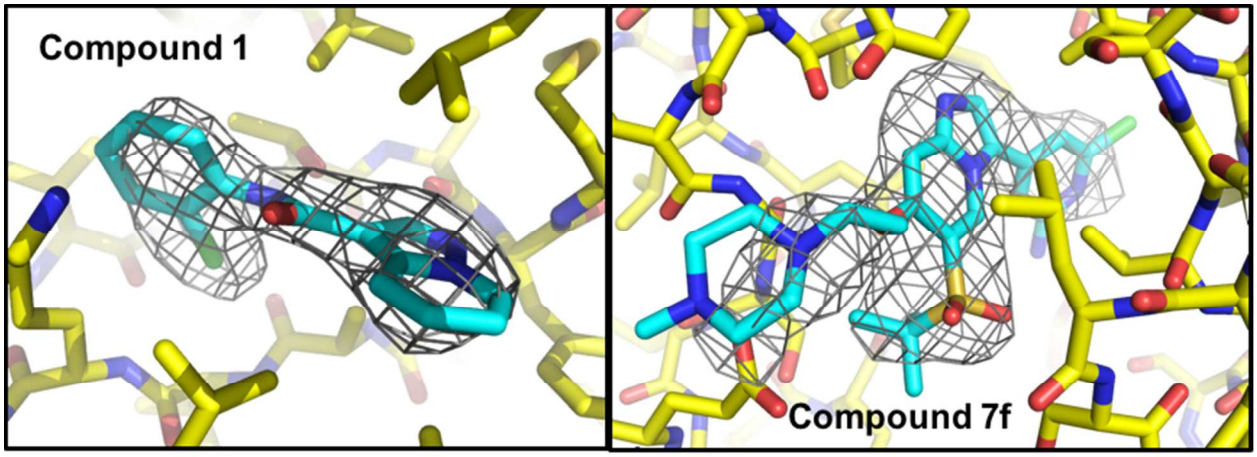


Figure SI-2 mFo-DFc omit maps contoured at 3σ above the mean electron density. Maps were calculated by removing the ligands and then utilizing a simulated annealing refinement protocol with the PHENIX {ref) package to reduce any phase bias enduced by the presence of the ligand. Both figures where made with PYMOL(REF) with, carbons colored cyan for the ligand, yellow for the protein. Electron density maps are displayed as grey meshes.

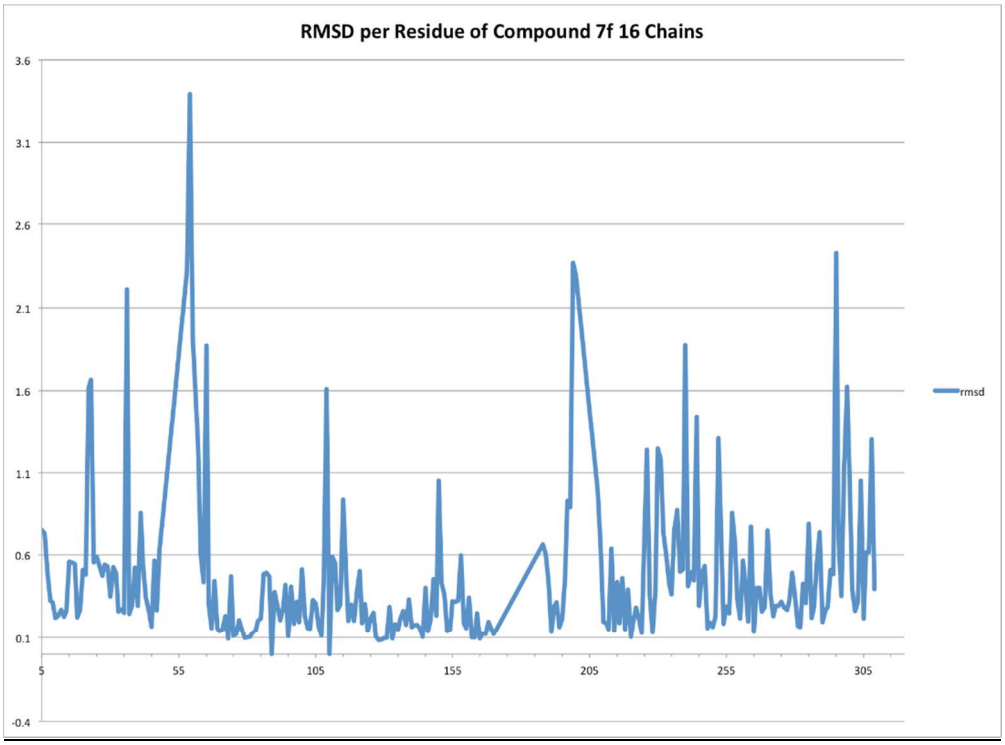


Figure SI-3 mean Root Mean Squared Deviation (RMSD) between all 16 chains in the asymmetric unit of the Compound **7f** dataset.

Molecular Docking Experiments.

Flexible ligand docking was performed using Glide 6.5 (Small-Molecule Drug Discovery Suite, Schrodinger Inc., Portland, OR, 2014) with standard precision protocol. The protein coordinates were taken from co-crystal structure of Compound **1** with RIPK2 kinase domain (PDB code 5W5J.pdb) and prepared for docking experiments with a help of Protein Preparation Wizard (Small-Molecule Drug Discovery Suite). The final pose was selected based on the lowest docking score using GlideScore.

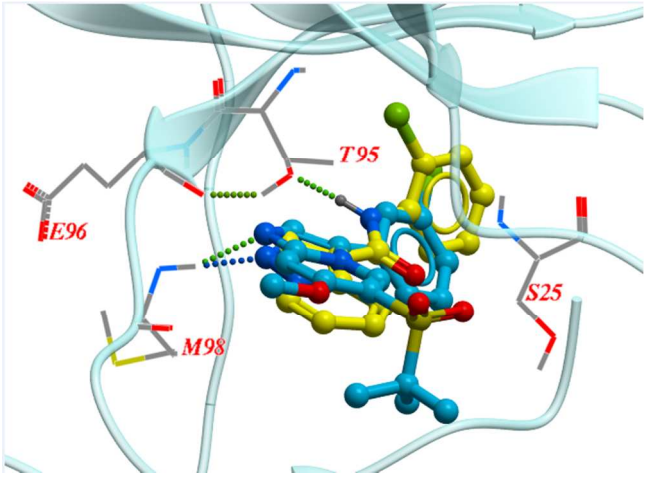


Figure SI-4. Docking of compound **4** (blue) in co-crystal structure of RIPK2 with compound **1** (yellow).

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