Delineating the distinct roles of TAK1 and IRAK-1/4 through the discovery of a super-selective IRAK-1/4 inhibitor

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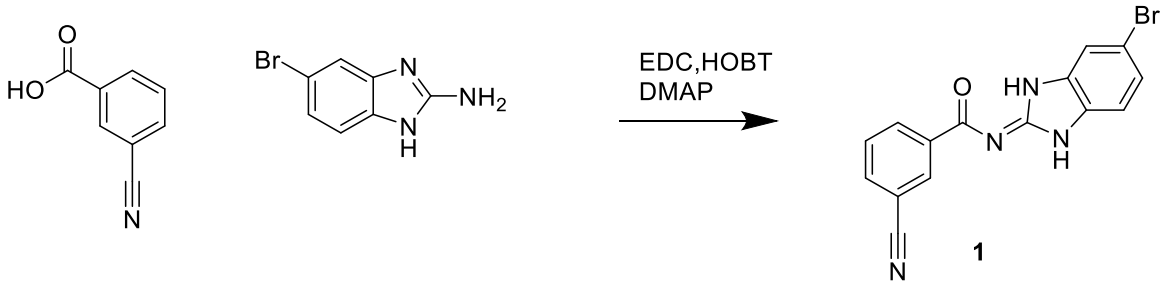
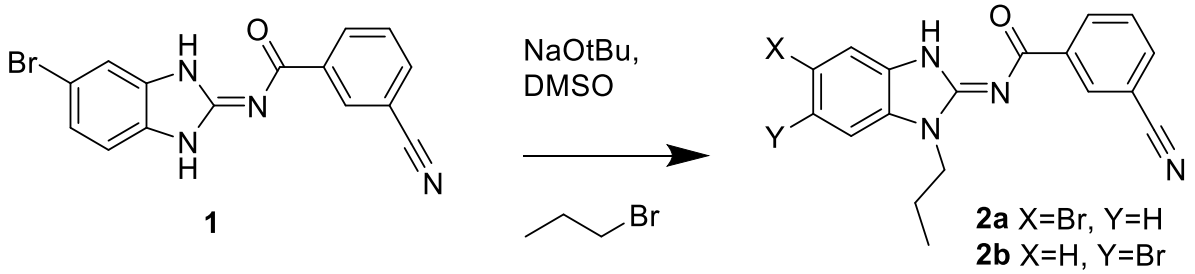
Synthesis of HS243 and other analogs.

Reagents were obtained from commercial sources and used without further purification. Proton NMR spectra were obtained on Varian 400 and 500 MHz spectrometers. LC/MS were obtained on an Agilent ion-trap LC/MS system.

Legend:
HS-231 mixture of **3a a**nd **3b**
HS-232 **9**
HS-233 **5b**
HS-234 **5a**
HS-238 **10a**
HS-242 **11a**
HS-243 **12**

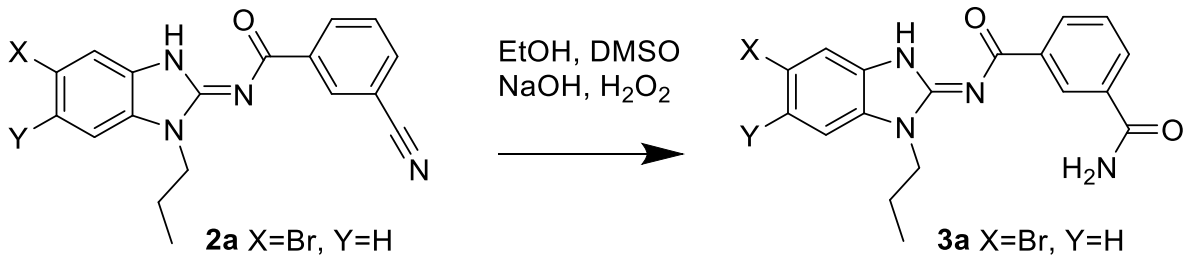


3-Cyanobenzoic acid (600 mg, 4.08 mmol) and 5-bromo-1H-benzo[d]imidazol-2-amine (865 mg, 4.08 mmol) were mixed with EDC (1.56 g, 8.16 mmol), HOBT (551 mg, 4.08 mmol) and DMAP (10 mg, 82 μmol), slurried in DMF (5 mL) and treated with Hunig' base (527 mg, 4.08 mmol). The mixture was stirred for 16 h and then concentrated. The solid residue was slurried in hot ethanol, cooled and a white powder was filtered off, washed with ethanol and air-dried to give nitrile **1** (980 mg, 70 %) as an off-white solid. LC/MS gave a single peak with m/z = 341.0 and 343.0, $[M+H]^+$.

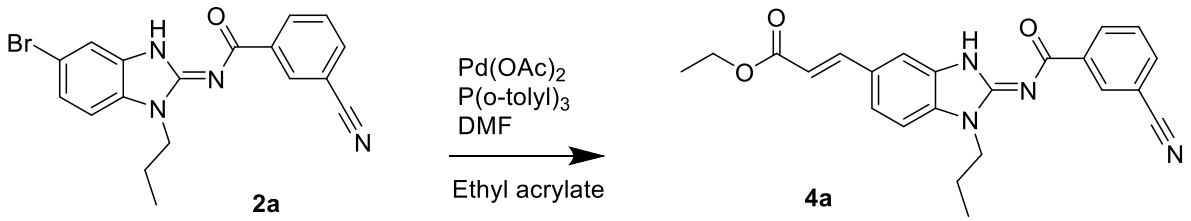


Nitrile **1** (980 mg, 2.97 mmol) was dissolved in THF (18 mL) and DMSO (7.5 mL) and treated with potassium t-butoxide (5.7 mL of 1M THF solution) followed by 1-bromopropane (333 μL, 706 mg, 5.7 mmol) and stirred at RT. After 2 h, the mixture was treated with a little acetic acid (200 μL) and stirred for an hour. The mixture was then poured into water (100 mL) giving rise to substantial precipitation. The slurry was stirred vigorously overnight then filtered and air-dried to give a mixture of **2a** and **2b** (838 mg, 76%) as a fluffy white powder. Some of the mixture (400 mg) was adsorbed onto silica and chromatographed (80 g Isco silica gel, 0 to 20% EtOAc in CH_2Cl_2) to give a partial separation of the two compounds. The clean fractions of the earlier eluting compound were combined to give **2a** (called upper bromide,184 mg). LC/MS shows a single tailing peak with m/z = 383.1 and 385.1, $[M+H]^+$. ^{1}H-NMR (dmso-d_6) δ 8.52 (s, 1H), 8.51 (d, J = 8 Hz, 1H), 8.00 (d, J = 8 Hz, 1H), 7.71, (t, J = 8 Hz, 1H), 7.70 (s, 1H), 7.55 (d, J = 8 Hz, 1H), 7.43 (d, J = 8 Hz, 1H), 4.25 (t, J = 7 Hz, 2H), 1.83 (p, J = 7 Hz, 2H), 0.91 (t, J = 7 Hz, 3H).
The clean fractions of the later eluting compound were combined to give **2b** (called lower bromide, 145 mg). LC/MS shows a single tailing peak with m/z = 383.1 and 385.1, ^{1}H-NMR (dmso-d_6) δ 8.52 (s, 1H), 8.51 (d, J = 8 Hz, 1H), 7.99 (d, J = 8 Hz, 1H), 7.87 (s, 1H), 7.71, (t, J = 8 Hz, 1H), 7.47 (d, J = 8 Hz, 1H), 7.40 (d, J = 8 Hz, 1H), 4.25 (t, J = 7 Hz, 2H), 1.83 (p, J = 7 Hz, 2H), 0.93 (t, J = 7 Hz, 3H).

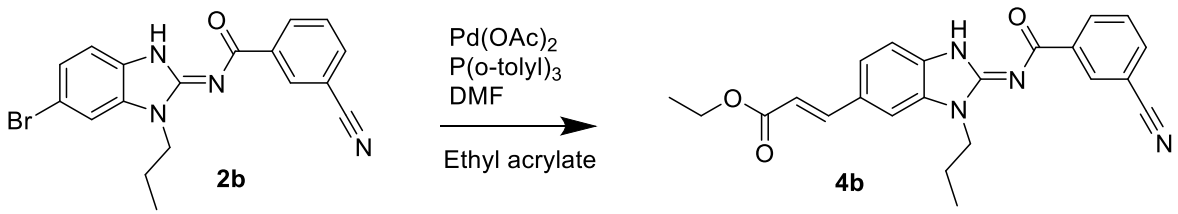
We were unable to obtain adequate NOe spectra to assign the structures. Identification of the isomeric structures was assigned later based on an unambiguous region-controlled synthesis of **2a** (see below).



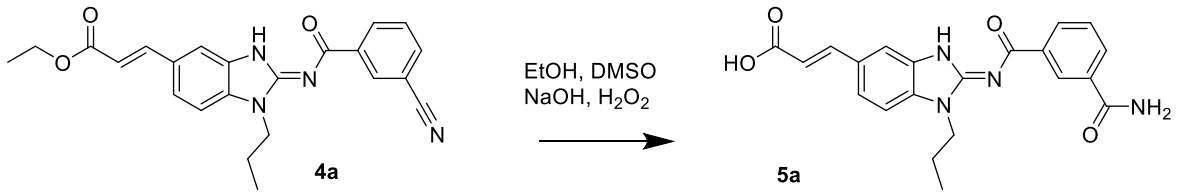
HS-231. A mixture of bromonitriles, 21a and 21b (22 mg, 57 μmol) was dissolved in DMSO (350 μL) and diluted with ethanol (500 μL). This was then treated with 50% NaOH (2 drops) followed by 30% hydrogen peroxide (3 drops). TLC indicated a new product, so the reaction mixture was adsorbed onto silica gel and chromatographed (silica gel, 0 to 10% MeOH in CH_2Cl_2) to give product in a single peak. The product was triturated in methanol to give the product, a mixture of bromide regioisomers, **3a** and **3b** (15.5 mg, 67%) as a white solid. LC/MS showed a single peak for the product (m/z = 401.1, $[M+H]^+$).



Nitrile **2a** (upper bromide, 100 mg, 261 μmol) was mixed with tri(o-tolyl)phosphine (9.5 mg, 31 μmol) and palladium(II) acetate (6 mg, 26 μmol) in DMF (1 mL) and treated with triethylamine (53 mg, 522 μmol) and ethyl acrylate (52 mg, 522 μmol), and stirred at RT with nitrogen bubbling. The mixture was then heated to 125 °C under nitrogen for 20 h and allowed to cool. The reaction mixture was diluted with DMSO (1 mL) and heated slightly to re-dissolve the product. The mixture was passed through a filter to remove palladium onto a column and chromatographed (43 g Isco C-18, 0 to 100% MeOH in 0.2% HCO_2H) to give **4a** (86 mg, 82%) as a white solid. LC/MS gave a single peak with m/z = 403.2 $[M+H]^+$. ^{1}H-NMR (dmso-d_6) δ 8.54 (s 1H), 8.52 (d, J = 8 Hz, 1H), 8.00 (d, J = 8 Hz, 1H), 7.78 (s, 1H), 7.73 (d, J = 16 Hz, 1H), 7.72 (t, J = 8 Hz, 1H), 7.69 (d, J = 8 Hz, 1H), 7.62 (d, J = 8 Hz, 1H), 6.53 (d, J = 16 Hz, 1H), 4.28 (t, J = 7 Hz, 2H), 4.20 (q, J = 7 Hz, 2H), 1.84 (hex, J = 7 Hz, 2H), 1.27 (t, J = 7 Hz, 3H), 0.93 (t, J = 7 Hz, 3H).

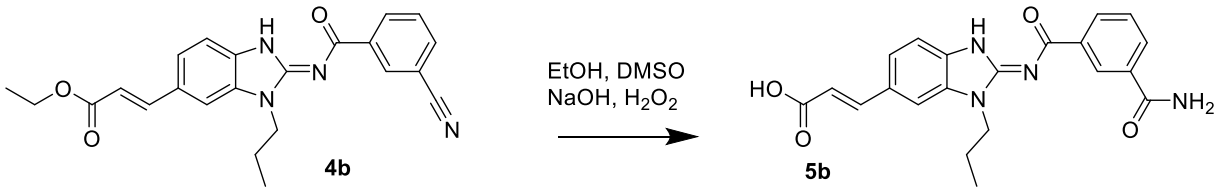


Nitrile **2b** (100 mg, 261 μmol) was reacted as described above for **2a** to give **4b** (103 mg, 98%) as a white solid. LC/MS gave a single peak with m/z = 403.3 $[M+H]^+$. ^{1}H-NMR (dmso-d_6) δ 8.53 (s 1H), 8.51 (d, J = 8 Hz, 1H), 8.06 (s, 1H), 8.00 (d, J = 8 Hz, 1H), 7.73 (d, J = 16 Hz, 1H), 7.71 (t, J = 8 Hz, 1H), 7.59 (d, J = 8 Hz, 1H), 7.54 (d, J = 8 Hz, 1H), 6.75 (d, J = 16 Hz, 1H), 4.28 (t, J = 7 Hz, 2H), 4.20 (q, J = 7 Hz, 2H), 1.86 (hex, J = 7 Hz, 2H), 1.27 (t, J = 7 Hz, 3H), 0.95 (t, J = 7 Hz, 3H).



HS-234. Nitrile **4a** (150 mg, 373 μmol) was slurried in ethanol (4 mL) and treated with 50% NaOH (12 drops, about 25 mg NaOH/drop, 300 mg) followed by 30% hydrogen peroxide (5 drops). The mixture was stirred at RT for 3 d. The mixture was then acidified by slow addition of 1N HCl to pH = 1, which led to precipitation of the product. The solid was then filtered off and washed with water and air dried overnight to give **5a** (141 mg, 96%) as an off white solid. LC/MS gave a single peak with m/z = 393.2 $[M+H]^+$. ^{1}H-NMR (dmso-d_6) δ 8.68 (s, 1H), 8.38 (d, J =

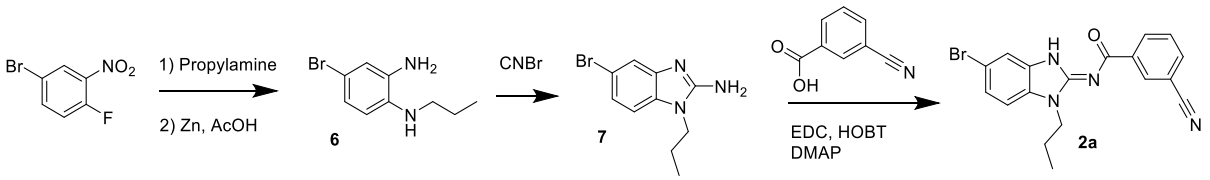
8 Hz, 1H), 8.11 (br s, 1H), 8.00 (d, J = 8 Hz, 1H), 7.77 (s,1H), 7.66 (d, J = 16 Hz, 1H), 7.57-7.63 (m, 2H), 7.55 (t, J = 8 Hz, 1H), 7.42 (br s, 1H), 6.43 (d, J =16 Hz, 1H), 4.27 (t, J = 7 Hz, 2H), 1.85 (hx, J = 7 Hz, 2H), 0.93 (t, J = 7 Hz, 3H).



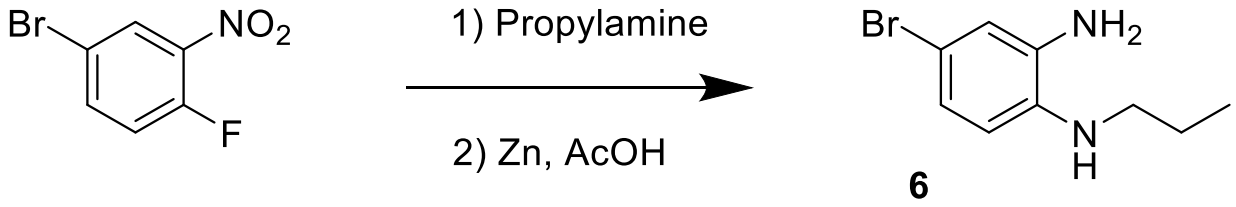
4b 5b

HS-233. Nitrile **4b** (50 mg, 124 μmol) was reacted as described above for **4a** to give **5b** (103 mg, 98%) as a white solid. LC/MS gave a little peak which trailed out in the MS forever with m/z = 393.2, $[M+H]^+$. NMR gave broad but consistent peaks in DMSO. $]^+$. ^{1}H-NMR (dmso-d_6) δ 8.68 (br s, 1H), 8.37 (br d, 1H), 7.91 (br d, 1H), 7.74 (br s, 1H), 7.65 (br d, J = 16 Hz, 1H), 7.42-7.53 (m, 3H), 6.51 (br d, J = 16 Hz, 1H), 4.26 (br ?, 2H), 1.86 (m, 2H), 0.94 (br ?, 3H).

Independent synthesis of **2a**

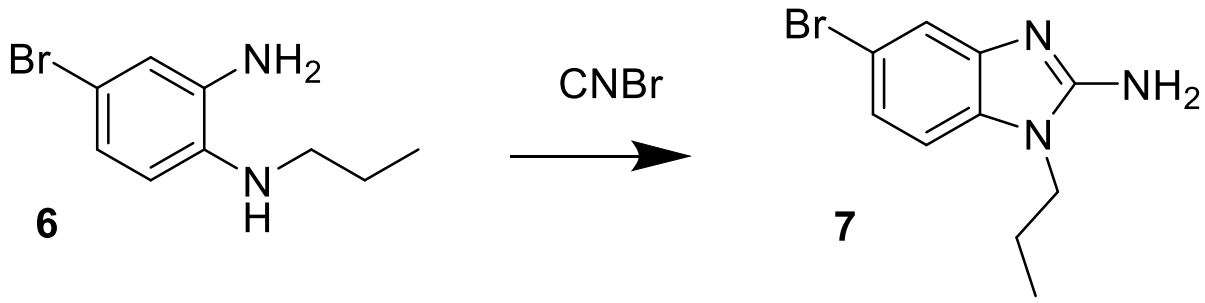


6 7 2a

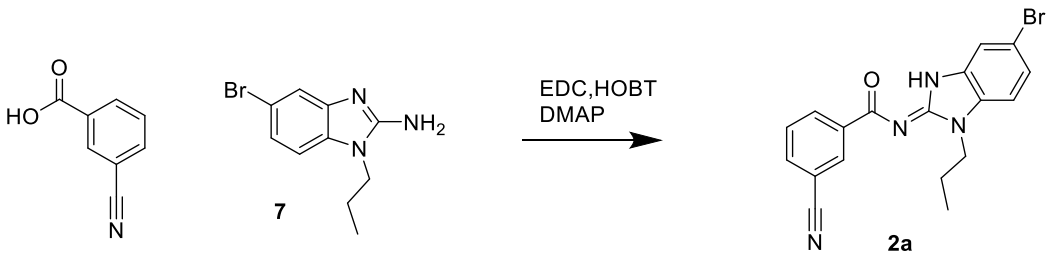


6

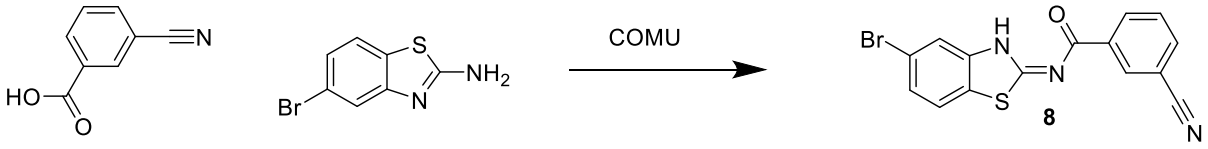
4-bromo-1-fluoro-2-nitrobenzene (1.5 g, 6.8 mmol) was dissolved in ethanol (10 mL) and treated with propyl amine (1.21 g, 1.68 mL, 20.4 mmol) and stirred at RT overnight. The next day, the reaction mixture was concentrated then re-dissolved in ethanol (20 mL) and acetic acid (5 mL). The mixture was treated with zinc powder (4 g) and heated to 80 °C with stirring for 1h. The reaction was allowed to cool and was filtered through Celite® with an ethanol wash. The eluant was concentrated then chromatographed (Silica gel, 100% CH_2Cl_2) to give the aniline **6** (1.49 g, 95%) as a clear oil, which solidified on standing. LC/MS gave a single peak with m/z = 229.0 for $[M+H]^+$. ^{1}H-NMR (dmso-d_6) δ 6.65 (d, J = 2.3 Hz, 1H), 6.57 (dd, J = 2.3, 8 Hz, 1H), 6.29 (d, J = 8 Hz, 1H), 4.83 (br s, 2H), 4.49 (br t, J = 5 Hz, 1H), 2.94 (br q, J = 6 Hz, 2H), 1.58 (hex, J = 7 Hz, 2H), 0.94 (t, J = 7 Hz, 3H).



Analogous to J. Med. Chem. **2012**, *55*, 6523−6540. Bromoaniline **6** (440mg, 1.93 μmol) was dissolved in ethanol (4 mL) and treated with fresh cyanogen bromide (306 mg, 2.89 μmol) and stirred at RT. After 1 h, the mixture was concentrated to give pink solid (728 mg). The pink solid was re-dissolved in ethanol, treated with xs 9/1 : MeOH/NH_4OH (2 mL) and concentrated onto silica gel (4 g) and chromatographed (40 g isco silica, 0 to 10% MeOH in CH_2Cl_2) to give 2-aminobenzimidazole **7** (421 mg, 86%) as a white solid. LC/MS gave a single peak with m/z = 254.0 for $[M+H]^+$. ^{1}H-NMR (dmso-d_6) δ 7.23 (br s, 1H), 7.1 (d, J = 8 Hz, 1H), 6.29 (br d, J = 8 Hz, 1H), 6.59 (br s, 2H), 3.91 (t, J = 7 Hz, 2H), 1.62 (hex, J = 7 Hz, 2H), 0.84 (t, J = 7 Hz, 3H).

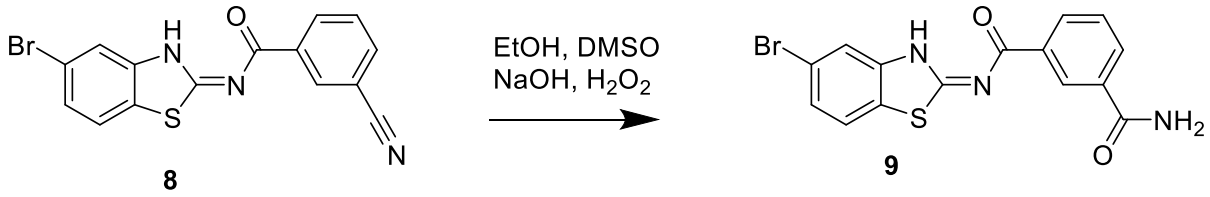


3-Cyanobenzoic acid (116 mg, 788 μmol) and 2-aminobenzimidazole **7** (200 mg, 788 μmol) were mixed with EDC (302 mg, 1.58 mmol), HOBT (106 mg, 788 μmol), DMAP (2 mg, 16 μmol) and slurried in DMF (2 mL) and treated with Hunig's base (102 mg, 788 μmol). The mixture was gently heated to dissolve everything and stirred at RT overnight. The next day, the reaction mixture was diluted with methanol (20 mL) and stirred as solids precipitated out. The solid was filtered off, washed with methanol, and air dried overnight to give nitrile **2a** (229 mg, 76%) as a white powder. Compound **2a**, prepared in this way, is identical to **2a** and different from **2b**, prepared by separation as described above.



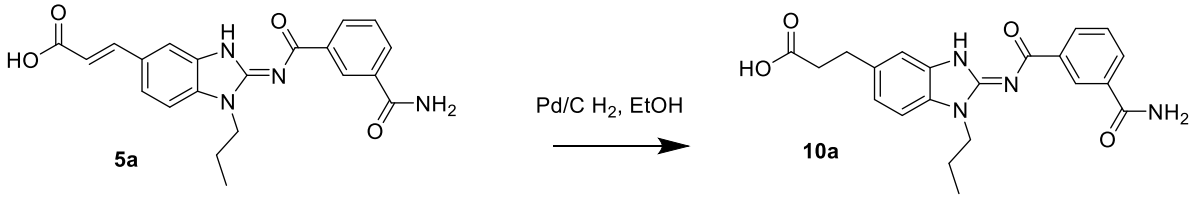
COMU (306 mg, 714 μmol) and 3-cyanobenzoic acid (100 mg., 680 μmol) were dissolved in DMF (1 mL) and treated with Hunig's base (88 mg, 680 μmol). After 5 m, 2-amino-5-bromobenzothiazole (156 mg, 680 μmol) was added along with more DMF (1 mL) and the mixture was stirred for 18 h. The mixture was treated with a little ethanol (2 mL) and heated to

clarity, then diluted with water (20 mL) which caused a lot of white solid to form. This was stirred vigorously for 2 hours then filtered and washed with water and air-dried to give nitrile **8** (243.9 mg, 100+%) as a white solid. LC/MS gave a major peak with m/z = 357.9 and 359.9 for $[M+H]^+$. There were 2 additional minor peaks in the UV trace. The material was used for the next step.



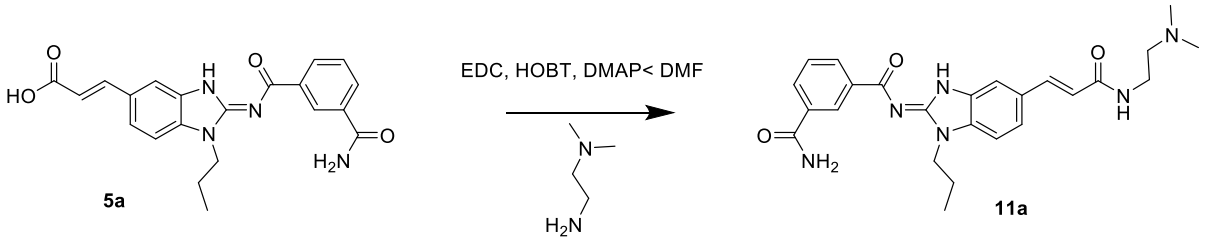
8 **9**

HS-232. Nitrile **8** (50 mg, 140 μmol) was dissolved in DMSO (100 μL) and diluted with ethanol (2 mL). This was then treated with 50% NaOH (5 drops). The mixture was stirred for 2 h, then treated with acetic acid (50 μL), then ethanol (2 ml), then water (2 mL). The mixture was stirred overnight. The next day, the solids were filtered off and air-dried to give the amide product **9** (41 mg, 78%) as a white powder. LC/MS shows a small single peak and a trailing m/z = 376.0 and 378.0 for $[M+H]^+$.

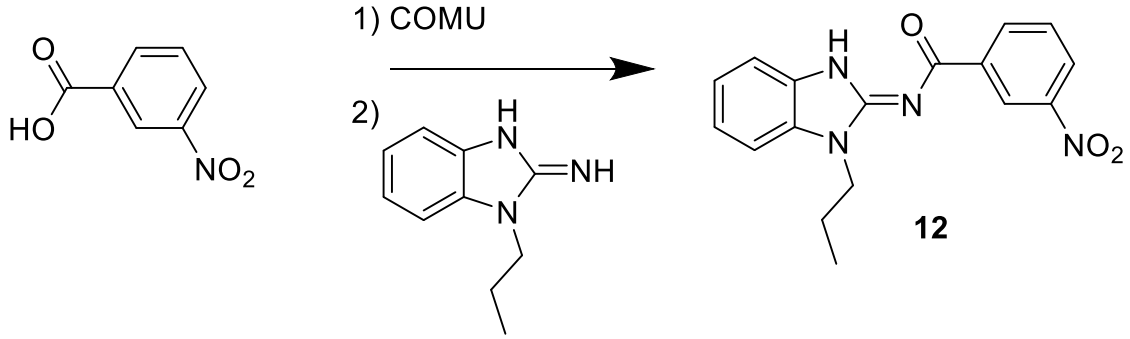


5a **10a**

HS-238. Acid **5a** (40 mg, 102 μmol) was slurried in ethanol (1 mL) and 10% Pd/C (5 mg) in EtOH (1 mL) and put under H_2 atmosphere with 3 vacuum flushes. After a week, LC/MS showed some progress. Acetic acid (1 mL) and more catalyst were added, and the reaction was mistakenly heated to reflux. LC/MS showed clean formation of product m/z = 395.1 and no sign of starting material. The reaction mixture, which contained substantial precipitate, was stirred under nitrogen overnight, then diluted with DMSO (to dissolve product, 2 mL) and filtered through Celite®, concentrated and chromatographed (50 g isco C-18, 0.2% formic in water to 100% MeOH) to give product in approx. 4/1 : methanol/water. Crystals formed in the fractions and were filtered off to give the saturated acid **10a** (18.2 mg, 45%) as a white solid. LC/MS gave a single peak with m/z = 395.2 for $[M+H]^+$. ^{1}H-NMR (dmso-d_6) δ 8.67 (s, 1H), 8.37 (d, J = 8 Hz, 1H), 8.09 (br s, 1H), 7.98 (d, J = 8 Hz, 1H), 7.53 (t, J = 8 Hz, 1H), 7.44 (d, J = 8 Hz, 1H), 7.41 (br s, 1H), 7.40 (s, 1H), 7.14 (d, J = 8Hz, 1H), 4.24 (t, J = 7 Hz, 2H), 2.90 (t, J = 7 Hz, 2H), 2.55 (t, J = 7 Hz, 1H), 1.83 (hx, J = 7 Hz, 2H), 0.92 (t, J = 7 Hz, 3H).



HS-242. Acid **5a** (100 mg, 0.25 μmol) was mixed with HOBT (34 mg, 0.25 μmol), DMAP (12 mg) and EDC (107 mg, 0.56 μmol) then slurried in DMF (1 mL). Diamine (90 mg, 1 mmol) was added and the mixture sonicated to dissolve everything. After one day, the entire reaction mixture was added to a column and chromatographed (50 g isco C-18, 0.2% formic in water to 100% MeOH) to **11a** (41 mg, 35%) as an off-white solid. LC/MS gave a single peak with m/z = 463.2 for $[M+H]^+$. ^{1}H-NMR (dmso-d_6) δ 12.81 (br s, 1H), 8.68 (s, 1H), 8.38 (d, J = 8 Hz, 1H), 8.20 (s, formate, 1H), 8.11-8.14 (m, 2H), 8.00 (d, J = 8 Hz, 1H0, 7.72 (s, 1H), 7.58 (d, j = 8 Hz, 1H), 7.55 (t, J = 8 Hz, 1H), 7.47 (d, J = 16 Hz, 1H), 7.47 (d, J = 8 Hz, 1H), 7.44 (br s, 1H), 6.62 (d, J = 16 Hz, 1H), 4.27 (t, J = 7 Hz, 2H), 3.30 (q, J = 7 Hz, 2H), 2.39 (t, J = 7 Hz, 2H), 2.20 (s, 6H), 1.85 (hx, J = 7 Hz, 2H), 0.93 (t, J = 7 Hz, 3H).



HS-243. COMU (270 mg, 629 mmol) and 3-nitrobenzoic acid (100 mg, 599 mmol) were combined in DMF (1 mL) and treated with Hunig's base (209 mL) followed by 1-propyl-2-aminobenzimidazole (105 mg, 599 mmol) in DMF (1 mL). After 1 h, the reaction mixture was added to a column and chromatographed (50 g isco C-18, 0.2% formic in water to 100% MeOH) to **12** (72 mg, 37%) as a light yellow fluffy solid. LC/MS gave a single peak with m/z = 325.1 for $[M+H]^+$. ^{1}H-NMR (dmso-d_6) δ 8.95 (s, 1H), 8.64 (d, J = 8 Hz, 1H), 8.38 (d, J = 8 Hz, 1H), 7.79 (t, J = 8 Hz, 1H) 7.58 (d, J = 8 Hz, 1H), 7.57 (d, J = 8 Hz, 1H), 7.28 (t, J = 8 Hz, 1H), 7.25 (t, J = 8 Hz, 1H), 4.28 (t, J = 7 Hz, 2H), 1.86 (hex, J = 7 Hz, 2H), 0.95 (t, J = 7 Hz, 3H).

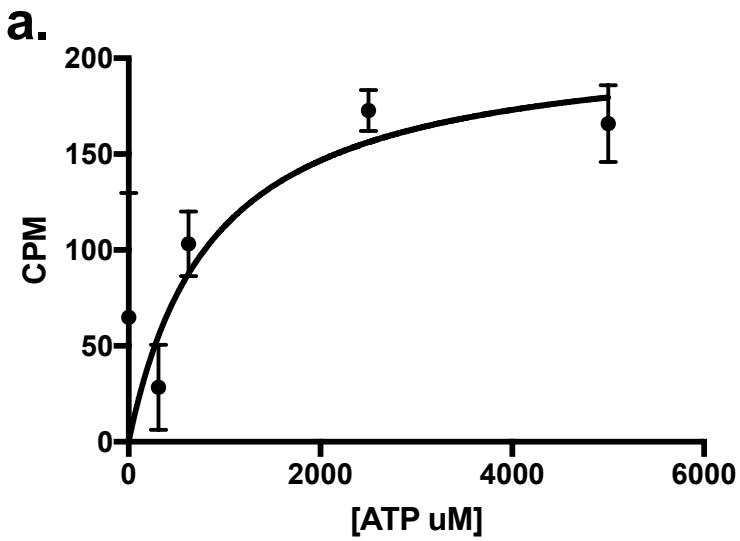


Figure 1s
Km determination of IRAK-4 for ATP. Km ~0.5 mM.

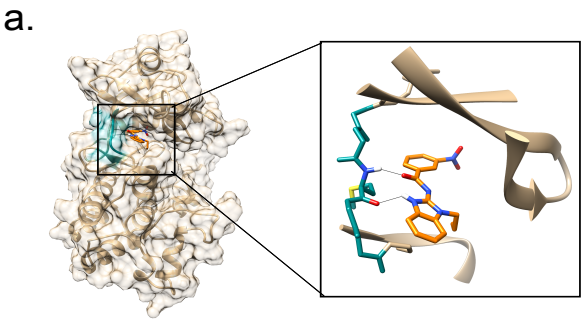


Figure 2s.
Modeling of HS-243 in the ATP binding pocket of IRAK-4. (a) Modeling of HS-243 binding in IRAK-4 ATP binding pocket indicated critical hydrogen binding of nitrogen and oxygen of HS-243 to a methionine residue of IRAK-4.

Kinase	Abbreviation	% Activity Remaining	Log (Inhibition)
AAK1	AAK1	86	1.934498451
ABL1(E255K)-phosphorylated	ABL1	100	2
ABL1(F317I)-nonphosphorylated	ABL1	100	2
ABL1(F317I)-phosphorylated	ABL1	100	2
ABL1(F317L)-nonphosphorylated	ABL1	100	2
ABL1(F317L)-phosphorylated	ABL1	100	2
ABL1(H396P)-nonphosphorylated	ABL1	89	1.949390007
ABL1(H396P)-phosphorylated	ABL1	100	2
ABL1(M351T)-phosphorylated	ABL1	100	2
ABL1(Q252H)-nonphosphorylated	ABL1	89	1.949390007
ABL1(Q252H)-phosphorylated	ABL1	100	2
ABL1(T315I)-nonphosphorylated	ABL1	100	2
ABL1(T315I)-phosphorylated	ABL1	100	2
ABL1(Y253F)-phosphorylated	ABL1	100	2
ABL1-nonphosphorylated	ABL1	92	1.963787827
ABL1-phosphorylated	ABL1	84	1.924279286
ABL2	ABL2	100	2
ACVR1	ACVR1	100	2
ACVR1B	ACVR1B	100	2
ACVR2A	ACVR2A	98	1.991226076
ACVR2B	ACVR2B	92	1.963787827
ACVRL1	ACVRL1	100	2
ADCK3	CABC1	100	2
ADCK4	ADCK4	99	1.995635195
AKT1	AKT1	92	1.963787827
AKT2	AKT2	100	2
AKT3	AKT3	90	1.954242509
ALK	ALK	100	2
ALK(C1156Y)	ALK	100	2
ALK(L1196M)	ALK	100	2
AMPK-alpha1	PRKAA1	95	1.977723605
AMPK-alpha2	PRKAA2	99	1.995635195
ANKK1	ANKK1	86	1.934498451
ARK5	NUAK1	99	1.995635195
ASK1	MAP3K5	100	2
ASK2	MAP3K6	100	2
AURKA	AURKA	100	2

AURKB	AURKB	100	2
AURKC	AURKC	81	1.908485019
AXL	AXL	80	1.903089987
BIKE	BMP2K	73	1.86332286
BLK	BLK	43	1.633468456
BMPR1A	BMPR1A	100	2
BMPR1B	BMPR1B	100	2
BMPR2	BMPR2	100	2
BMX	BMX	100	2
BRAF	BRAF	100	2
BRAF(V600E)	BRAF	90	1.954242509
BRK	PTK6	100	2
BRSK1	BRSK1	89	1.949390007
BRSK2	BRSK2	94	1.973127854
BTK	BTK	100	2
BUB1	BUB1	100	2
CAMK1	CAMK1	100	2
CAMK1B	PNCK	100	2
CAMK1D	CAMK1D	100	2
CAMK1G	CAMK1G	100	2
CAMK2A	CAMK2A	100	2
CAMK2B	CAMK2B	100	2
CAMK2D	CAMK2D	100	2
CAMK2G	CAMK2G	99	1.995635195
CAMK4	CAMK4	99	1.995635195
CAMKK1	CAMKK1	77	1.886490725
CAMKK2	CAMKK2	72	1.857332496
CASK	CASK	100	2
CDC2L1	CDK11B	89	1.949390007
CDC2L2	CDC2L2	100	2
CDC2L5	CDK13	100	2
CDK11	CDK19	94	1.973127854
CDK2	CDK2	98	1.991226076
CDK3	CDK3	88	1.944482672
CDK4	CDK4	100	2
CDK4-cyclinD1	CDK4	86	1.934498451
CDK4-cyclinD3	CDK4	98	1.991226076
CDK5	CDK5	100	2
CDK7	CDK7	87	1.939519253
CDK8	CDK8	97	1.986771734
CDK9	CDK9	83	1.919078092

CDKL1	CDKL1	83	1.919078092
CDKL2	CDKL2	88	1.944482672
CDKL3	CDKL3	95	1.977723605
CDKL5	CDKL5	100	2
CHEK1	CHEK1	94	1.973127854
CHEK2	CHEK2	100	2
CIT	CIT	72	1.857332496
CLK1	CLK1	29	1.462397998
CLK2	CLK2	88	1.944482672
CLK3	CLK3	83	1.919078092
CLK4	CLK4	28	1.447158031
CSF1R	CSF1R	86	1.934498451
CSF1R-autoinhibited	CSF1R	53	1.72427587
CSK	CSK	100	2
CSNK1A1	CSNK1A1	60	1.77815125
CSNK1A1L	CSNK1A1L	78	1.892094603
CSNK1D	CSNK1D	43	1.633468456
CSNK1E	CSNK1E	44	1.643452676
CSNK1G1	CSNK1G1	63	1.799340549
CSNK1G2	CSNK1G2	49	1.69019608
CSNK1G3	CSNK1G3	75	1.875061263
CSNK2A1	CSNK2A1	100	2
CSNK2A2	CSNK2A2	91	1.959041392
CTK	MATK	100	2
DAPK1	DAPK1	91	1.959041392
DAPK2	DAPK2	97	1.986771734
DAPK3	DAPK3	100	2
DCAMKL1	DCLK1	91	1.959041392
DCAMKL2	DCLK2	100	2
DCAMKL3	DCLK3	100	2
DDR1	DDR1	92	1.963787827
DDR2	DDR2	100	2
DLK	MAP3K12	100	2
DMPK	DMPK	94	1.973127854
DMPK2	CDC42BPG	100	2
DRAK1	STK17A	88	1.944482672
DRAK2	STK17B	88	1.944482672
DYRK1A	DYRK1A	90	1.954242509
DYRK1B	DYRK1B	25	1.397940009
DYRK2	DYRK2	100	2
EGFR	EGFR	90	1.954242509

EGFR(E746-A750del)	EGFR	98	1.991226076
EGFR(G719C)	EGFR	89	1.949390007
EGFR(G719S)	EGFR	90	1.954242509
EGFR(L747-E749del, A750P)	EGFR	99	1.995635195
EGFR(L747-S752del, P753S)	EGFR	100	2
EGFR(L747-T751del,Sins)	EGFR	96	1.982271233
EGFR(L858R)	EGFR	81	1.908485019
EGFR(L858R,T790M)	EGFR	100	2
EGFR(L861Q)	EGFR	80	1.903089987
EGFR(S752-I759del)	EGFR	96	1.982271233
EGFR(T790M)	EGFR	92	1.963787827
EIF2AK1	EIF2AK1	93	1.968482949
EPHA1	EPHA1	100	2
EPHA2	EPHA2	100	2
EPHA3	EPHA3	93	1.968482949
EPHA4	EPHA4	100	2
EPHA5	EPHA5	100	2
EPHA6	EPHA6	100	2
EPHA7	EPHA7	100	2
EPHA8	EPHA8	100	2
EPHB1	EPHB1	100	2
EPHB2	EPHB2	83	1.919078092
EPHB3	EPHB3	100	2
EPHB4	EPHB4	100	2
EPHB6	EPHB6	93	1.968482949
ERBB2	ERBB2	100	2
ERBB3	ERBB3	100	2
ERBB4	ERBB4	79	1.897627091
ERK1	MAPK3	100	2
ERK2	MAPK1	100	2
ERK3	MAPK6	100	2
ERK4	MAPK4	93	1.968482949
ERK5	MAPK7	100	2
ERK8	MAPK15	82	1.913813852
ERN1	ERN1	92	1.963787827
FAK	PTK2	100	2
FER	FER	95	1.977723605
FES	FES	99	1.995635195
FGFR1	FGFR1	94	1.973127854
FGFR2	FGFR2	100	2
FGFR3	FGFR3	100	2

FGFR3(G697C)	FGFR3	98	1.991226076
FGFR4	FGFR4	93	1.968482949
FGR	FGR	95	1.977723605
FLT1	FLT1	73	1.86332286
FLT3	FLT3	40	1.602059991
FLT3(D835H)	FLT3	48	1.681241237
FLT3(D835V)	FLT3	93	1.968482949
FLT3(D835Y)	FLT3	26	1.414973348
FLT3(ITD)	FLT3	24	1.380211242
FLT3(ITD,D835V)	FLT3	43	1.633468456
FLT3(ITD,F691L)	FLT3	85	1.929418926
FLT3(K663Q)	FLT3	35	1.544068044
FLT3(N841I)	FLT3	77	1.886490725
FLT3(R834Q)	FLT3	100	2
FLT3-autoinhibited	FLT3	68	1.832508913
FLT4	FLT4	96	1.982271233
FRK	FRK	100	2
FYN	FYN	100	2
GAK	GAK	82	1.913813852
GCN2(Kin.Dom.2,S808G)	EIF2AK4	100	2
GRK1	GRK1	100	2
GRK2	ADRBK1	78	1.892094603
GRK3	ADRBK2	100	2
GRK4	GRK4	71	1.851258349
GRK7	GRK7	100	2
GSK3A	GSK3A	95	1.977723605
GSK3B	GSK3B	100	2
HASPIN	GSG2	100	2
HCK	HCK	87	1.939519253
HIPK1	HIPK1	78	1.892094603
HIPK2	HIPK2	100	2
HIPK3	HIPK3	100	2
HIPK4	HIPK4	95	1.977723605
HPK1	MAP4K1	94	1.973127854
HUNK	HUNK	100	2
ICK	ICK	100	2
IGF1R	IGF1R	100	2
IKK-alpha	CHUK	100	2
IKK-beta	IKBKB	100	2
IKK-epsilon	IKBKE	100	2
INSR	INSR	90	1.954242509

INSRR	INSRR	94	1.973127854
IRAK1	IRAK1	0.65	-0.187086643
IRAK3	IRAK3	78	1.892094603
IRAK4	IRAK4	1.6	0.204119983
ITK	ITK	75	1.875061263
JAK1(JH1domain-catalytic)	JAK1	100	2
JAK1(JH2domain-pseudokinase)	JAK1	93	1.968482949
JAK2(JH1domain-catalytic)	JAK2	100	2
JAK3(JH1domain-catalytic)	JAK3	100	2
JNK1	MAPK8	100	2
JNK2	MAPK9	100	2
JNK3	MAPK10	100	2
KIT	KIT	38	1.579783597
KIT(A829P)	KIT	100	2
KIT(D816H)	KIT	100	2
KIT(D816V)	KIT	100	2
KIT(L576P)	KIT	28	1.447158031
KIT(V559D)	KIT	23	1.361727836
KIT(V559D,T670I)	KIT	50	1.698970004
KIT(V559D,V654A)	KIT	37	1.568201724
KIT-autoinhibited	KIT	57	1.755874856
LATS1	LATS1	100	2
LATS2	LATS2	100	2
LCK	LCK	100	2
LIMK1	LIMK1	100	2
LIMK2	LIMK2	96	1.982271233
LKB1	STK11	100	2
LOK	STK10	87	1.939519253
LRRK2	LRRK2	100	2
LRRK2(G2019S)	LRRK2	100	2
LTK	LTK	79	1.897627091
LYN	LYN	93	1.968482949
LZK	MAP3K13	100	2
MAK	MAK	100	2
MAP3K1	MAP3K1	100	2
MAP3K15	MAP3K15	100	2
MAP3K2	MAP3K2	100	2
MAP3K3	MAP3K3	94	1.973127854
MAP3K4	MAP3K4	100	2
MAP4K2	MAP4K2	100	2
MAP4K3	MAP4K3	98	1.991226076

MAP4K4	MAP4K4	83	1.919078092
MAP4K5	MAP4K5	97	1.986771734
MAPKAPK2	MAPKAPK2	95	1.977723605
MAPKAPK5	MAPKAPK5	100	2
MARK1	MARK1	89	1.949390007
MARK2	MARK2	100	2
MARK3	MARK3	91	1.959041392
MARK4	MARK4	85	1.929418926
MAST1	MAST1	96	1.982271233
MEK1	MAP2K1	100	2
MEK2	MAP2K2	96	1.982271233
MEK3	MAP2K3	100	2
MEK4	MAP2K4	100	2
MEK5	MAP2K5	87	1.939519253
MEK6	MAP2K6	100	2
MELK	MELK	100	2
MERTK	MERTK	86	1.934498451
MET	MET	100	2
MET(M1250T)	MET	97	1.986771734
MET(Y1235D)	MET	95	1.977723605
MINK	MINK1	58	1.763427994
MKK7	MAP2K7	100	2
MKNK1	MKNK1	100	2
MKNK2	MKNK2	100	2
MLCK	MYLK3	89	1.949390007
MLK1	MAP3K9	97	1.986771734
MLK2	MAP3K10	78	1.892094603
MLK3	MAP3K11	96	1.982271233
MRCKA	CDC42BPA	86	1.934498451
MRCKB	CDC42BPB	100	2
MST1	STK4	80	1.903089987
MST1R	MST1R	100	2
MST2	STK3	82	1.913813852
MST3	STK24	100	2
MST4	MST4	100	2
MTOR	MTOR	100	2
MUSK	MUSK	100	2
MYLK	MYLK	97	1.986771734
MYLK2	MYLK2	94	1.973127854
MYLK4	MYLK4	100	2
MYO3A	MYO3A	95	1.977723605

MYO3B	MYO3B	93	1.968482949
NDR1	STK38	94	1.973127854
NDR2	STK38L	88	1.944482672
NEK1	NEK1	100	2
NEK10	NEK10	100	2
NEK11	NEK11	100	2
NEK2	NEK2	88	1.944482672
NEK3	NEK3	98	1.991226076
NEK4	NEK4	100	2
NEK5	NEK5	100	2
NEK6	NEK6	100	2
NEK7	NEK7	91	1.959041392
NEK9	NEK9	86	1.934498451
NIK	MAP3K14	97	1.986771734
NIM1	MGC42105	100	2
NLK	NLK	100	2
OSR1	OXSR1	100	2
p38-alpha	MAPK14	100	2
p38-beta	MAPK11	100	2
p38-delta	MAPK13	99	1.995635195
p38-gamma	MAPK12	98	1.991226076
PAK1	PAK1	100	2
PAK2	PAK2	100	2
PAK3	PAK3	73	1.86332286
PAK4	PAK4	100	2
PAK6	PAK6	100	2
PAK7	PAK7	84	1.924279286
PCTK1	CDK16	100	2
PCTK2	CDK17	97	1.986771734
PCTK3	CDK18	99	1.995635195
PDGFRA	PDGFRA	97	1.986771734
PDGFRB	PDGFRB	74	1.86923172
PDPK1	PDPK1	95	1.977723605
PFCDPK1(P.falciparum)	CDPK1	100	2
PFPK5(P.falciparum)	MAL13P1.279	100	2
PFTAIRE2	CDK15	94	1.973127854
PFTK1	CDK14	100	2
PHKG1	PHKG1	97	1.986771734
PHKG2	PHKG2	86	1.934498451
PIK3C2B	PIK3C2B	97	1.986771734
PIK3C2G	PIK3C2G	99	1.995635195

PIK3CA	PIK3CA	100	2
PIK3CA(C420R)	PIK3CA	100	2
PIK3CA(E542K)	PIK3CA	81	1.908485019
PIK3CA(E545A)	PIK3CA	99	1.995635195
PIK3CA(E545K)	PIK3CA	100	2
PIK3CA(H1047L)	PIK3CA	100	2
PIK3CA(H1047Y)	PIK3CA	100	2
PIK3CA(I800L)	PIK3CA	100	2
PIK3CA(M1043I)	PIK3CA	100	2
PIK3CA(Q546K)	PIK3CA	52	1.716003344
PIK3CB	PIK3CB	100	2
PIK3CD	PIK3CD	81	1.908485019
PIK3CG	PIK3CG	100	2
PIK4CB	PI4KB	100	2
PIKFYVE	PIKFYVE	100	2
PIM1	PIM1	100	2
PIM2	PIM2	100	2
PIM3	PIM3	100	2
PIP5K1A	PIP5K1A	73	1.86332286
PIP5K1C	PIP5K1C	100	2
PIP5K2B	PIP4K2B	68	1.832508913
PIP5K2C	PIP4K2C	42	1.62324929
PKAC-alpha	PRKACA	100	2
PKAC-beta	PRKACB	87	1.939519253
PKMYT1	PKMYT1	100	2
PKN1	PKN1	97	1.986771734
PKN2	PKN2	95	1.977723605
PKNB(M.tuberculosis)	pknB	94	1.973127854
PLK1	PLK1	100	2
PLK2	PLK2	100	2
PLK3	PLK3	100	2
PLK4	PLK4	94	1.973127854
PRKCD	PRKCD	88	1.944482672
PRKCE	PRKCE	100	2
PRKCH	PRKCH	96	1.982271233
PRKCI	PRKCI	100	2
PRKCQ	PRKCQ	90	1.954242509
PRKD1	PRKD1	92	1.963787827
PRKD2	PRKD2	100	2
PRKD3	PRKD3	93	1.968482949
PRKG1	PRKG1	90	1.954242509

PRKG2	PRKG2	94	1.973127854
PRKR	EIF2AK2	100	2
PRKX	PRKX	96	1.982271233
PRP4	PRPF4B	100	2
PYK2	PTK2B	89	1.949390007
QSK	KIAA0999	100	2
RAF1	RAF1	100	2
RET	RET	96	1.982271233
RET(M918T)	RET	91	1.959041392
RET(V804L)	RET	85	1.929418926
RET(V804M)	RET	82	1.913813852
RIOK1	RIOK1	56	1.748188027
RIOK2	RIOK2	100	2
RIOK3	RIOK3	80	1.903089987
RIPK1	RIPK1	93	1.968482949
RIPK2	RIPK2	100	2
RIPK4	RIPK4	94	1.973127854
RIPK5	DSTYK	100	2
ROCK1	ROCK1	100	2
ROCK2	ROCK2	100	2
ROS1	ROS1	99	1.995635195
RPS6KA4(Kin.Dom.1-N-terminal)	RPS6KA4	89	1.949390007
RPS6KA4(Kin.Dom.2-C-terminal)	RPS6KA4	100	2
RPS6KA5(Kin.Dom.1-N-terminal)	RPS6KA5	97	1.986771734
RPS6KA5(Kin.Dom.2-C-terminal)	RPS6KA5	90	1.954242509
RSK1(Kin.Dom.1-N-terminal)	RPS6KA1	89	1.949390007
RSK1(Kin.Dom.2-C-terminal)	RPS6KA1	100	2
RSK2(Kin.Dom.1-N-terminal)	RPS6KA3	100	2
RSK2(Kin.Dom.2-C-terminal)	RPS6KA3	100	2
RSK3(Kin.Dom.1-N-terminal)	RPS6KA2	92	1.963787827
RSK3(Kin.Dom.2-C-terminal)	RPS6KA2	100	2
RSK4(Kin.Dom.1-N-terminal)	RPS6KA6	100	2
RSK4(Kin.Dom.2-C-terminal)	RPS6KA6	100	2
S6K1	RPS6KB1	100	2
SBK1	SBK1	100	2
SGK	SGK1	100	2
SgK110	SgK110	100	2
SGK2	SGK2	100	2
SGK3	SGK3	100	2
SIK	SIK1	100	2
SIK2	SIK2	100	2

SLK	SLK	100	2
SNARK	NUAK2	94	1.973127854
SNRK	SNRK	100	2
SRC	SRC	100	2
SRMS	SRMS	100	2
SRPK1	SRPK1	93	1.968482949
SRPK2	SRPK2	100	2
SRPK3	SRPK3	79	1.897627091
STK16	STK16	43	1.633468456
STK33	STK33	96	1.982271233
STK35	STK35	100	2
STK36	STK36	87	1.939519253
STK39	STK39	100	2
SYK	SYK	97	1.986771734
TAK1	MAP3K7	73	1.86332286
TAOK1	TAOK1	100	2
TAOK2	TAOK2	100	2
TAOK3	TAOK3	58	1.763427994
TBK1	TBK1	85	1.929418926
TEC	TEC	100	2
TESK1	TESK1	100	2
TGFBR1	TGFBR1	95	1.977723605
TGFBR2	TGFBR2	100	2
TIE1	TIE1	100	2
TIE2	TEK	91	1.959041392
TLK1	TLK1	94	1.973127854
TLK2	TLK2	100	2
TNIK	TNIK	62	1.792391689
TNK1	TNK1	100	2
TNK2	TNK2	100	2
TNNI3K	TNNI3K	100	2
TRKA	NTRK1	80	1.903089987
TRKB	NTRK2	87	1.939519253
TRKC	NTRK3	100	2
TRPM6	TRPM6	100	2
TSSK1B	TSSK1B	100	2
TSSK3	TSSK3	100	2
TTK	TTK	100	2
TXK	TXK	100	2
TYK2(JH1domain-catalytic)	TYK2	100	2
TYK2(JH2domain-pseudokinase)	TYK2	100	2

TYRO3	TYRO3	93	1.968482949
ULK1	ULK1	100	2
ULK2	ULK2	100	2
ULK3	ULK3	100	2
VEGFR2	KDR	85	1.929418926
VPS34	PIK3C3	100	2
VRK2	VRK2	100	2
WEE1	WEE1	98	1.991226076
WEE2	WEE2	93	1.968482949
WNK1	WNK1	100	2
WNK2	WNK2	99	1.995635195
WNK3	WNK3	100	2
WNK4	WNK4	100	2
YANK1	STK32A	100	2
YANK2	STK32B	100	2
YANK3	STK32C	98	1.991226076
YES	YES1	100	2
YSK1	STK25	100	2
YSK4	MAP3K19	89	1.949390007
ZAK	ZAK	100	2
ZAP70	ZAP70	100	2

Table 1s - 468 kinase profile of HS-243 (10 μM) inhibition.

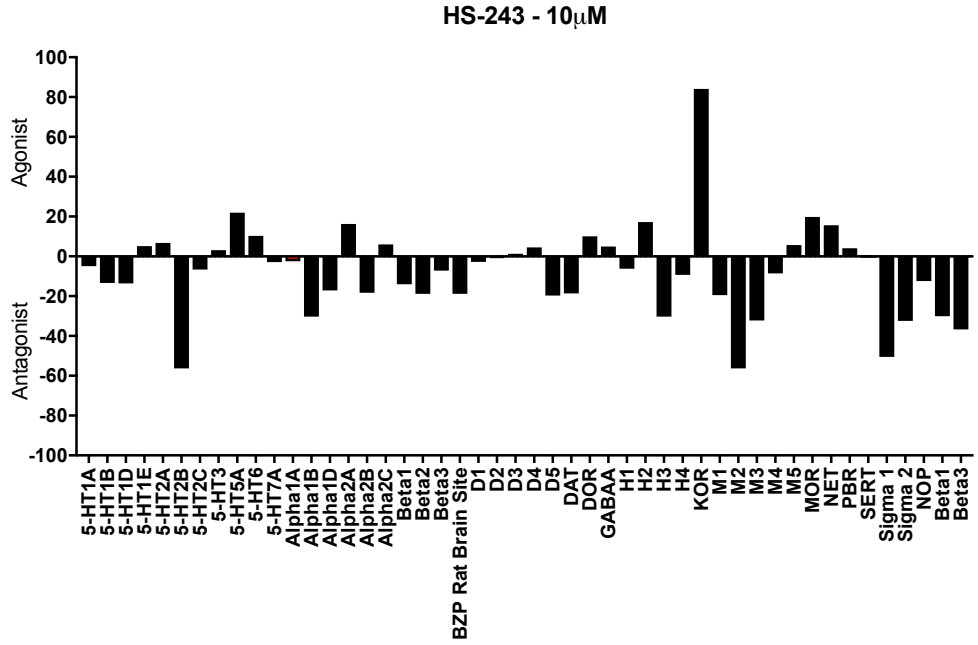


Figure 3s.

Selectivity of HS-243 within the purinome. HS-243 was tested at 10μM against various ATP binding enzymes for potency. Positive values indicate agonist like potential, whereas negative values indicated antagonist activity. Compounds with greater than 50% agonist or antagonist activity were further tested in a dose dependent manner for K_i determination. KOR and 5-HT2A were determined to have a $K_i > 10\mu M$.

Analyte	Mean- Vehicle	SEM -Vehicle	Mean - HS-243	SEM HS-243
Adiponectin	13.22775	0.294100925	12.3845	0.07081196
Apolopoprotein A-1	13.456	0.490706931	12.952	0.28823789
Angiogenin	12.906625	0.411065302	12.63533333	0.2094567
Angiopoietin-1	12.378625	0.387344882	12.15033333	0.21631311
Angiopoietin-2	12.747125	0.287566469	12.35316667	0.20520972
BAFF	11.453625	0.303157713	12.91166667	0.06289829
BDNF	11.791375	0.215968205	13.156	0.22924387
C5a	11.83	0.15124084	13.42116667	0.17822091
CD14	48.410375	3.889079167	22.26366667	0.53464672
CD30	12.5345	0.53344146	17.20733333	2.41499141
CD40 Ligand	12.811	0.174579137	10.83833333	2.35169925
Chitinase-3 like 1	159.176	8.905261748	83.737	33.5927755
Compliment Factor D	47.173	4.62320498	19.50283333	3.50623291
C Reactive Protein	12.506875	0.623595167	12.1965	0.11445669
Cripto-1	11.648375	0.136303334	12.68933333	0.07845292
Cystatin C	13.97625	0.478888187	13.58733333	0.08662868
Dkk-1	11.8115	0.087466422	13.476	0.29549746
DPPIV	11.2285	0.239322568	13.71283333	0.24818346
EGF	11.457375	0.443380545	14.01	0.24513619
Emmprin	16.20825	1.413423039	14.93983333	0.56492804
ENA-78	12.53175	0.218484696	14.4315	0.68737441
Endoglin	14.347125	1.014321086	13.99916667	0.9575984
Fas Ligand	12.104125	0.398350316	13.20816667	0.70662398
FGF Basic	12.243	0.444221698	12.716	0.04902635
FGF-7	12.14825	0.525512944	12.87516667	0.1549092
FGF-19	17.833375	0.701041379	13.755	0.7780354
Flt-3 Ligand	11.44825	0.211259862	13.59283333	0.30533893
G-CSF	11.359	0.311966411	14.00266667	0.25511767
GDF-15	118.37425	9.747109526	66.001	3.09631648
GM-CSF	11.327875	0.231288904	14.167	0.10284616
GRO-a	40.35775	2.992682224	18.75033333	1.04978924
Growth Hormone	12.728875	0.271807405	12.31266667	0.35549383
HGF	12.176	0.361705854	12.531	0.07264698
ICAM-1	21.35	1.925750103	16.2305	0.29539648
IFN-y	12.284375	0.126687341	12.6975	0.04850344
IGFBP-2	12.046625	0.281491295	12.99233333	0.19891923
IGFBP-3	12.841875	0.348857981	13.40483333	0.16208494
IL-1a	12.257125	0.361315154	13.73816667	0.45837342
IL-1B	12.431125	0.413694602	14.17566667	0.30727598
IL-1ra	26.07325	7.015823391	16.60983333	0.76687821

IL-2	12.904125	1.005976875	14.20583333	0.1565315
IL-3	11.195625	0.313998233	13.95916667	0.33563526
IL-4	12.220875	0.155155349	12.08983333	0.2458052
IL-5	12.414375	0.20322272	12.17	0.11326849
IL_6	18.659375	4.875526751	14.059	0.81225555
IL-8	186.629	6.482806918	140.9326667	5.22513274
IL-10	12.546125	0.323865287	12.99516667	0.29403009
IL-11	12.864625	0.409037202	13.12683333	0.12046622
IL-12p70	11.9635	0.186759315	13.2115	0.10418773
IL-13	11.983875	0.31101992	13.23783333	0.26994665
IL-15	12.186	0.440678785	14.02216667	0.3160715
IL-16	12.2935	0.151442921	14.192	0.06221535
IL-17a	25.127	2.340444019	15.67416667	0.38310055
IL18 Bpa	13.308625	0.468685812	14.37266667	0.23473466
IL-19	12.177625	0.129918624	12.01133333	0.39865653
IL-22	13.28125	0.258711591	12.40316667	0.13243153
IL-23	12.502625	0.173419335	12.39616667	0.13979072
IL-24	11.798375	0.410034824	12.98266667	0.35840856
IL-27	12.195375	0.3447092	12.84716667	0.49056773
IL-31	12.1105	0.414044533	13.12783333	0.26825941
IL-32	11.682125	0.362018725	13.415	0.35678985
IL-33	11.8375	0.350393648	13.68383333	0.32257226
IL-34	13.414125	0.628874834	14.53083333	0.38739862
IP-10	190.299	8.316666542	146.102	8.30823858
I-TAC	208.62375	4.19121857	148.6381667	0.92154112
Kallikrein 3	13.302625	0.160894553	14.96716667	0.57885126
Leptin	12.4635	0.236778113	12.2375	0.46406366
LIF	12.920125	0.237618161	12.28066667	0.28996987
Lipocalin-2	12.7525	0.248897904	12.872	0.13821843
MCP-1	84.72475	8.335310268	44.072	4.14330059
MCP-3	12.924875	0.682612365	13.07766667	0.65138937
M-CSF	12.241875	0.296985576	13.60283333	0.41760591
MIF	31.285375	3.699317557	18.79916667	1.09027039
MIG	22.10425	2.560970637	14.9165	0.15250109
MIP-1a/MIP-1B	140.236875	11.29332363	78.98566667	6.16136068
MIP-3a	147.45825	9.71111566	82.41166667	3.55793007
MIP-3B	13.288375	0.85405536	13.83816667	0.20165241
MMP-9	80.219	5.597329479	26.43466667	2.48240247
Myeloperoxidase	12.636625	0.144204192	12.48316667	0.3475848
Osteopontin	116.299375	8.335600154	80.44533333	3.86825758
PDGF-AA	55.15525	6.662486327	42.88	1.66955713

PDGF-AB/BB	11.733875	0.309258682	12.317	0.67811448
Pentraxin 3	17.318875	1.131816191	13.30816667	0.55383582
PF4	12.45	0.225323194	13.04083333	0.36993291
RAGE	12.187125	0.293961759	13.30883333	0.47052651
RANTES	108.090875	11.1092317	57.869	5.7401955
RBP-4	12.10025	0.074075553	13.79916667	0.36737814
Relaxin-2	10.685375	0.376761108	13.5815	0.35881135
Resistin	11.818625	0.535817381	13.881	0.40083205
SDF-1a	14.72825	0.658666124	14.516	0.08474816
Serpin E1	14.5955	0.475074073	13.0295	0.18599888
SHBG	12.51325	0.300484643	12.19316667	0.42732953
ST2	12.356	0.278630206	12.2965	0.26722041
TARC	11.953375	0.322700866	12.36783333	0.51375305
TFF-3	12.598625	0.278451774	12.73566667	0.54748557
TfR	12.811125	0.345806688	13.12233333	0.38368285
TGF-alpha	12.55725	0.342744712	13.49116667	0.68970147
Thrombosponidin-1	17.24675	1.186240183	14.654	0.30048128
TNF-alpha	90.283125	9.304893908	98.70133333	2.2278062
uPAR	65.289625	8.507473287	28.4885	0.58055125
VEGF	12.047125	0.577673941	13.87583333	0.27171099
Vitamin DBP	12.9335	0.378149827	12.482	0.43565018
CD31	25.0875	3.268090466	20.112	0.3854025
Tim-3	111.803125	9.545909991	70.137	3.59899119
VCAM-1	14.625625	0.819188022	13.20483333	0.38688977

Table 2s

THP-1 cells were challenged with LPS for 24 hours in the presence of vehicle or HS-243. All 105 cytokine analytes presented as mean, and standard error of the mean (SEM).