SUPPORTING INFORMATION.

Using ovality to predict non-mutagenic, orally efficacious pyridazine amides as cell specific spleen tyrosine kinase inhibitors

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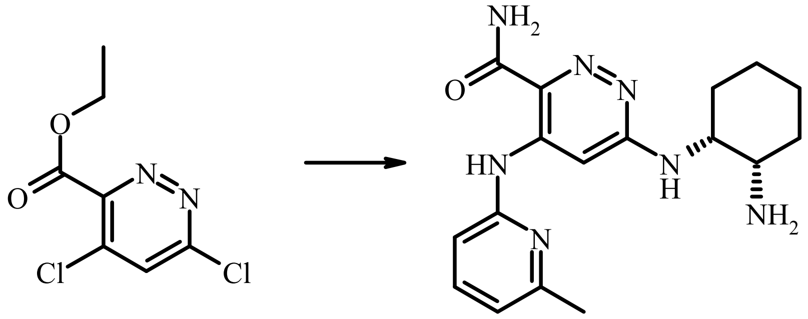
CONTENTS.

EXPERIMENTAL SECTION

General Methods. All reagents and solvents were purchased from commercial sources and used without further purification. ^{1}H NMR spectra were measured using a Bruker NMR Avance 400MHz or 300MHz spectrometer, and chemical shifts were expressed in δ (ppm) units using tetramethylsilane as an internal standard (in ^{1}H NMR description, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad peak). All compounds were analyzed by LC/MS (liquid chromatography/mass spectrometry) using a Waters ZQ mass detector and Waters LC system. Ionization was generally achieved via electron spray (ES). The LC fraction detection consisted of both a diode array detector and an evaporative light scattering detector, and all tested compounds had purity greater than 95%.

6-((1R,2S)-2-Amino-cyclohexylamino)-4-(6-methyl-pyridin-2-ylamino)-pyridazine-3-carboxylic acid amide [**9**]



Step 1

6-Chloro-4-(6-methyl-pyridin-2-ylamino)-pyridazine-3-carboxylic acid ethyl ester

4,6-Dichloro-pyridazine-3-carboxylic acid ethyl ester (300 mg, 1.36 mmol) and 6-methylpyridin-2-amine (176 mg, 1.63 mmol) were dissolved in acetonitrile (4.1 mL), then heated at 140 °C for 16 h. A second portion of 6-methylpyridin-2-amine (73 mg, 0.67 mmol) was added. After a further 2 d at 140 °C the reaction mixture was cooled anc concentrated *in vacuo*. Purification by chromatography (silica, 5 to 40 % ethyl acetate in hexanes) gave 6-chloro-4-(6-methyl-pyridin-2-ylamino)-pyridazine-3-carboxylic acid ethyl ester (146 mg, 36 %) as a light yellow oil. ^{1}H NMR (300 MHz, CHLOROFORM-*d*) δ ppm 10.68 (br. s., 1 H) 9.21 (s, 1 H) 7.59 (t, *J*=7.7 Hz, 1 H) 6.90 (d, *J*=7.6 Hz, 1 H) 6.76 (d, *J*=7.9 Hz, 1 H) 4.57 (q, *J*=7.2 Hz, 2 H) 2.57 (s, 3 H) 1.51 (t, *J*=7.0 Hz, 3 H); LCMS (EI/CI) *m/z*: 315 [M + Na].

Step 2

6-Chloro-4-(6-methyl-pyridin-2-ylamino)-pyridazine-3-carboxylic acid amide

6-Chloro-4-(6-methyl-pyridin-2-ylamino)-pyridazine-3-carboxylic acid ethyl ester (137 mg, 0.468 mmol) was suspended in 7M ammonia in methanol (3 mL). After 4 h, the reaction mixture was concentrated *in vacuo* to give 6-chloro-4-(6-methyl-pyridin-2-ylamino)-pyridazine-3-carboxylic acid amide (123 mg, 100 %) as an off-white solid which was used directly in the next step without purification. LCMS (EI/CI) *m/z*: 264 [M + H].

Step 3

{(1S,2R)-2-[6-Carbamoyl-5-(6-methyl-pyridin-2-ylamino)-pyridazin-3-ylamino]-cyclohexyl}-carbamic acid *tert*-butyl ester

6-Chloro-4-(6-methyl-pyridin-2-ylamino)-pyridazine-3-carboxylic acid amide (130 mg, 0.49 mmol), N,N-diisopropylethylamine (0.17 mL, 0.986 mmol) and *tert*-butyl (1S,2R)-2-aminocyclohexylcarbamate (211 mg, 0.986 mmol) were dissolved in N-methylpyrrolidinone (2 mL) and heated at 150 °C for 2 d. The reaction mixture was cooled and concentrated *in vacuo*, then diluted with water, and extracted with ethyl acetate. The combined organic extracts were washed with brine, dried (sodium sulfate), filtered and concentrated in vacuo.

Purification by chromatography (silica, 0 to 5 % methanol in dichloromethane) gave {(1S,2R)-2-[6-carbamoyl-5-(6-methyl

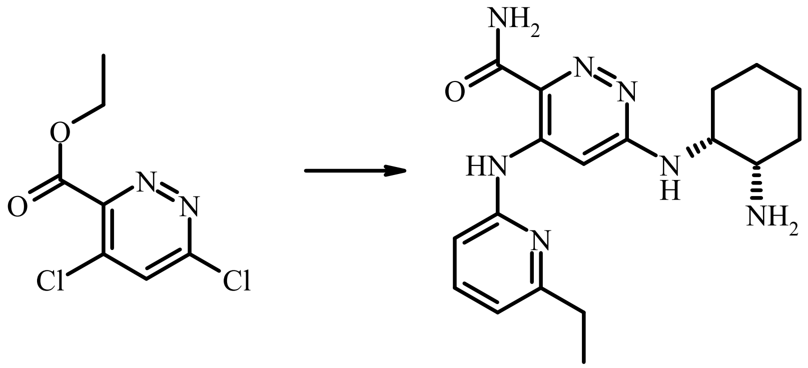
-pyridin-2-ylamino)-pyridazin-3-ylamino]-cyclohexyl}-carbamic acid *tert*-butyl ester (78 mg, 35 %) as a light brown amorphous residue. LCMS (EI/CI) *m/z*: 442 [M + H].

Step 4

6-((1R,2S)-2-amino-cyclohexylamino)-4-(6-methyl-pyridin-2-ylamino)-pyridazine-3-carboxylic acid amide [**9**]

{(1S,2R)-2-[6-Carbamoyl-5-(6-methyl-pyridin-2-ylamino)-pyridazin-3-ylamino]-cyclohexyl}-carbamic acid *tert*-butyl ester (188 mg, 0.426 mmol) was dissolved in dichloromethane (4 mL) then cooled to 0 °C. Trifluoroacetic acid (2 mL, 25 mmol) was added drop-wise then the reaction mixture was warmed to 25 °C. After 4 h, the mixture was cooled in an ice bath and neutralized with sodium bicarbonate solution. The mixture was extracted with ethyl acetate, and the combined organic layers washed with brine, dried (sodium sulfate), filtered, and concentrated *in vacuo*. Purification by chromatography (silica, 0 to 100 % of a 0.1:0.01 solution of methanol:NH_4OH in dichloromethane) gave **9** (61 mg, 42 %) as a light brown solid. ^1H NMR (400 MHz, DMSO-d_6) d ppm 11.61 (s, 1 H) 8.35 - 8.44 (m, 1 H) 8.10 (s, 1 H) 7.61 (br. s, 1 H) 7.61 (t, *J*=7.3 Hz, 1 H) 6.81 - 6.89 (m, 1 H) 6.85 (d, *J*=7.3 Hz, 1 H) 6.76 (d, *J*=8.1 Hz, 1 H) 3.76 - 3.99 (m, 2 H) 3.11 - 3.18 (m, 1 H) 2.48 - 2.49 (m, 3 H) 1.49 - 1.77 (m, 7 H) 1.27 - 1.38 (m, 2 H); LCMS (EI/CI) *m/z*: 342 [M + H].

6-((1R,2S)-2-Amino-cyclohexylamino)-4-(6-ethyl-pyridin-2-ylamino)-pyridazine-3-carboxylic acid amide [**14**]



Step 1

6-Chloro-4-(6-ethyl-pyridin-2-ylamino)-pyridazine-3-carboxylic acid ethyl ester

4,6-Dichloro-pyridazine-3-carboxylic acid ethyl ester (500 mg, 2.26 mmol) and 6-ethylpyridin-2-amine 415 mg, 3.39 mmol) were dissolved in acetonitrile (7 mL), then heated at 140 °C for 3 d. The reaction mixture was cooled and concentrated *in vacuo*. Purification by chromatography (silica, 5 to 50 % ethyl acetate in hexanes) gave 6-chloro-4-(6-ethyl-pyridin-2-ylamino)-pyridazine-3-carboxylic acid ethyl ester (246 mg, 35 %) as a light brown solid. ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 10.68 (br. s., 1 H) 9.30 (s, 1 H) 7.61 (t, *J*=7.8 Hz, 1 H) 6.91 (d, *J*=7.3 Hz, 1 H) 6.77 (d, *J*=7.8 Hz, 1 H) 4.57 (q, *J*=7.2 Hz, 2 H) 2.86 (q, *J*=7.6 Hz, 2 H) 1.52 (t, *J*=7.1 Hz, 3 H) 1.38 (t, *J*=7.5 Hz, 3 H); LCMS (EI/CI) *m/z*: 307 [M + H].

Step 2

6-Chloro-4-(6-ethyl-pyridin-2-ylamino)-pyridazine-3-carboxylic acid amide

6-Chloro-4-(6-ethyl-pyridin-2-ylamino)-pyridazine-3-carboxylic acid ethyl ester (241 mg, 0.786 mmol) was suspended in 7M ammonia in methanol (9 mL). After 3 h, the reaction mixture was concentrated *in vacuo* to give 6-chloro-4-(6-ethyl-pyridin-2-ylamino)-pyridazine-3-carboxylic acid amide (218 mg, 100 %) as an off-white solid which was used directly in the next step without purification. LCMS (EI/CI) *m/z*: 278 [M + H].

Step 3

{(1S,2R)-2-[6-Carbamoyl-5-(6-ethyl-pyridin-2-ylamino)-pyridazin-3-ylamino]-cyclohexyl}-carbamic acid *tert*-butyl ester

6-Chloro-4-(6-ethyl-pyridin-2-ylamino)-pyridazine-3-carboxylic acid amide (218 mg, 0.785 mmol),and *tert*-butyl (1S,2R)-2-aminocyclohexylcarbamate (336 mg, 1.57 mmol) were dissolved in N-methylpyrrolidinone (4 mL) and heated at 150 °C for 40 h. Additional *tert*-butyl (1S,2R)-2-aminocyclohexylcarbamate (168 mg, 0.78 mmol) was added and the heating continued for 16 h more. The reaction mixture was cooled and concentrated *in vacuo*, then diluted with water, and extracted with ethyl acetate. The combined organic extracts were washed with brine, dried (sodium sulfate), filtered and concentrated in vacuo. Purification by chromatography (silica, 0 to 5 % methanol in dichloromethane) gave {(1S,2R)-2-[6-carbamoyl-5-(6-ethyl-pyridin-2-ylamino)-pyridazin-3-ylamino]-cyclohexyl}-carbamic acid *tert*-butyl ester (167 mg, 46 %) as a light brown amorphous residue. ^{1}H NMR (300 MHz, CHLOROFORM-*d*) δ ppm 11.44 (s, 1 H) 8.47 (s, 1 H) 7.97 - 8.03 (m, 1 H) 7.52 (t, *J*=7.6 Hz, 1 H) 7.22 - 7.26 (m, 1 H) 6.78 (d, *J*=7.2 Hz, 1 H) 6.70 (d, *J*=7.9 Hz, 1 H) 5.38 - 5.43 (m, 1 H) 4.92 - 5.00 (m, 1 H) 3.97 - 4.08 (m, 2 H) 2.80 (q, *J*=7.6 Hz, 2 H) 1.58 - 1.96 (m, 8 H) 1.41 (s, 9 H) 1.31 - 1.39 (m, 3 H); LCMS (EI/CI) *m/z*: 456 [M + H].

Step 4

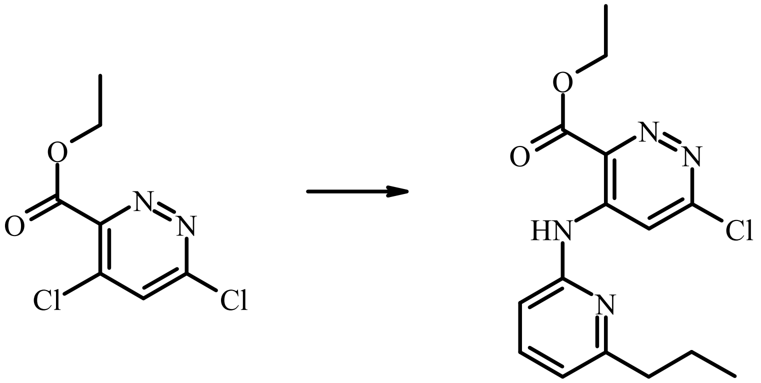
6-((1R,2S)-2-Amino-cyclohexylamino)-4-(6-ethyl-pyridin-2-ylamino)-pyridazine-3-carboxylic acid amide [**14**]

{(1S,2R)-2-[6-Carbamoyl-5-(6-ethyl-pyridin-2-ylamino)-pyridazin-3-ylamino]-cyclohexyl}-carbamic acid *tert*-butyl ester (167 mg, 0.367 mmol) was dissolved in dichloromethane (4 mL) then cooled to 0 °C. Trifluoroacetic acid (2 mL, 26 mmol) was added drop-wise then the reaction mixture was warmed to 25 °C. After 5 h, the mixture was cooled in an ice bath and neutralized with sodium bicarbonate solution. The mixture was extracted with ethyl acetate, and the combined organic layers washed with brine, dried (sodium sulfate), filtered, and concentrated *in vacuo*. Purification by chromatography (silica, 5 to 80 % of a 0.1:0.01 solution of methanol:NH_4OH in dichloromethane) gave **14** (66 mg, 50 %) as a light brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.66 (s, 1 H) 8.35 - 8.40 (m, 1 H) 8.09 (s, 1 H) 7.63 (dd, *J*=8.2, 7.5 Hz, 1 H) 7.62 (br. s., 1 H) 6.86 (d, *J*=7.6 Hz, 1 H) 6.79 (d, *J*=7.6 Hz, 1 H) 6.76 (d, *J*=7.8 Hz, 1 H) 3.73 - 3.86 (m, 1 H) 3.10 - 3.15 (m, 1 H) 2.75 (q, *J*=7.6 Hz, 2 H) 1.48 - 1.73 (m, 7 H) 1.27 (t, *J*=7.7 Hz, 3 H) 1.24 - 1.37 (m, 2 H); LCMS (EI/CI) *m/z*: 356 [M + H].

6-((1R,2S)-2-aminocyclohexylamino)-4-(6-propylpyridin-2-ylamino)pyridazine-3-carboxamide [**15**]

Step 1

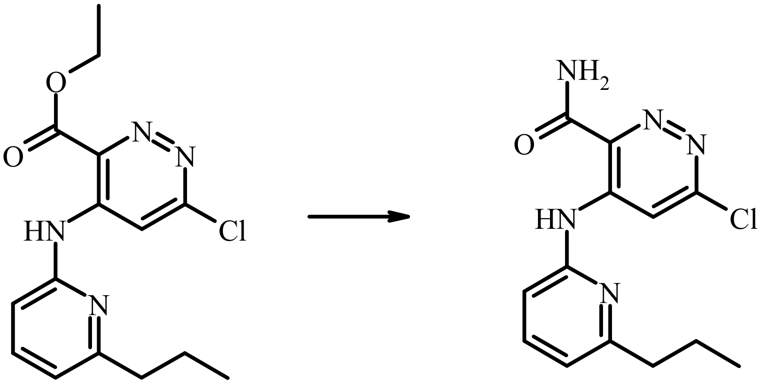
Ethyl 6-chloro-4-(6-propylpyridin-2-ylamino)pyridazine-3-carboxylate



A mixture of ethyl 4,6-dichloropyridazine-3-carboxylate (0.80 g, 3.62 mmol) and 6-propylpyridin-2-amine (739 mg, 5.43 mmol) was dissolved in acetonitrile (4 mL) and heated at 130°C. After 24 h, the reaction mixture was cooled and concentrated *in vacuo*, then purified by chromatography (silica, 80 g, 0 to 3 % acetone in dichloromethane over 15 min, then 3 to 10 % acetone in dichloromethane over another 15 min) to give ethyl 6-chloro-4-(6-propylpyridin-2-ylamino)pyridazine-3-carboxylate (335 mg, 1.04 mmol, 29 %) as a pale yellow crystalline solid after drying under high vacuum at room temperature. LC-MS 321.1 $[M+H]^+$.

Step 2

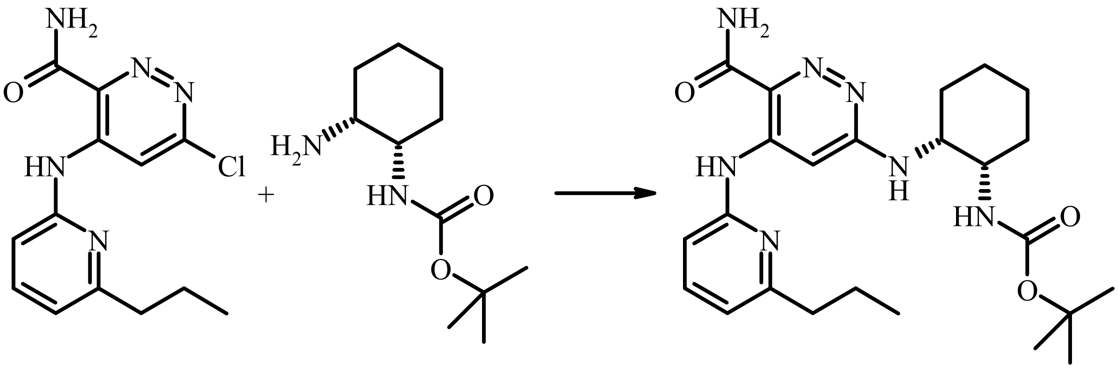
6-Chloro-4-(6-propylpyridin-2-ylamino)pyridazine-3-carboxamide



Ethyl 6-chloro-4-(6-propylpyridin-2-ylamino)pyridazine-3-carboxylate (340 mg, 1.06 mmol) was stirred with 7N methanolic ammonia (15.1 mL, 106 mmol). A colorless precipitate formed within a few minutes. After 18 h, the mixture was concentrated *in vacuo* to give 6-chloro-4-(6-propylpyridin-2-ylamino)pyridazine-3-carboxamide (289 mg, 991 µmol, 94 %) as an off-white powder. LC-MS 292.2 $[M+H]^+$. This material was used directly without further purification.

Step 3

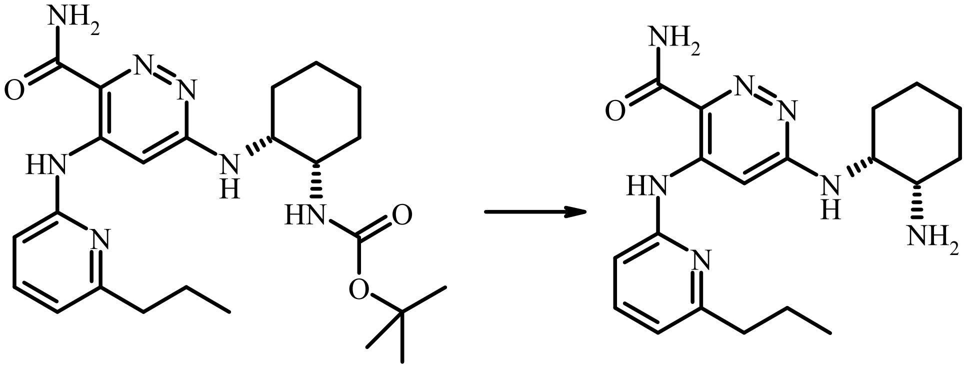
tert-Butyl (1S,2R)-2-(6-carbamoyl-5-(6-propylpyridin-2-ylamino)pyridazin-3-ylamino)cyclohexylcarbamate



6-Chloro-4-(6-propylpyridin-2-ylamino)pyridazine-3-carboxamide (289 mg, 991 µmol) and *tert*-butyl (1S,2R)-2-aminocyclohexylcarbamate (425 mg, 1.98 mmol) were dissolved in N-methyl-2-pyrrolidinone (2 mL) and heated at 130 °C for 24 h. LCMS showed desired product mass, as well as starting material. A third equivalent of *tert*-butyl (1S,2R)-2-aminocyclohexylcarbamate was added (213 mg, 0.991 mmol) and heating continued. After 8 h, a third portion of *tert*-butyl (1S,2R)-2-aminocyclohexylcarbamate (0.310 g) was added. After 24 h, the mixture was cooled and concentrated *in vacuo* (high vacuum, 100 °C) to a brown solid residue. Purification by chromatography (silica, 0 to 10 % of a 9:1 MeOH:NH_4OH solution in dichloromethane, 20 min) gave *tert*-butyl (1S,2R)-2-(6-carbamoyl-5-(6-propylpyridin-2-ylamino)pyridazin-3-ylamino)cyclohexylcarbamate (271 mg, 577 µmol, 58 %) as a light brown solid. . LC-MS 470.2 $[M+H]^+$.

Step 4

<u>6-((1R,2S)-2-aminocyclohexylamino)-4-(6-propylpyridin-2-ylamino)pyridazine-3-carboxamide [**15**]</u>

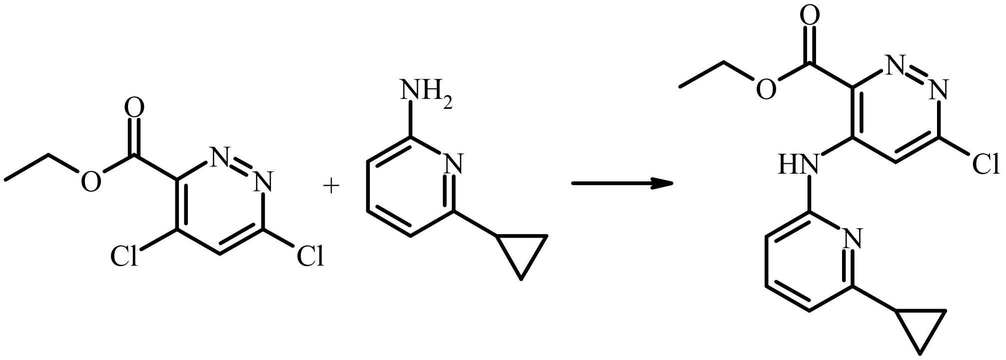


tert-Butyl (1S,2R)-2-(6-carbamoyl-5-(6-propylpyridin-2-ylamino)pyridazin-3-ylamino)cyclohexylcarbamate (260 mg, 554 µmol) was dissolved in dichloromethane (4 mL) then TFA (3.16 g, 2.13 mL, 27.7 mmol) was added. The mixture was stirred at room temperature for 12 h then the mixture was concentrated *in vacuo* to a brown oil, and purified by chromatography (silica, 40 g, 0 to 20 % of a 9:1 MeOH:NH_4OH solution in dichloromethane, 30 min) to give a pale yellow foam (140 mg). This was dissolved in ethanol and concentrated *in vacuo* (3 x 20 mL) to give the desired product, pure by H nmr and LCMS except for traces of isopropyl alcohol. This was dissolved in absolute ethanol, concentrated *in vacuo*, and then triturated twice with ethanol (2 x 0.5 mL) and then dried to give **15** (110 mg, 295 μmol, 53 %) as a white powder. ^{1}H NMR (DMSO-d_6) δ: 11.67 (s, 1H), 8.39 (br. s., 1H), 8.08 (br. s., 1H), 7.63 (t, J = 7.7 Hz, 2H), 6.85 (d, J = 7.6 Hz, 1H), 6.72 - 6.82 (m, 2H), 3.74 (br. s., 1H), 3.12 (d, J = 3.0 Hz, 1H), 2.65 - 2.76 (m, 2H), 1.45 - 1.81 (m, 10H), 1.21 - 1.39 (m, 2H), 0.94 (t, J = 7.3 Hz, 3H); LC-MS 370.2 $[M+H]^+$.

6-((1R,2S)-2-Aminocyclohexylamino)-4-(6-cyclopropylpyridin-2-ylamino)pyridazine-3-carboxamide [**16**]

Step 1

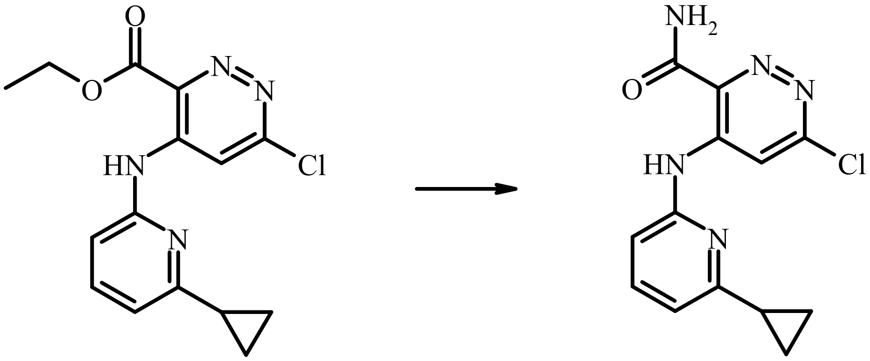
Ethyl 6-chloro-4-(6-cyclopropylpyridin-2-ylamino) pyridazine-3-carboxylate



A pressure tube charged with ethyl 4,6-dichloropyridazine-3-carboxylate (300 mg, 1.36 mmol), 6-cyclopropylpyridin-2-amine (273 mg, 2.04 mmol), and acetonitrile (8 mL) was heated at 140 °C for 20 h. After cooling to room temperature, the mixture was concentrated *in vacuo* and the residue obtained was purified by chromatography (silica, 50 µm, 80 g, Analogix, 0 to 10 % acetone in dichloromethane, 20 min) to give ethyl 6-chloro-4-(6-cyclopropylpyridin-2-ylamino)pyridazine-3-carboxylate (145 mg, 34 %) as a yellow solid. ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 10.64 (br. s., 1 H) 9.39 (s, 1 H) 7.63 (t, *J*=7.83 Hz, 1 H) 6.94 (d, *J*=7.58 Hz, 1 H) 6.78 (d, *J*=8.08 Hz, 1 H) 4.12 (s, 3 H) 1.99 – 2.10 (m, 1 H) 1.06 - 1.12 (m, 4 H). LCMS (EI/CI) *m/z*: 319 [M + H].

Step 2

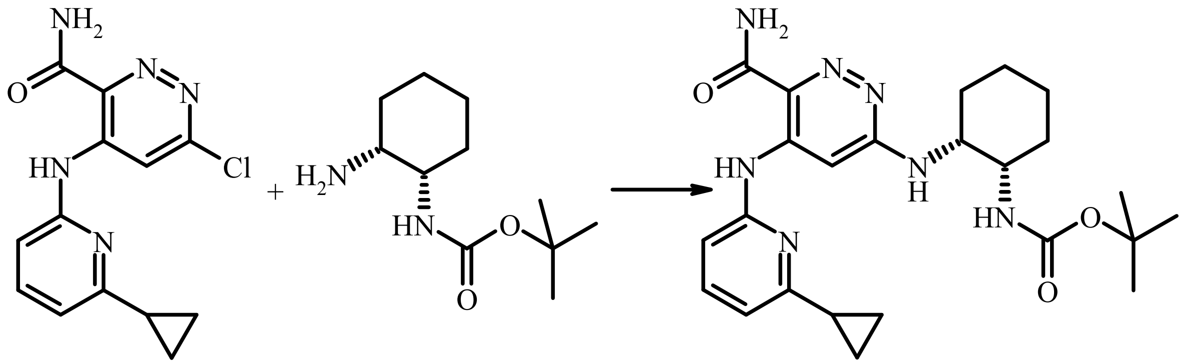
6-Chloro-4-(6-cyclopropylpyridin-2-ylamino) pyridazine-3-carboxamide



Ethyl 6-chloro-4-(6-cyclopropylpyridin-2-ylamino)pyridazine-3-carboxylate (140 mg, 439 µmol) and ammonia (7M in methanol, 9.44 g, 12 mL, 84.0 mmol) were heated at 50 ^{o}C in a sealed tube for 21 h. After cooling to room temperature, concentration in vacuo gave 6-chloro-4-(6-cyclopropylpyridin-2-ylamino)pyridazine-3-carboxamide (112 mg, 88 %) as a yellow solid. ^{1}H NMR (300 MHz, CHLOROFORM-*d*) δ ppm 11.51 (br. s., 1 H) 9.16 (s, 1 H) 8.16 (br. s., 1 H) 7.52 (t, *J*=7.74 Hz, 1 H) 6.86 - 6.96 (m, 1 H) 6.68 (d, *J*=7.93 Hz, 1 H) 5.65 (br. s., 1 H) 1.99 - 2.10 (m, 1 H) 1.06 - 1.12 (m, 4 H). LCMS (EI/CI) *m/z*: 290 [M + H]. The crude product was used directly in the next step without further purification.

Step 3

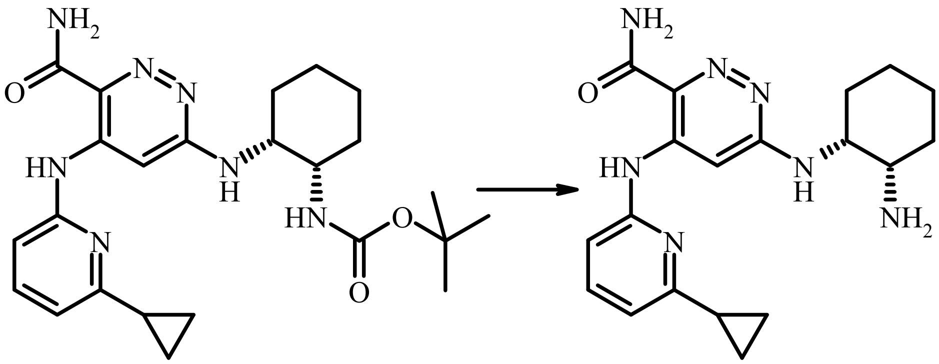
tert-Butyl (1S,2R)-2-(6-carbamoyl-5-(6-cyclopropylpyridin-2-ylamino)pyridazin-3-ylamino)cyclohexylcarbamate



To a stirred solution of 6-chloro-4-(6-cyclopropylpyridin-2-ylamino) pyridazine-3-carboxamide (104 mg, 359 µmol) in *N*-methyl-2-pyrrolidinone (5 mL) was added DMAP (47 mg, 377 µmol) and *tert*-butyl (1S,2R)-2-aminocyclohexylcarbamate (154 mg, 718 µmol).. The mixture was heated to 150 ^{o}C for 1.5 days, then a stream of N_2 was blown into the flask while heating at 140^{o}C to evaporate the volatile solvents. The residue obtained was then purified by chromatography (silica, 50 µm, 60 g, Analogix, 97:2.75:0.15 to 84:15.2: 0.8 dichloromethane:MeOH:NH_4OH, 30 min) to afford *tert*-butyl (1S,2R)-2-(6-carbamoyl-5-(6-cyclopropylpyridin-2-ylamino)pyridazin-3 ylamino)cyclohexylcarbamate (126 mg, containing some residual NMP) as a brown viscous oil. ^{1}H NMR (300 MHz, CHLOROFORM-*d*) δ ppm 11.43 (br. s., 1 H) 8.17 (br. s., 1 H) 8.03 (br. s., 1 H) 7.44 (br. s., 1 H) 7.03 (br. s., 1 H) 6.80 (br. s., 1 H) 6.60 (br. s., 1 H) 5.76 (br. s., 1 H) 5.49 (br. s., 1 H) 3.82 (br. s., 1 H) 3.24 (br. s., 1 H) 2.03 (br. s., 1 H) 1.32 - 1.91 (m, 8 H) 1.45 (s., 9 H) 1.05 (d, *J*=16.62 Hz, 4 H). LCMS (EI/CI) *m/z*: 468 [M + H]. This was used directly in the next step without further purification.

Step 4

6-((1R,2S)-2-Aminocyclohexylamino)-4-(6-cyclopropylpyridin-2-ylamino)pyridazine-3-carboxamide [**16**]

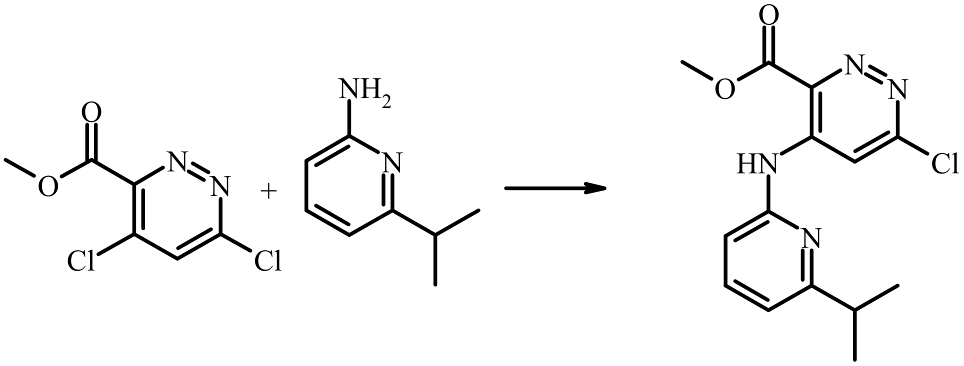


To a solution of *tert*-butyl (1S,2R)-2-(6-carbamoyl-5-(6-cyclopropylpyridin-2-ylamino)pyridazin-3-ylamino)cyclohexylcarbamate (25 mg, 53.5 μmol) in dichloromethane (4 mL) was added TFA (370 μg, 0.25 μL, 3.24 μmol). After 6 h, the mixture was concentrated *in vacuo*, then purified by flash chromatography (spherical silica, 20-45 μm, 25 g, Versaflash from Supelco, 97:2.75:0.15 to 84:15.2:0.8 dichloromethane:MeOH:NH_4OH, 30 min) to give **16** (13 mg, 10 %, two steps) as a brown solid. ^1H NMR (300 MHz, CHLOROFORM-*d*) δ ppm 11.43 (br. s., 1 H) 8.17 (br. s., 1 H) 8.03 (br. s., 1 H) 7.44 (br. s., 1 H) 7.03 (br. s., 1 H) 6.80 (br. s., 1 H) 6.60 (br. s., 1 H) 5.76 (br. s., 1 H) 5.49 (br. s., 1 H) 3.82 (br. s., 1 H) 3.24 (br. s., 1 H) 2.03 (br. s., 1 H) 1.32 - 1.91 (m, 8 H) 1.05 (d, *J*=16.62 Hz, 4 H). LCMS (EI/CI) *m/z*: 368 [M + H].

6-((1R,2S)-2-Amino-cyclohexylamino)-4-(6-isopropyl-pyridin-2-ylamino)-pyridazine-3-carboxylic acid amide [**17**]

Step 1

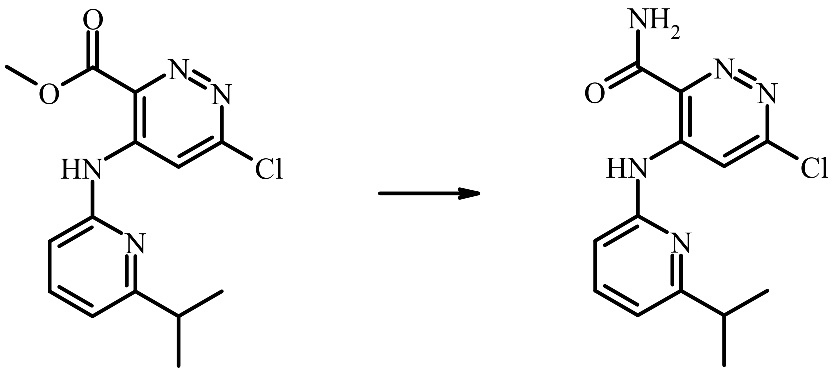
Methyl 6-chloro-4-(6-isopropylpyridin-2-ylamino)pyridazine-3-carboxylate



A stirred solution of methyl 4, 6-dichloropyridazine-3-carboxylate (255 mg, 1.23 mmol) and 6-isopropylpyridin-2-amine (252 mg, 1.85 mmol) in acetonitrile (8.0 mL) was heated at 140 °C for 21 h. After cooling to room temperature the reaction mixture was concentrated *in vacuo*, then the residue purified by chromatography (silica, 20-45 μm, 80 g, Thomson, 0 to 10 % acetone in dichloromethane, 20 min) to give methyl 6-chloro-4-(6-isopropylpyridin-2-ylamino)pyridazine-3-carboxylate (113 mg, 30 %) as a yellow solid. ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 10.64 (br. s., 1 H) 9.39 (s, 1 H) 7.63 (t, *J*=7.83 Hz, 1 H) 6.94 (d, *J*=7.58 Hz, 1 H) 6.78 (d, *J*=8.08 Hz, 1 H) 4.12 (s, 3 H) 3.01 - 3.19 (m, 1 H) 1.31 - 1.42 (m, 6 H). LCMS (EI/CI) *m/z*: 307 [M + H].

Step 2

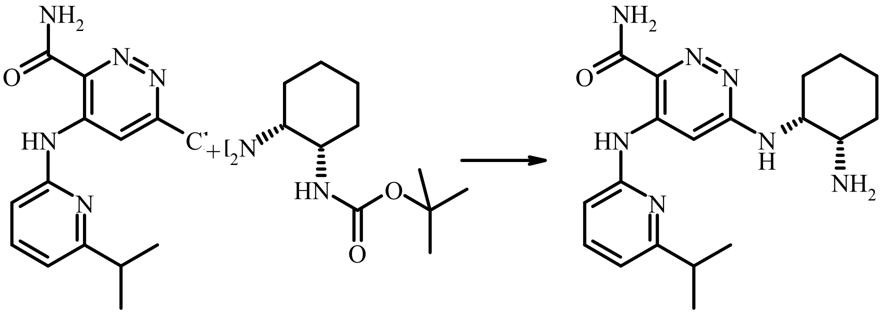
6-Chloro-4-(6-isopropylpyridin-2-ylamino)pyridazine-3-carboxamide



Methyl 6-chloro-4-(6-isopropylpyridin-2-ylamino)pyridazine-3-carboxylate (99 mg, 322 µmol) was added to a pressure tube followed by ammonia (7M in methanol, 3.94 g, 5 mL, 35.0 mmol). The mixture was heated to 50 °C for 18 h, and then additional 7 N ammonia in methanol (7.5 mL) was added. After 24 h, the mixture was cooled, filtered and dried to afford 6-chloro-4-(6-isopropylpyridin-2-ylamino)pyridazine-3-carboxamide (103 mg, 100 %) as a yellow solid. ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 11.58 (br. s., 1 H) 9.41 (s, 1 H) 8.20 (br. s., 1 H) 7.60 (t, *J*=7.83 Hz, 1 H) 6.90 (d, *J*=7.33 Hz, 1 H) 6.77 (d, *J*=8.08 Hz, 1 H) 5.72 (br. s., 1 H) 3.01 - 3.16 (m, 1 H) 1.37 (d, *J*=6.82 Hz, 6 H). LCMS (EI/CI) *m/z*: 292 [M + H].

Step 3

6-((1R,2S)-2-Aminocyclohexylamino)-4-(6-isopropylpyridin-2-ylamino)pyridazine-3-carboxamide [**17**]

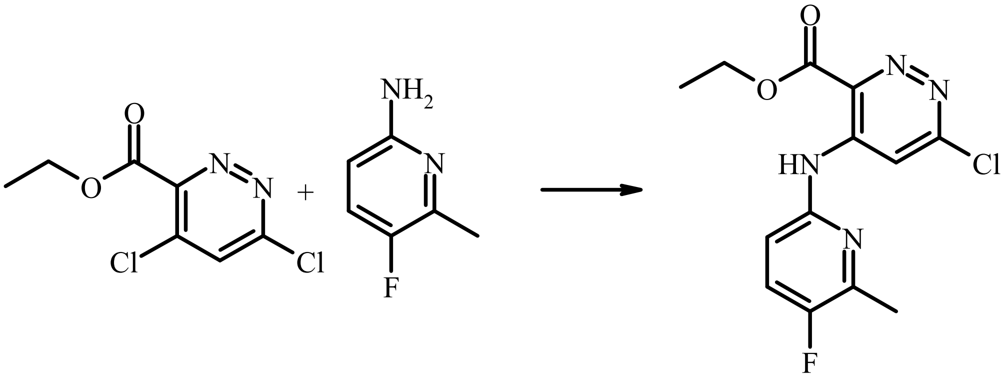


To a solution of 6-chloro-4-(6-isopropylpyridin-2-ylamino)pyridazine-3-carboxamide (102 mg, 350 μmol) in *N*-methyl-2-pyrrolidinone (6 ml) was added *tert*-butyl (1S,2R)-2-aminocyclohexylcarbamate (150 mg, 699 μmol) and the mixture heated to 150 °C for 3 d. Additional *tert*-butyl (1S,2R)-2-aminocyclohexylcarbamate (150 mg, 699 μmol) was added and the reaction heated to 150 °C for an additional 2 d, then cooled, concentrated, and purified by chromatography (silica, 50 μm, 40 g, Analogix, 96:3.8:0.2 to 84:15.2:0.8; dichloromethane:MeOH:NH_4OH) to give a mixture of *tert*-butyl (1S,2R)-2-(6-carbamoyl-5-(6-isopropylpyridin-2-ylamino)pyridazin-3-ylamino)cyclohexylcarbamate and 6-((1R,2S)-2-aminocyclohexylamino)-4-(6-isopropylpyridin-2-ylamino)pyridazine-3-carboxamide (33 mg). This mixture was dissolved in dichloromethane (2 mL) then TFA (370 mg, 250 μL, 3.24 mmol) was added. After 18 h, the mixture was concentrated *in vacuo* and the residue obtained was purified by chromatography (spherical silica, 20-45 μm, 25 g, Versaflash Supelco, 96:3.8:0.2 to 84:15.2:0.8 dichloromethane:MeOH:NH_4OH, 25 min gradient to afford **17** (12 mg, 9 %) as a brown solid. ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 11.35 (br. s., 1 H) 8.33 (s, 1 H) 7.96 (br. s., 1 H) 7.44 (t, *J*=7.83 Hz, 1 H) 6.70 (d, *J*=7.58 Hz, 1 H) 6.61 (d, *J*=8.08 Hz, 1 H) 5.61 (br. s., 1 H) 5.31 (br. s., 1 H) 3.75 (br. s., 1 H) 3.19 (d, *J*=3.28 Hz, 1 H) 2.89 - 3.01 (m, 1 H) 1.32 - 1.80 (m, 8 H) 1.27 (d, *J*=6.82 Hz, 6 H). LCMS (EI/CI) *m/z*: 370 [M + H].

6-((1R,2S)-2-Aminocyclohexylamino)-4-(5-fluoro-6-methylpyridin-2-ylamino pyridazine-3-carboxamide [**18**]

Step 1

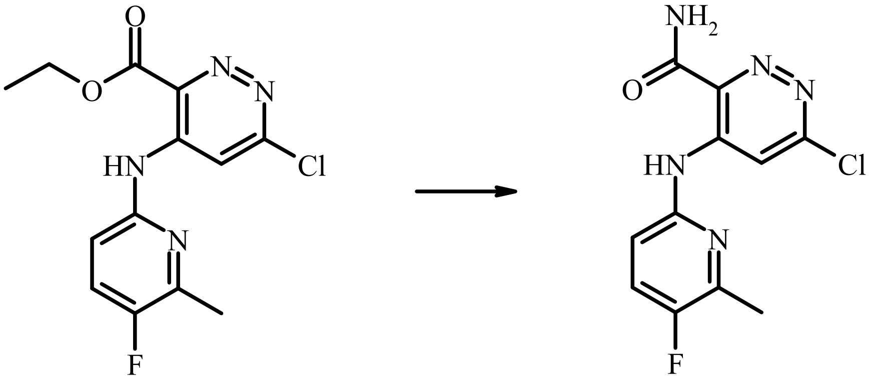
Ethyl 6-chloro-4-(5-fluoro-6-methylpyridin-2-ylamino) pyridazine-3-carboxylate



A pressure tube was charged with ethyl 4,6-dichloropyridazine-3-carboxylate (300 mg, 1.36 mmol), 5-fluoro-6-methylpyridin-2-amine (257 mg, 2.04 mmol), and acetonitrile (8 mL) and then heated at 140 °C for 3 d. After cooling to room temperature the reaction mixture was concentrated *in vacuo* and the residue purified by chromatography (silica, 50 μm, 80 g, Analogix, 0 to 10 % acetone in dichloromethane, 25 min) to afford ethyl 6-chloro-4-(5-fluoro-6-methylpyridin-2-ylamino)pyridazine-3-carboxylate (69 mg, 16 %) as a yellow solid. ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 10.72 (br. s., 1 H) 9.06 (s, 1 H) 7.38 (s, 1 H) 6.74 - 6.85 (m, 1 H) 4.58 (q, *J*=7.07 Hz, 2 H) 2.56 (d, *J*=3.03 Hz, 3 H) 1.53 (t, *J*=7.07 Hz, 3 H) 1.27 (s, 1 H). LCMS (EI/CI) *m/z*: 311 [M + H].

Step 2

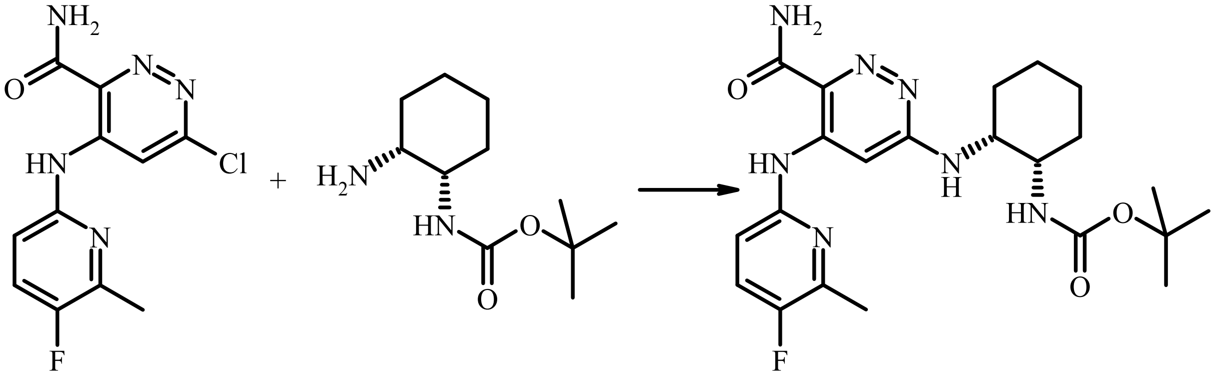
6-Chloro-4-(5-fluoro-6-methylpyridin-2-ylamino) pyridazine-3-carboxamide



Ethyl 6-chloro-4-(5-fluoro-6-methylpyridin-2-ylamino) pyridazine-3-carboxylate (111.2 mg, 358 μmol) was added to a pressure tube containing ammonia 7M in methanol (9.44 g, 12 mL, 84.0 mmol). The reaction mixture was heated to 50 ^{o}C for 6 h, then was concentrated *in vacuo* to give 6-chloro-4-(5-fluoro-6-methylpyridin-2-ylamino) pyridazine-3-carboxamide (110 mg 100 %) as a yellow solid. ^{1}H NMR (300 MHz, CHLOROFORM-*d*) δ ppm 11.67 (br. s., 1 H) 9.10 (s, 1 H) 8.17 (br. s., 1 H) 7.35 (t, *J*=8.50 Hz, 1 H) 6.79 (dd, *J*=8.69, 3.02 Hz, 1 H) 5.67 (br. s., 1 H) 2.54 (d, *J*=3.02 Hz, 3 H). LCMS (EI/CI) *m/z*: 282 [M + H]. The crude product was used in the next step without further purification.

Step 3

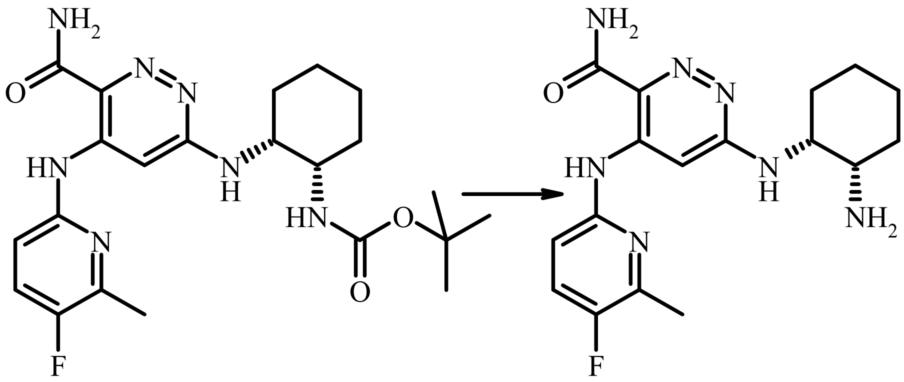
tert-Butyl (1S,2R)-2-(6-carbamoyl-5-(5-fluoro-6-methylpyridin-2-ylamino)pyridazin-3-ylamino)cyclohexylcarbamate



To a solution of 6-chloro-4-(5-fluoro-6-methylpyridin-2-ylamino) pyridazine-3-carboxamide (109 mg, 387 µmol) in *N*-methyl-2-pyrrolidinone (2.4 mL) was added DMAP (50.7 mg, 406 µmol) and *tert*-butyl (1S, 2R)-2-aminocyclohexylcarbamate (166 mg, 774 µmol) and the mixture was heated to 150 °C for 1.5 d. A steam of N_2 was blown into the mixture while still heating at 140 °C to evaporate the NMP, then the reaction mixture was purified by chromatography (silica, 50 µm, 40 g, Analogix, 97:2.75:0.15 to 84:15.2:0.8 dichloromethane:MeOH:NH_4OH, 30 min) to provide *tert*-butyl (1S,2R)-2-(6-carbamoyl-5-(5-fluoro-6-methylpyridin-2-ylamino)pyridazin-3-ylamino)cyclohexylcarbamate (82 mg, 23 %) as a brown oil. ^1H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 11.43 (s, 1 H) 8.24 (s, 1 H) 8.08 (br. s., 2 H) 7.22 - 7.37 (m, 1 H) 6.66 - 6.77 (m, 1 H) 5.64 (d, *J*=3.28 Hz, 1 H) 4.02 (br. s., 2 H) 2.49 (d, *J*=3.03 Hz, 3 H) 1.67 - 1.92 (m, 2 H) 1.35 - 1.65 (m, 8 H). LCMS (EI/CI) *m/z*: 460 [M + H].

Step 4

6-((1R,2S)-2-Aminocyclohexylamino)-4-(5-fluoro-6-methylpyridin-2-ylamino pyridazine-3-carboxamide [**18**]

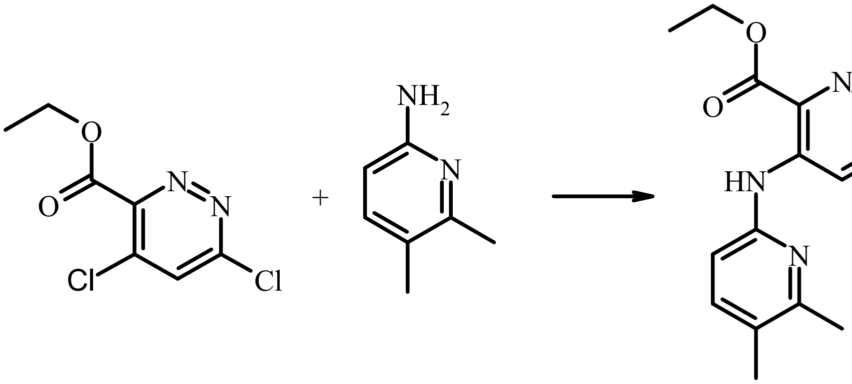


To a solution of *tert*-butyl (1S, 2R)-2-(6-carbamoyl-5-(5-fluoro-6-methylpyridin-2-ylamino) pyridazin-3-ylamino) cyclohexylcarbamate (40 mg, 87.0 µmol) in dichloromethane (3.2 mL) was added TFA (592 µg, 0.400 µL, 5.19 µmol) and the mixture was stirred at room temperature for 3 h. The mixture was concentrated *in vacuo* then purified by chromatography (spherical silica, 20-45 µm, 23 g, Versaflash from Supelco, 99:0.95:0.05 to 90:9.5:0.5 dichloromethane:MeOH:NH_4OH, 25 min) to give **18** (10 mg, 32 % yield) as a light brown solid. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 11.65 (s, 1 H) 8.38 (br. s., 1 H) 7.97 (s, 1 H) 7.54 - 7.69 (m, 2 H) 6.75 - 6.94 (m, 2 H) 3.15 (d, *J*=3.03 Hz, 1 H) 2.47 (d, *J*=2.78 Hz, 3 H) 1.44 - 1.84 (m, 8 H) 1.33 (br. s., 2 H). LCMS (EI/CI) *m/z*: 360 [M + H].

6-((1R,2S)-2-Amino-cyclohexylamino)-4-(5,6-dimethyl-pyridin-2-ylamino)-pyridazine-3-carboxylic acid amide [**19**]

Step 1

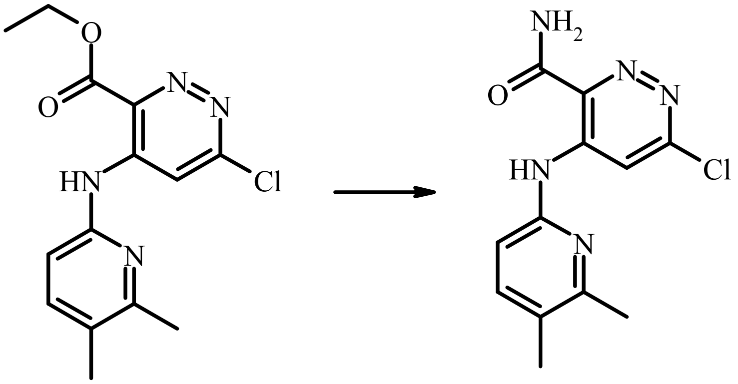
Ethyl 6-chloro-4-(5,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxylate



A heavy walled sealable tube was charged with ethyl 4,6-dichloropyridazine-3-carboxylate (300 mg, 1.36 mmol) and 5,6-dimethylpyridin-2-amine (249 mg, 2.04 mmol). To the mixture was added acetonitrile (8.00 mL) and the reaction mixture was heated with stirring in an oil bath at 140 ^{o}C for 20h. After cooling to room temperature the residue was suspended in dichloromethane and purified by flash chromatography (silica 20-45 μM, 40g,Thomson) eluting with 0 to 10% over 20 min, acetone/dichloromethane to give ethyl 6-chloro-4-(5,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxylate (195 mg, 46.8 %) as an off-white solid. ^{1}H NMR (CHLOROFORM-d) δ: 10.54 (s, 1H), 9.14 (s, 1H), 7.41 (d, J = 8.3 Hz, 1H), 6.70 (d, J = 8.3 Hz, 1H), 4.55 (q, J = 7.2 Hz, 2H), 2.50 (s, 3H), 2.26 (s, 3H), 1.50 (t, J = 7.2 Hz, 3H); LC-MS 307.0 $[M+H]^{+}$.

Step 2

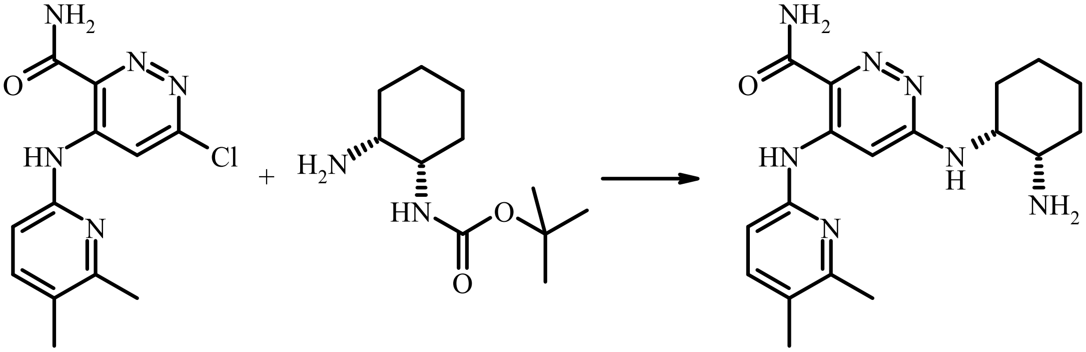
6-Chloro-4-(5,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxamide



In a 50 mL round bottom flask, ethyl 6-chloro-4-(5,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxylate (929 mg, 3.03 mmol) was suspended in ammonia 7M in methanol (7.87 g, 10.0 mL, 70.0 mmol). The flask was sealed and stirred for 2 h at room temperature. Solvents evaporated and solid residue dried in high vacuum to give 6-chloro-4-(5,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxamide (841 mg, 100 % yield) as an off-white solid. ^{1}H NMR (CHLOROFORM-d) δ: 11.37 (br. s., 1H), 9.11 (s, 1H), 8.09 (br. s., 1H), 7.32 (d, J = 8.3 Hz, 1H), 6.63 (d, J = 8.1 Hz, 1H), 5.59 (br. s., 1H), 2.43 (s, 3H), 2.19 (s, 3H); LC-MS 278.0 $[M+H]^+$.

Step 3

<u>6-((1R,2S)-2-Aminocyclohexylamino)-4-(5,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxamide [**19**]</u>

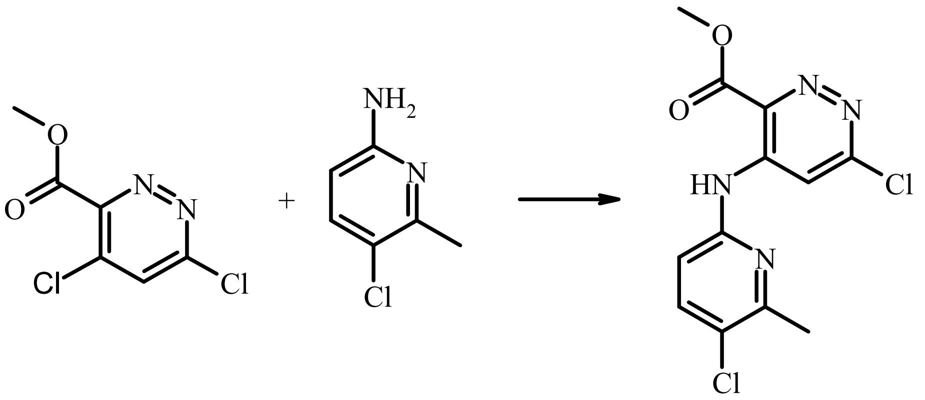


6-Chloro-4-(5,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxamide (841 mg, 3.03 mmol) was dissolved in NMP (2 mL) and to this solution was added *tert*-butyl (1S,2R)-2-aminocyclohexylcarbamate (649 mg, 3.03 mmol) and the reaction mixture heated in an oil bath with stirring at 120 ^{o}C for 24 h. A second equivalent of *tert*-butyl (1S,2R)-2-aminocyclohexylcarbamate (649 mg, 3.03 mmol) was added and reaction continued for 72 h. The mixture was cooled, then the NMP solvent was distilled off under high vacuum. The residue was dissolved in dichloromethane containing few drops of methanol and passed through a silica plug eluting with 5% of a 9:1 MeOH:NH_4OH solution in CH_2Cl_2 to yield *tert*-butyl (1S,2R)-2-(6-carbamoyl-5-(5,6-dimethylpyridin-2-ylamino)pyridazin-3-ylamino)cyclohexylcarbamate mixed with 6-chloro-4-(5,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxamide. The mixture was suspended in CH_2Cl_2 (3 mL) then TFA (1.48 g, 13.0 mmol) was added and mixture stirred at room temperature for 18 h. The mixture was concentrated in vacuo and the residue obtained was purified by chromatography (spherical silica 20-45 uM, 50g, Versaflash Supelco, 0 to 10 % of a 9:1 MeOH:NH_4OH solution in CH_2Cl_2, 20 min) to give **19** (550 mg, 49 % yield over two steps) as an off-white solid. ^{1}H NMR (DMSO-d_6) δ: 11.48 (s, 1H), 8.36 (br. s., 1H), 8.05 (s, 1H), 7.60 (br. s., 1H), 7.47 (d, J = 8.1 Hz, 1H), 6.80 (d, J = 8.1 Hz, 1H), 6.73 (d, J = 8.1 Hz, 1H), 3.87 (br. s., 1H), 3.15 (br. s., 1H), 2.45 (s, 3H), 2.20 (s, 3H), 1.44 - 1.79 (m, 8H), 1.32 (br. s., 2H); LC-MS 356.1 $[M+H]^+$.

6-((1R,2S)-2-Amino-cyclohexylamino)-4-(5-chloro-6-methyl-pyridin-2-ylamino)-pyridazine-3-carboxylic acid amide [**20**]

Step 1

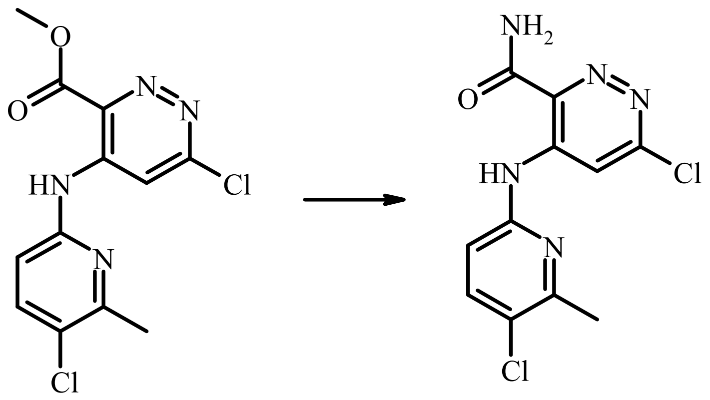
Methyl 6-chloro-4-(5-chloro-6-methylpyridin-2-ylamino)pyridazine-3-carboxylate



A pressure tube was charged with methyl 4,6-dichloropyridazine-3-carboxylate (2.00 g, 9.66 mmol) and 5-chloro-6-methylpyridin-2-amine (2.76 g, 19.3 mmol). To the mixture was added acetonitrile (12.0 mLl) and the reaction mixture heated with stirring at 130 °C for 1.5 days. After cooling to room temperature, the acetonitrile was removed *in vacuo*. The residue obtained was purified by chromatography (silica, 80 g, 50 μm from Analogix, eluting with 0% to 5% acetone in dichloromethane over 20 min., holding at 5 % for 5 min, then increasing the gradient from 5% to 10% over the next 20min) to give methyl 6-chloro-4-(5-chloro-6-methylpyridin-2-ylamino)pyridazine-3-carboxylate (618 mg, 20 %) as an orange solid. ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 10.70 (br. s., 1 H), 9.18 (s, 1 H), 7.63 (d, *J*=8.34 Hz, 1 H), 6.78 (d, *J*=8.34 Hz, 1 H), 4.12 (s, 3 H), 2.66 (s, 3 H), 1.58 (br. s., 1 H); LC-MS 313 $[M+H]^+$.

Step 2

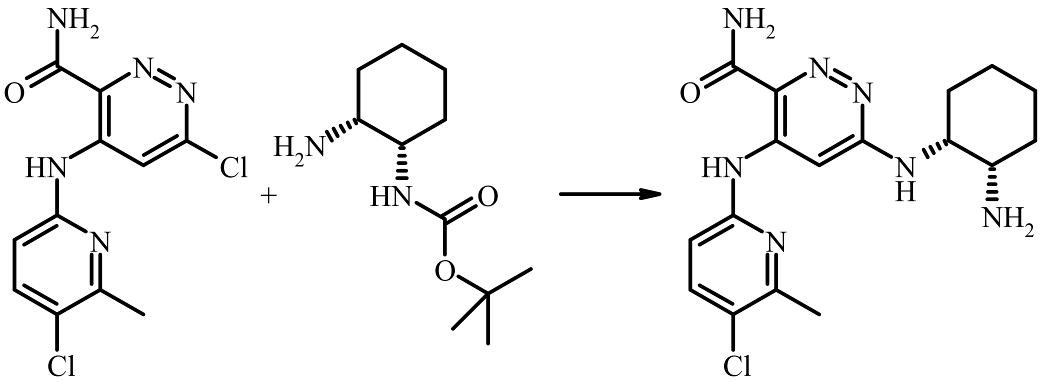
6-Chloro-4-(5-chloro-6-methylpyridin-2-ylamino)pyridazine-3-carboxamide



A pressure tube was charged with methyl 6-chloro-4-(5-chloro-6-methylpyridin-2-ylamino)pyridazine -3-carboxylate (600 mg, 1.92 mmol) and an ammonia solution in methanol (7M, 20 mL, 140 mmol). The reaction mixture was heated to 50 °C and was stirred for 18 h. The mixture was cooled and concentrated *in vacuo* to give 6-chloro-4-(5-chloro-6-methylpyridin-2-ylamino)pyridazine-3-carboxamide (665 mg crude, 116%) as a yellow solid. ^{1}H NMR (400 MHz, CHLOROFORM-*d*) d ppm 11.69 (br. s., 1 H), 9.18 (s, 1 H), 8.20 (br. s., 1 H), 7.61 (d, *J*=8.59 Hz, 1 H), 6.77 (d, *J*=8.59 Hz, 1 H), 5.71 (br. s., 1 H), 2.65 (s, 3 H). LC-MS 298 $[M+H]^+$. This material was used directly in the next step without further purification.

Step 3

6-((1R,2S)-2-Aminocyclohexylamino)-4-(5-chloro-6-methylpyridin-2-ylamino)pyridazine-3-carboxamide [**20**]

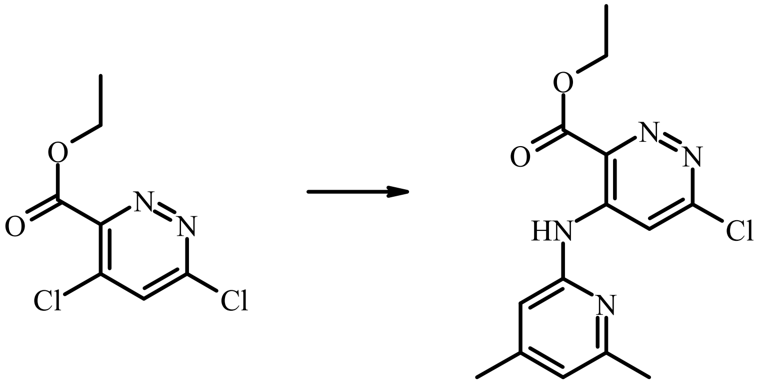


A pressure tube was charged with 6-chloro-4-(5-chloro-6-methylpyridin-2-ylamino)pyridazine-3-carboxamide (600 mg, 2.01 mmol), *tert*-butyl (1S,2R)-2-aminocyclohexylcarbamate (863 mg, 4.03 mmol) and NMP (6 mL) to give a yellow suspension. The reaction mixture was stirred at 140 °C for 2.5 days. After cooling to room temperature, the NMP was distilled off using a Kugelrohr to afford a brown, viscous oil. This crude material was dissolved in dichloromethane and methanol and adsorbed onto silica gel. Purification by chromatography (spherical silica 20-45 µm, 50 g, Versaflash column from Supelco, eluting from 100% dichloromethane to 84:15.2:0.8 dichloromethane/methanol/NH_4OH), to give *tert-b*utyl (1S,2R)-2-(6-carbamoyl-5-(5-chloro-6-methylpyridin-2-ylamino)pyridazin-3-ylamino)cyclohexylcarbamate (242.3 mg) as a brown solid. The product was then dissolved in dichloromethane (3 mL) and TFA (2.96 g, 2 mL, 26.0 mmol) was added. The mixture was stirred at room temperature for 2 h. The excess TFA and the dichloromethane were removed *in vacuo* and the residue obtained was purified by chromatography (spherical silica 20-45 µm, 50 g, Versaflash from Supelco, eluting with a gradient of 0.05:0.95:99 NH_4OH:methanol:dichloromethane up to 0.6:11.4:88 NH_4OH:methanol:dichloromethane over 40 min) to give [**20**] (152 mg, 4 %) as a light brown solid. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 11.77 (br. s., 1 H), 8.42 (br. s., 1 H), 8.05 (br. s., 1 H), 7.59 - 7.80 (m, 2 H), 6.78 - 6.99 (m, 2 H), 3.18 (br. s., 1 H), 2.57 (br. s., 4 H), 1.21 - 1.42 (m, 2 H), 1.20 - 1.81 (m, 8 H), . LC-MS 376 $[M+H]^+$.

6-((1R,2S)-2-aminocyclohexylamino)-4-(4,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxamide [**21**]

Step 1

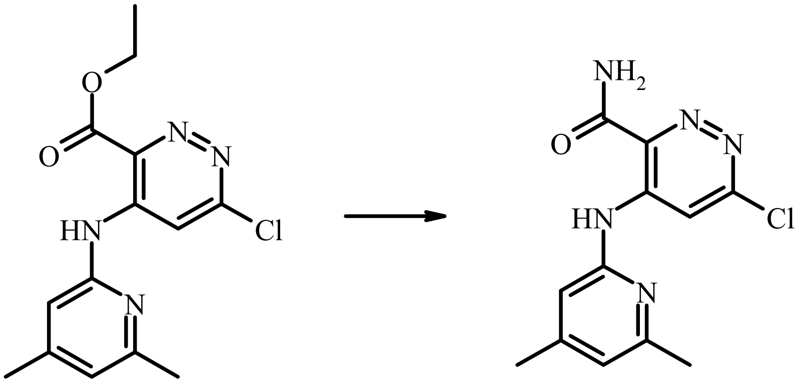
Ethyl 6-chloro-4-(4,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxylate



To a solution of ethyl 4,6-dichloropyridazine-3-carboxylate (790 mg, 3.57 mmol) in acetonitrile (11.9 mL) was added 4,6-dimethylpyridin-2-amine (873 mg, 7.15 mmol) and heated at 140°C in a sealed vial for 20 h. The mixture was concentrated *in vacuo* then purified by chromatography (silica, 0-8 % acetone in dichloromethane) to give ethyl 6-chloro-4-(4,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxylate (165 mg, 538 µmol, 15 %) as an off-white solid. ^{1}H NMR (400 MHz, CHLOROFORM-d) δ ppm 10.60 (s, 1 H), 9.24 (s, 1 H), 8.75 (s, 1 H), 8.59 (s, 1 H), 4.58 (q, *J*=7.1 Hz, 2 H), 2.53 (s, 3 H), 2.33 (s, 3 H), 1.52 (t, *J*=7.1 Hz, 3 H); MS (EI/CI) *m/z*: 306.1 [M + H].

Step 2

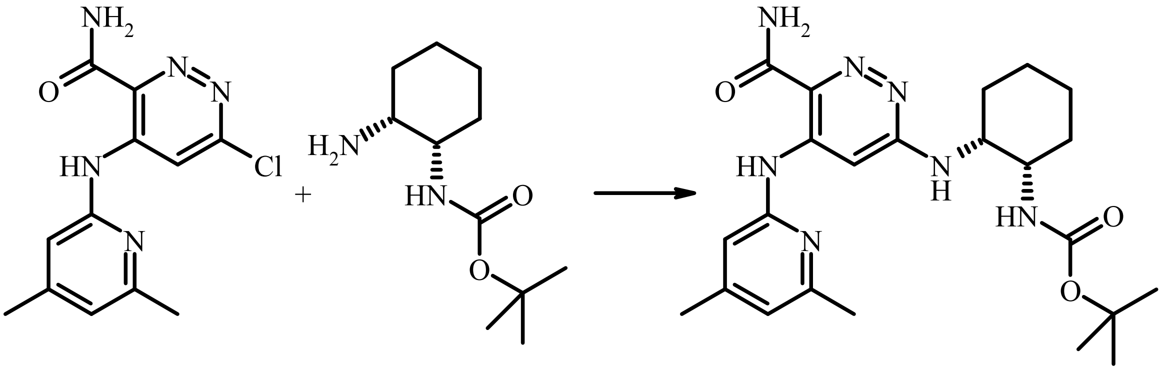
6-Chloro-4-(4,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxamide



To a solution of ethyl 6-chloro-4-(4,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxylate (165 mg, 538 µmol) in methanol (1 mL) was added ammonia in methanol (4.72 g, 6 mL, 42.0 mmol) and the mixture stirred at 50°C for 16 h. The mixture was then concentrated *in vacuo* to give 6-chloro-4-(4,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxamide (140 mg, 504 µmol, 94 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-*d*) δ ppm 11.92 (s, 1 H), 9.13 (s, 1 H), 8.86 (s, 1 H), 8.21 (s, 1 H), 6.84 (s, 1 H), 6.76 (s, 1 H), 2.45 (s, 3 H), 2.29 (s, 3 H); MS (EI/CI) *m/z*: 277.9 [M + H].

Step 3

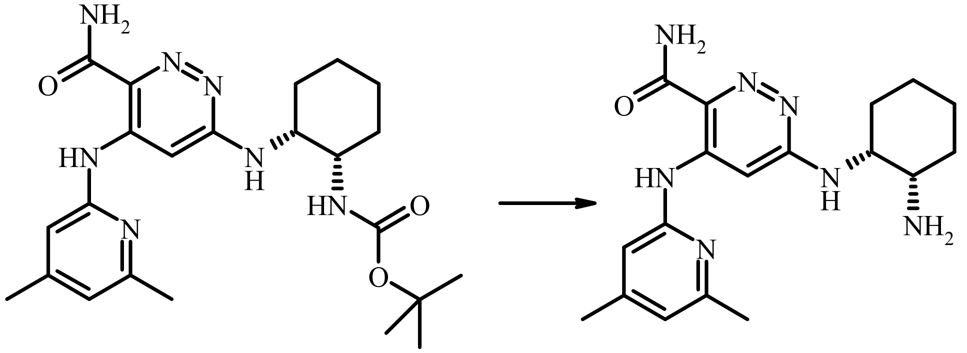
tert-Butyl-(1S,2R)-2-(6-carbamoyl-5-(4,6-dimethylpyridin-2-ylamino)pyridazin-3-ylamino)cyclohexylcarbamate



To a solution of 6-chloro-4-(4,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxamide (140 mg, 504 μmol) in NMP (2.52 mL) was added *tert*-butyl (1S,2R)-2-aminocyclohexylcarbamate (216 mg, 1.01 mmol) and the mixture heated at 130°C for 72 h. The reaction mixture was cooled and diluted with ethyl acetate and brine. The organic phase was washed with brine (2x) and water (1x), then the organic phase was concentrated *in vacuo* then purified by chromatography (silica, 40-100 % ethyl acetate in hexanes) to give *tert*-butyl (1S,2R)-2-(6-carbamoyl-5-(4,6-dimethylpyridin-2-ylamino)pyridazin-3-ylamino)cyclohexylcarbamate (75 mg, 98.8 μmol, 20 %) as an orange solid. MS (EI/CI) *m/z*: 456.2 [M + H].

Step 4

6-((1R,2S)-2-aminocyclohexylamino)-4-(4,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxamide 2,2,2-trifluoroacetate

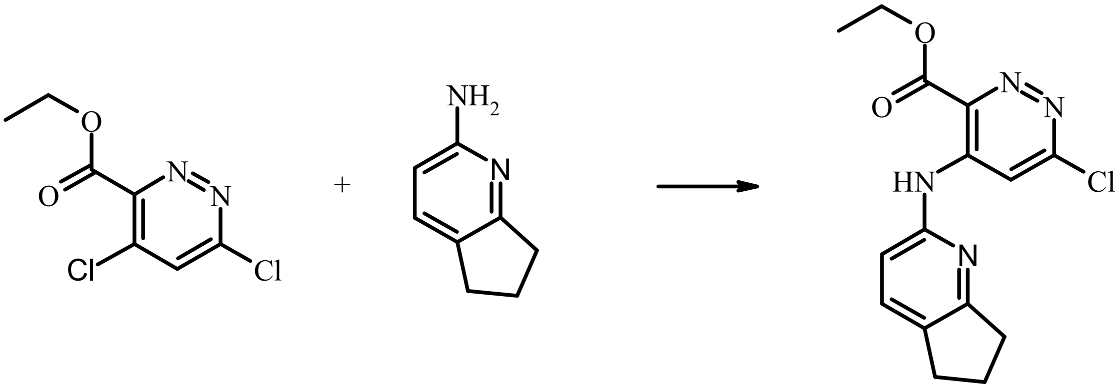


To a solution of *tert*-butyl (1S,2R)-2-(6-carbamoyl-5-(4,6-dimethylpyridin-2-ylamino)pyridazin-3-ylamino)cyclohexylcarbamate (75 mg, 165 μmol) in dichloromethane (5 mL) was added trifluoroacetic acid (1.88 g, 1.27 mL, 16.5 mmol) and the mixture stirred at room temperature for 16 h. The mixture was concentrated and then the residue was diluted with dichloromethane and neutralized with NH_4OH. Purification by chromatography (silica, 0-100 % of a 10:0.5:89.5 methanol:NH_4OH:dichloromethane in dichloromethane) followed by further purification by HPLC (C-18, 10-100% water in acetonitrile gradient) gave 6-((1R,2S)-2-aminocyclohexylamino)-4-(4,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxamide 2,2,2-trifluoroacetate (33 mg, 70.3 μmol, 43 %) as a fluffy off white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.66 (s, 1 H), 8.38 (s, 1 H), 8.25 (s, 1 H), 7.86 (br. s, 1 H), 7.40 (br. s, 2 H), 6.76 (s, 1 H), 6.65 (s, 1 H), 4.40 (s., 1 H), 3.63 (s, 1 H), 2.48 (s, 3 H), 1.58 - 1.86 (m, 8 H), 1.46 (m, 2 H); MS (EI/CI) *m/z*: 356.1 [M + H].

6-((1R,2S)-2-aminocyclohexylamino)-4-(6,7-dihydro-5H-cyclopenta[b]pyridin-2-ylamino)pyridazine-3-carboxamide [**22**]

Step 1

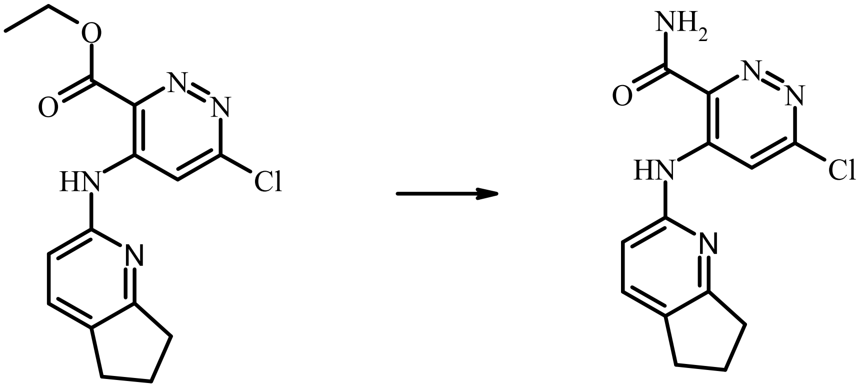
Ethyl 6-chloro-4-(6,7-dihydro-5H-cyclopenta[b]pyridin-2-ylamino)pyridazine-3-carboxylate



A heavy walled resealable tube was charged with ethyl 4,6-dichloropyridazine-3-carboxylate (1.5 g, 6.79 mmol), 6,7-dihydro-5H-cyclopenta[b]pyridin-2-amine (1.09 g, 8.14 mmol), acetonitrile (10 mL) and Hunig's base (877 mg, 1.19 mL, 6.79 mmol). The mixture was heated at 80 °C with stirring for 3d, after cooling to room temperature, the solvents were distilled off and the crude residue obtained was purified by chromatography (80g column, 50 μM from Thomson, 0 to 10 % acetone in dichloromethane, 20 min) to give ethyl 6-chloro-4-(6,7-dihydro-5H-cyclopenta[b]pyridin-2-ylamino)pyridazine-3-carboxylate (554 mg, 26 %) as a light brown solid. ^{1}H NMR (CHLOROFORM-d) δ: 10.51 (s, 1H), 8.94 (s, 1H), 7.41 (d, J = 8.3 Hz, 1H), 6.60 (d, J = 8.3 Hz, 1H), 4.46 (q, J = 7.2 Hz, 2H), 2.92 (t, J = 7.7 Hz, 2H), 2.83 (t, J = 7.4 Hz, 2H), 1.87 - 2.24 (m, 2H), 1.42 (t, J = 7.2 Hz, 3H); LC-MS 319.0 $[M+H]^+$.

Step 2

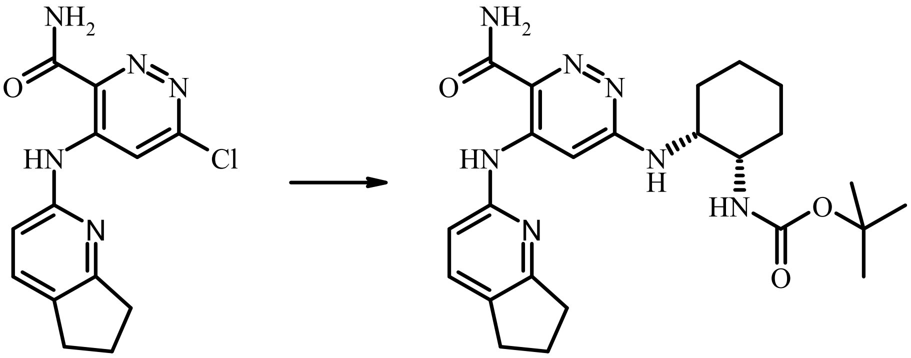
6-Chloro-4-(6,7-dihydro-5H-cyclopenta[b]pyridin-2-ylamino)pyridazine-3-carboxamide



Ethyl 6-chloro-4-(6,7-dihydro-5H-cyclopenta[b]pyridin-2-ylamino)pyridazine-3-carboxylate (554 mg, 1.74 mmol) was suspended in ammonia 7M in methanol (11.8 g, 15.0 mL, 105 mmol). The flask containing the mixture was sealed and stirred at room temperature for 18 h. The precipitated off-white solid was separated by filtration and dried under high vacuum to give 6-chloro-4-(6,7-dihydro-5H-cyclopenta[b]pyridin-2-ylamino)pyridazine-3-carboxamide (375 mg, 75 %) as an off-white solid. ^{1}H NMR (DMSO-d_6) δ: 11.92 (s, 1H), 8.95 (s, 1H), 8.83 (s, 1H), 8.18 (s, 1H), 7.63 (d, J = 7.9 Hz, 1H), 6.84 (d, J = 7.9 Hz, 1H), 2.78 - 3.00 (m, 4H), 2.07 (quin, J = 7.6 Hz, 2H); LC-MS 290.0 $[M+H]^+$.

Step 3

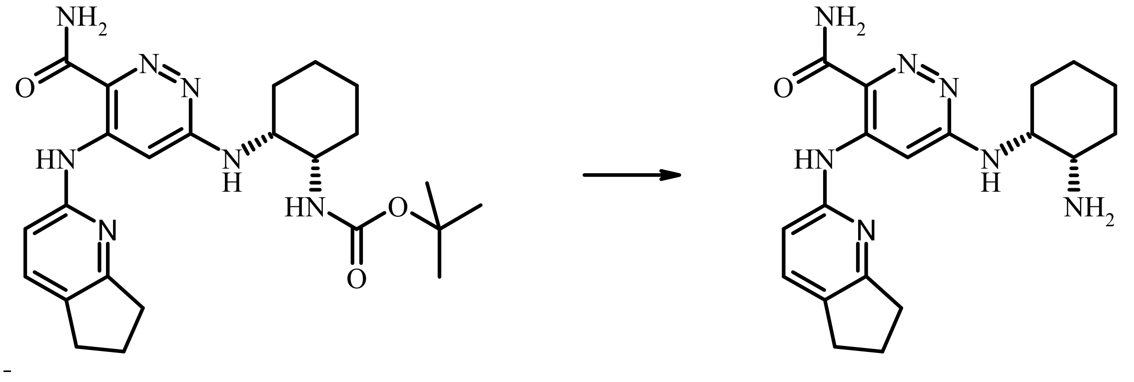
tert-Butyl (1S,2R)-2-(6-carbamoyl-5-(6,7-dihydro-5H-cyclopenta[b]pyridin-2-ylamino)pyridazin-3-ylamino)cyclohexylcarbamate



A solution of 6-chloro-4-(6,7-dihydro-5H-cyclopenta[b]pyridin-2-ylamino)pyridazine-3-carboxamide (375 mg, 1.29 mmol) and tert-butyl (1S,2R)-2-aminocyclohexylcarbamate (555 mg, 2.59 mmol) in N-methyl-2-pyrrolidinone (3 mL) was treated with *tert*-butyl (1S,2R)-2-aminocyclohexylcarbamate (555 mg, 2.59 mmol), The reaction mixture was heated at 120 °C for 72 h, then cooled to room temperature, concentrated under high vacuum and the residue obtained was purified by chromatography (spherical silica 20-45 µM, 80g, Versaflash Supelco, 0 to 5% of a 9:1 MeOH:NH_4OH solution in CH_2Cl_2, 20 min) to give *tert*-butyl (1S,2R)-2-(6-carbamoyl-5-(6,7-dihydro-5H-cyclopenta[b]pyridin-2-ylamino)pyridazin-3-ylamino)cyclohexylcarbamate (444 mg, 73 %) as a light brown foam. ^1H NMR (CHLOROFORM-d) δ: 11.30 (s, 1H), 8.19 (s, 1H), 8.06 (d, J = 3.4 Hz, 1H), 7.40 (d, J = 8.3 Hz, 1H), 6.75 - 7.10 (m, 1H), 6.61 (d, J = 8.3 Hz, 1H), 5.77 - 6.14 (m, 2H), 3.99 (m, 1H), 3.55 - 3.74 (m, 1H), 2.67 - 3.05 (m, 4H), 2.11 (quin, J = 7.5 Hz, 2H), 1.18 - 2.01 (m, 17H); LC-MS 468.2 $[M+H]^+$.

Step 4

6-((1R,2S)-2-Aminocyclohexylamino)-4-(6,7-dihydro-5H-cyclopenta[b]pyridin-2-ylamino)pyridazine-3-carboxamide [**22**]



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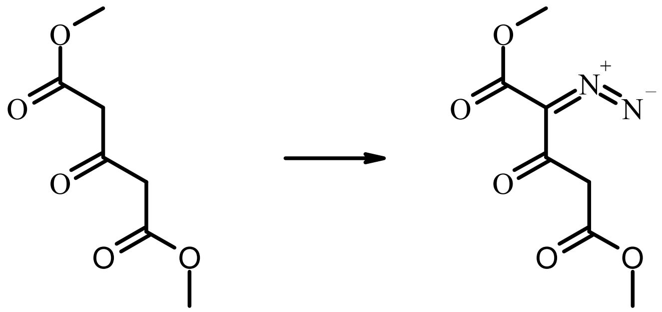
A solution of *tert*-butyl (1S,2R)-2-(6-carbamoyl-5-(6,7-dihydro-5H-cyclopenta[b]pyridin-2-ylamino)pyridazin-3-ylamino)cyclohexylcarbamate (444 mg, 950 µmol) in CH_2Cl_2 (5 mL) was treated with TFA (4.08 g, 2.76 mL, 35.8 mmol) and the mixture stirred at room temperature for 18 h. The solvents were evaporated and the residue purified by flash chromatography (spherical silica 20-45 μM, 80g, Versaflash Supelco) eluting with 0 to 5 % of a 9:1 MeOH:NH_4OH solution in CH_2Cl_2, 15 min) to give **22** (80 mg, 23 %) as an off-white solid. ^1H NMR (DMSO-d_6) δ: 11.54 (br. s., 1H), 8.35 (br. s., 1H), 7.95 (br. s., 1H), 7.46 - 7.69 (m, 2H), 6.80 (d, J = 7.2 Hz, 1H), 6.69 (d, J = 7.6 Hz, 1H), 3.89 (br. s., 1H), 3.12 (br. s., 1H), 2.75 - 2.98 (m, 4H), 1.96 - 2.17 (m, 2H), 1.61 (d, J = 11.7 Hz, 8H), 1.31 (br. s., 2H); LC-MS 368.3 $[M+H]^+$.

6-((1R,2S)-2-Amino-cyclohexylamino)-4-(5-chloro-6-methyl-pyridin-2-ylamino)-pyridazine-3-carboxylic acid amide [**23**]

Step 1

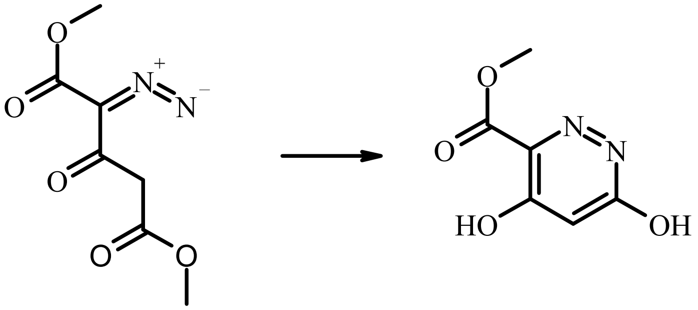
2-Diazo-3-oxo-pentanedioic acid dimethyl ester



To a stirred solution of dimethyl 3-oxopentanedioate (50.0 g, 287.1 mmol) and triethylamine (47.8 mL, 344.5 mmol) in acetonitrile (1.2 L) at 0°C was added 4-acetamidobenzene sulfonyl azide (69.0 g, 287.1 mmol) portion wise. After addition was completed, the reaction mixture was stirred at room tempertature for 1 h (silica TLC; ethyl acetate: hexane = 1: 4, Rf = 0.3; showed KMnO4 activity). The reaction mixture was filtered, and the filtrate was concentrated to get a viscous mass, which was diluted with n-hexane (3 L). The unwanted solid byproduct was removed by filtration then filtrate was concentrated under reduced pressure to give 2-diazo-3-oxo-pentanedioic acid dimethyl ester (50.0 g, 87 % crude yield) as a light yellow liquid, which was directly used for next step without further purification.

Step 2

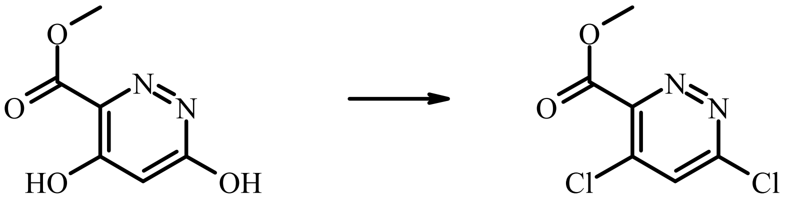
4,6-Dihydroxy-pyridazine-3-carboxylic acid methyl ester



A mixture of 2-diazo-3-oxo-pentanedioic acid dimethyl ester (50.0 g, 249.8 mmol) and triphenylphosphine (65.5 g, 249.8 mmol) in diethyl ether (500 mL) was stirred at room temperature for 24 h. The organic solvent was removed under vacuum and then acetic acid (500 mL) and water (50 mL) were added to the residue and the mixture was refluxed for 10 h. The reaction mixture was concentrated under reduced pressure to obtain a viscous residue. Trituration with ethyl acetate generated a yellow solid that was purified by chromatography (silica, 100-200 mesh, 1-5 % methanol in dichloromethane) to give 4,6-dihydroxy-pyridazine-3-carboxylic acid methyl ester (12.8 g, 30 %) as a yellow solid. LC-MS 169.2 $[M+H]^+$.

Step 3

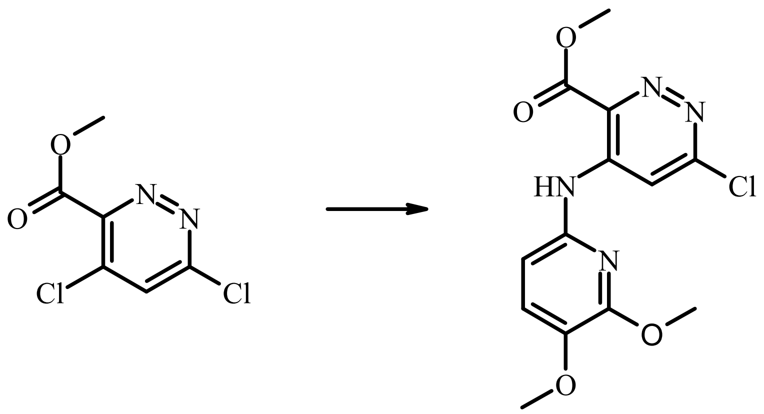
4,6-Dichloro-pyridazine-3-carboxylic acid methyl ester



A mixture of 4,6-dihydroxy-pyridazine-3-carboxylic acid methyl ester (10.5 g, 61.7 mmol) and $POCl_3$ (70 mL) was heated to 95°C for 5 h. The excess $POCl_3$ was removed under reduced pressure, then the crude residue was added to ice-water (250 mL) and extracted with ethyl acetate (3 x 100 mL). The combined extracts were dried then concentrated to give a crude residue which was purified by chromatography (silica, 100-200 mesh, 30 % ethyl acetate in hexane) to give 4,6-dichloro-pyridazine-3-carboxylic acid methyl ester (9.2 g, 72 %) as an off white solid. LC-MS: 207.0 $[M+H]^+$.

Step 4

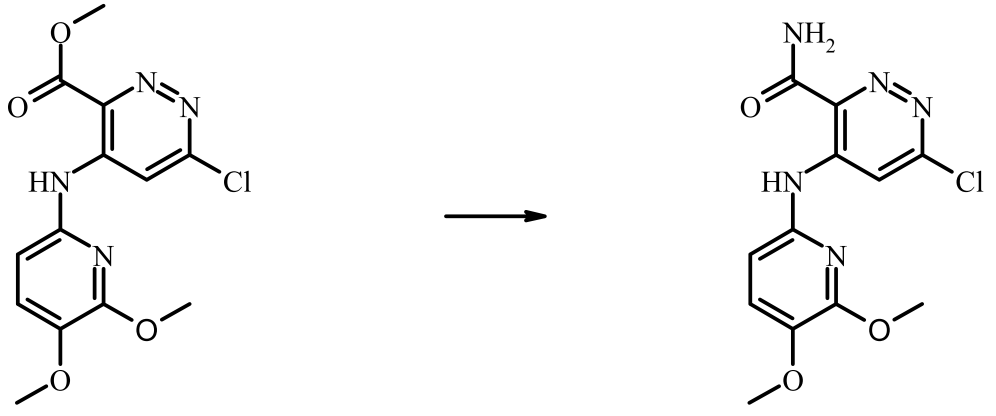
6-Chloro-4-(5,6-dimethoxy-pyridin-2-ylamino)-pyridazine-3-carboxylic acid methyl ester



A mixture of 4,6-dichloro-pyridazine-3-carboxylic acid methyl ester (1.0 g, 4.83 mmol) and 5,6-dimethoxypyridin-2-amine (968 mg, 6.28 mmol) was dissolved in acetonitrile (5 mL) and heated at 70°C for 16 h. The mixture was concentrated *in vacuo* and the crude mass obtained was purified by chromatography (silica, 100-200 mesh, 10-70 % ethyl acetate in hexane) to give 6-chloro-4-(5,6-dimethoxy-pyridin-2-ylamino)-pyridazine-3-carboxylic acid methyl ester (830 mg, 53 %) as a yellow solid. LC-MS: 325.2 $[M+H]^+$.

Step 5

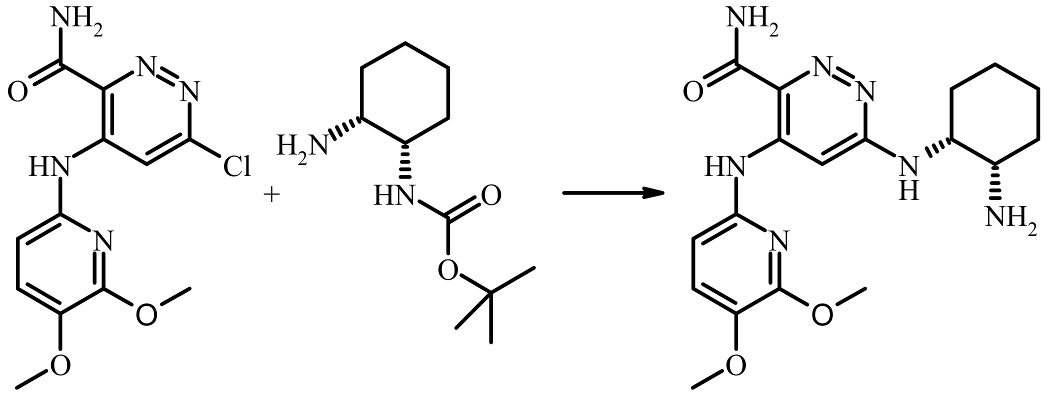
6-Chloro-4-(5,6-dimethoxy-pyridin-2-ylamino)-pyridazine-3-carboxylic acid amide



6-Chloro-4-(5,6-dimethoxy-pyridin-2-ylamino)-pyridazine-3-carboxylic acid methyl ester (1.7 g, 5.2 mmol) was suspended in ammonia in methanol (7N, 30.0 mL, 210 mmol) and the flask sealed. After stirring for 6 h at room temperature the mixture was concentrated *in vacuo* to give 6-chloro-4-(5,6-dimethoxy-pyridin-2-ylamino)-pyridazine-3-carboxylic acid amide (1.55 g, 96 %) as a yellow powder. LC-MS: 310.4 $[M+H]^+$.

Step 6

6-((1R,2S)-2-Aminocyclohexylamino)-4-(5,6-dimethoxypyridin-2-ylamino)pyridazine-3-carboxamide [**23**]



Method A

A pressure tube was charged with 6-chloro-4-(5,6-dimethoxypyridin-2-ylamino)pyridazine-3-carboxamide (310 mg, 1.00 mmol), *tert*-butyl (1S,2R)-2-aminocyclohexylcarbamate (429 mg, 2.00 mmol) and NMP (4 mL). The reaction mixture was stirred at 140 °C for 18 h, then the NMP was distilled using a Kugelrohr under high vacuum and at 120 °C to afford a brown viscous oil. The crude oil was dissolved in dichloromethane and methanol then adsorbed onto silica and purified by chromatography (spherical silica 20-45 μm, 11g, Versaflash from Supelco, eluting with 100 % dichloromethane to 88:11.4:0.6 dichloromethane:methanol:NH_4OH, 40 min) to give *tert*-butyl (1S,2R)-2-(6-carbamoyl-5-(5,6-dimethoxypyridin-2-ylamino)pyridazin-3-ylamino)cyclohexylcarbamate as a brown viscous oil (291.3 mg). This was dissolved in dichloromethane (2 mL) and TFA (740 mg, 500 μL, 6.49 mmol) was added. The reaction mixture was stirred at room temperature for 3 h. The TFA and the dichloromethane were removed *in vacuo* and the residue obtained was purified by chromatography (Spherical silica 20-45 μm, 50 g, Versaflash from Supelco, eluting with 0.1:1.9:98 NH_4OH:methanol:dichloromethane to 0.6:11.4:88 NH_4OH:methanol:dichloromethane, 40 min) to give **23** (81 mg, 21 %) as a light brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.33 (br. s., 1 H), 8.34 (br. s., 1 H), 7.59 (br. s., 1 H), 7.46 (s, 1 H), 7.35 (d, *J*=8.08 Hz, 1 H), 6.70 (d, *J*=7.83 Hz, 1 H), 6.57 (d, *J*=8.34 Hz, 1 H), 3.95 (s, 3 H), 3.76 (s, 3 H), 3.10 (br. s., 1 H), 2.03 (br. s., 2 H), 1.18 - 1.78 (m, 8 H), 0.98 - 1.12 (m, 1 H); LC-MS 388 $[M+H]^+$.

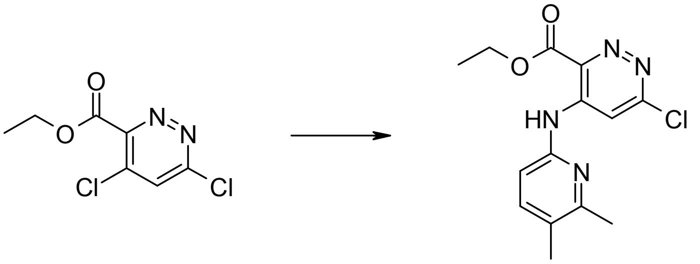
Method B

To 6-chloro-4-(5,6-dimethoxypyridin-2-ylamino)pyridazine-3-carboxamide (500 mg, 1.6 mmol) and *tert*-butyl (1S, 2R)-2-aminocyclohexylcarbamate (692 mg, 3.2 mmol) in NMP (1.5 mL), methoxy trimethylsilane (0.5 mL, 3.6 mmol) was added and the mixture heated to 150°C for 65 h. The mixture was concentrated under high vacuum to obtain a sticky crude mass that was purified by chromatography (silica, 1 – 10 % methanol in dichloromethane) to give **23** (220 mg 35 %) as a yellow solid. LC-MS 388 $[M+H]^+$.

6-(2-Aminoethylamino)-4-(5,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxamide [**24**]

Step 1

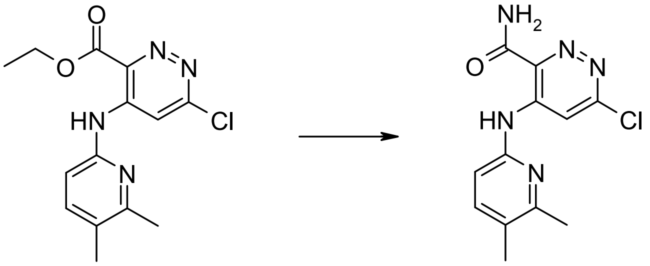
Ethyl 6-chloro-4-(5,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxylate



A pressure tube was charged with ethyl 4,6-dichloropyridazine-3-carboxylate (3.00 g, 13.6 mmol), 5,6-dimethylpyridin-2-amine (2.49 g, 20.4 mmol) and acetonitrile (8 mL), then sealed and heated with stirring in an oil bath (130 ^{o}C) for 18 h. After cooling to room temperature, the mixture was concentrated *in vacuo* and the residue obtained was purified by chromatography (silica, 0 % to 10 % acetone in dichloromethane, 20 min) to afford ethyl 6-chloro-4-(5,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxylate **(**2.45 g, 59 %) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.20 (s, 1 H); 8.86 (s, 1 H); 7.57 (d, *J*=8.08 Hz, 1 H); 6.97 (d, *J*=8.08 Hz, 1 H); 4.40 (q, *J*=7.24 Hz, 2 H); 2.42 (s, 3 H); 2.23 (s, 3 H); 1.35 (t, *J*=7.20 Hz, 3 H); LC-MS 307 [M+H]$^{+}$.

Step 2

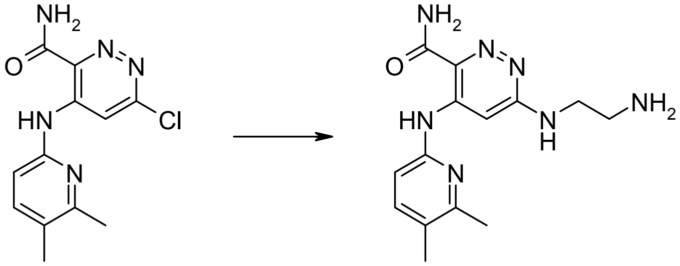
6-Chloro-4-(5,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxamide



A pressure tube was charged ethyl 6-chloro-4-(5,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxylate (2.44 g, 8.0 mmol) and 7N ammonia in MeOH (60 mL, 420 mmol). The mixture was heated with stirring in an oil bath at 50 °C for 18 h and then cooled to room temperature. The mixture was filtered to collect a white solid that was dried under high vacuum to give 6-chloro-4-(5,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxamide (2.12 g, 96 %) that was used directly in the next step without further purification. ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 11.48 (br. s., 1 H); 9.18 (s, 1 H); 8.17 (br. s., 1 H); 7.43 (d, *J*=8.34 Hz, 1 H); 6.74 (d, *J*=7.83 Hz, 1 H); 5.67 (br. s., 1 H); 2.53 (s, 3 H); 2.28 (s, 3 H); LC-MS 278 $[M+H]^+$.

Step 3

<u>6-(2-Aminoethylamino)-4-(5,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxamide [**24**]</u>



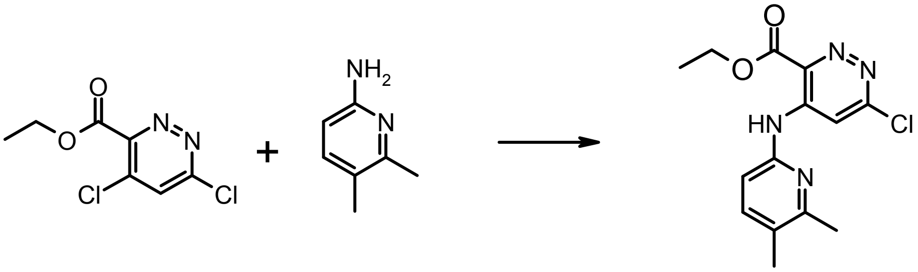
A pressure tube was charged with 6-chloro-4-(5,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxamide (150 mg, 540 μmol) and NMP (2 mL). To this solution was added ethylenediamine (325 mg, 365 μL, 5.4 mmol) and the reaction mixture was stirred at 140 °C for 1.5 h. After cooling to room temperature, the NMP and the ethylenediamine were distilled using a Kugelrohr apparatus to afford a light brown solid. This solid was purified by chromatography (spherical silica, 11g, 0.3:5.7:94 NH_4OH:MeOH:dichloromethane to 0.6:11.4:88 NH_4OH:MeOH:dichloromethane, 40 min) to give **24** (114 mg, 70 %) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ ppm 11.49 (br. s., 1 H); 8.35 (br. s., 1 H); 8.00 -

8.16 (m, 1 H); 7.61 (br. s., 1 H); 7.46 (d, *J*=7.83 Hz, 1 H); 7.20 (br. s., 1 H); 6.71 (d, *J*=8.08 Hz, 1 H); 3.36 (d, *J*=4.29 Hz, 2 H); 2.78 (t, *J*=5.81 Hz, 2 H); 2.45 (s, 3 H); 2.19 (s, 3 H); LC-MS 302 $[M+H]^+$.

6-(2-Aminoethoxy)-4-(5,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxamide [**25**]

Step 1

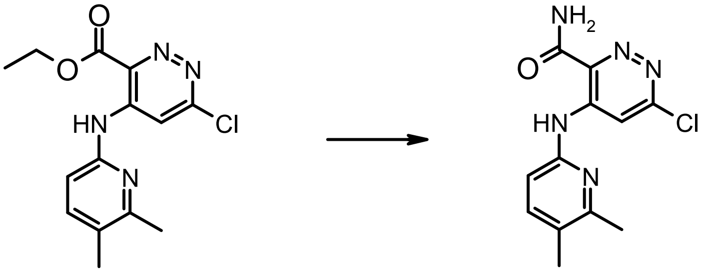
Ethyl 6-chloro-4-(5,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxylate



A pressure tube was charged with ethyl 4,6-dichloropyridazine-3-carboxylate (3000 mg, 13.6 mmol), 5,6-dimethylpyridin-2-amine (2.49 g, 20.4 mmol) and acetonitrile (8 mL). The mixture was heated with stirring in an oil bath at 130 °C for 18 h. After cooling to room temperature, the solvent was evaporated and the residue was suspended in dichloromethane, adsorbed on silica gel and then purified by flash chromatography (silica gel, 50 µm, 80 g column from Analogix, 0 to 10 % acetone in dichloromethane, 20 min) to afford ethyl 6-chloro-4-(5,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxylate **(**2.45 g, 59 %) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.20 (s, 1 H), 8.86 (s, 1 H), 7.57 (d, *J*=8.08 Hz, 1 H), 6.97 (d, *J*=8.08 Hz, 1 H), 4.40 (q, *J*=7.24 Hz, 2 H), 2.42 (s, 3 H), 2.23 (s, 3 H), 1.35 (t, *J*=7.20 Hz, 3 H); MS (EI/CI) *m/z*: 307 $[M+H]^+$.

Step 2

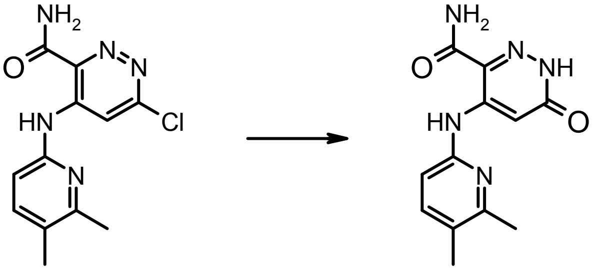
6-Chloro-4-(5,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxamide



A pressure tube was charged ethyl 6-chloro-4-(5,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxylate (2.44 g, 7.95 mmol) and ammonia in MeOH (7M, 60 mL, 420 mmol). This mixture was heated with stirring at 50 °C for 18 h. After cooling to room temperature the reaction mixture, a yellow suspension, was filtered and the white residue obtained was dried under high vacuum to give 6-chloro-4-(5,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxamide (2.12 g, 96 %). This was used in the next step without further purification. ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 11.48 (br. s., 1 H), 9.18 (s, 1 H), 8.17 (br. s., 1 H), 7.43 (d, *J*=8.34 Hz, 1 H), 6.74 (d, *J*=7.83 Hz, 1 H), 5.67 (br. s., 1 H), 2.53 (s, 3 H), 2.28 (s, 3 H); MS (EI/CI) *m/z*: 278 [M+H]$^+$.

Step 3

4-(5,6-Dimethylpyridin-2-ylamino)-6-oxo-1,6-dihydropyridazine-3-carboxamide

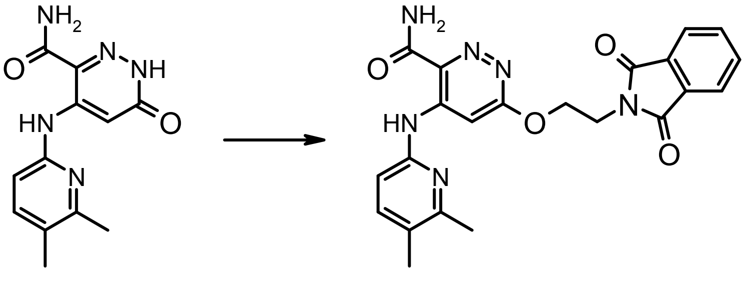


A microwave vial was charged with 6-chloro-4-(5,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxamide (185 mg, 666 μmol), AcOH (3.99 g, 3.8 mL, 66.4 mmol), sodium acetate

(82.0 mg, 0.999 mmol) and water (1.52 g, 1.52 mL, 84.4 mmol). The reaction mixture was stirred at 140 °C for 2 h in a microwave. After cooling to room temperature, the reaction mixture was concentrated in vacuo and then the crude residue purified by chromatography (spherical silica 20-45 50 µm, 11g, Versaflash from Supelco, eluting with 0.1:1.9:98 NH_4OH:MeOH:dichloromethane to 0.6:11.4:88 NH_4OH:MeOH:dichloromethane over 40 min) to give 4-(5,6-dimethylpyridin-2-ylamino)-6-oxo-1,6-dihydropyridazine-3-carboxamide (45 mg, 26 %) as a yellow solid ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.89 (s, 1 H), 11.28 (s, 1 H), 8.12 (s, 1 H), 7.93 (s, 1 H), 7.81 (s, 1 H), 7.49 (d, *J*=8.08 Hz, 1 H), 6.76 (d, *J*=8.08 Hz, 1 H), 2.38 - 2.45 (m, 3 H), 2.21 (s, 3 H); MS (EI/CI) *m/z*: 260 $[M+H]^+$.

Step 4

4-(5,6-Dimethylpyridin-2-ylamino)-6-(2-(1,3-dioxoisoindolin-2-yl)ethoxy)pyridazine-3-carboxamide

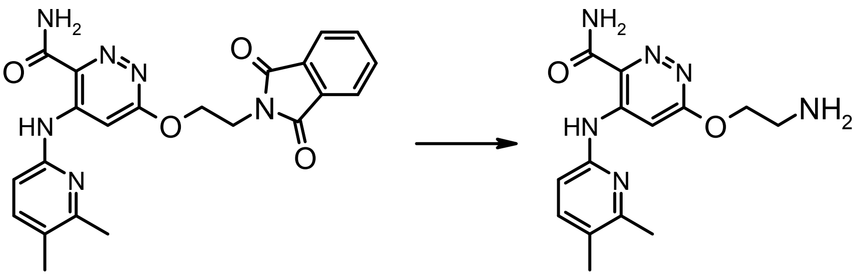


To a stirred solution of 4-(5,6-dimethylpyridin-2-ylamino)-6-oxo-1,6-dihydropyridazine-3-carboxamide (35 mg, 135 µmol) in DMF (3 mL) was added sodium hydride (6.48 mg, 270 µmol) and the reaction mixture was stirred for 30 min at room temperature. 2-(2-Bromoethyl)isoindoline-1,3-dione (51.5 mg, 202 µmol) was added and the mixture (a yellow suspension) was stirred for 16 h at room temperature. The reaction mixture was quenched with water (20 mL) and diethyl ether (20 mL). The precipitate was collected by filtration, the

the filtrates were extracted with ether. The organic extracts were combined with the collected solid and purified by chromatography (spherical silica, 11g, Versaflash from Supelco, 0 % to 10% MeOH in dichloromethane) to give 4-(5,6-dimethylpyridin-2-ylamino)-6-(2-(1,3-dioxoisoindolin-2-yl)ethoxy)pyridazine-3-carboxamide (49 mg, 84 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ ppm 11.26 - 11.33 (m, 1 H), 8.16 (br. s., 1 H), 7.83 (br. s., 4 H), 7.79 (br. s., 2 H), 7.46 - 7.54 (m, 1 H), 6.71 - 6.80 (m, 1 H), 4.28 - 4.37 (m, 2 H), 3.96 - 4.06 (m, 2 H), 2.39 (s, 3 H), 2.20 (s, 3 H); MS (EI/CI) *m/z*: 433 $[M+H]^+$.

Step 5

6-(2-Aminoethoxy)-4-(5,6-dimethylpyridin-2-ylamino)pyridazine-3-carboxamide [**25**]

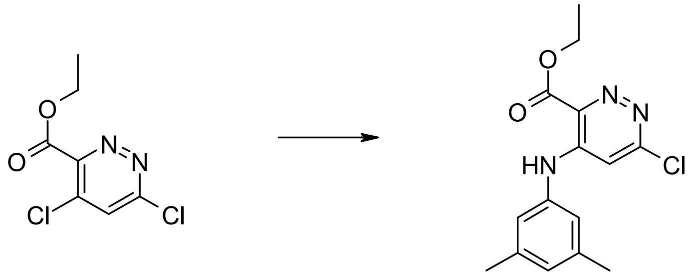


A pressure tube was charged with 4-(5,6-dimethylpyridin-2-ylamino)-6-(2-(1,3-dioxoisoindolin-2-yl)ethoxy)pyridazine-3-carboxamide (30 mg, 69.4 µmol) and EtOH (2 mL). To this solution was added hydrazine (2.67 mg, 2.61 µL, 83.3 µmol) and the reaction mixture was stirred for 3 h at room temperature, then warmed to 50 °C. After 2 h, a second portion of hydrazine (2.2 mg, 69.4 µmol) was added and the reaction mixture was stirred overnight at 40 °C. The mixture was cooled and concentrated *in vacuo* to afford a white solid. Purification by chromatography (spherical silica, 11g, Versaflash from Supelco, eluting with 0.1:1.9:98 NH_4OH:MeOH:dichloromethane to 0.6:11.4:88 NH_4OH:MeOH:dichloromethane over 40 min) gave **25** (20 mg, 10 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) d ppm 11.26 (s, 1 H), 8.24 (br. s., 1 H), 7.96 (br. s., 1 H), 7.87 (s, 1 H), 7.49 (d, *J*=8.08 Hz, 1 H), 6.77 (d, *J*=8.08 Hz, 1 H), 4.07 (t, *J*=6.44 Hz, 2 H), 2.94 (t, *J*=6.32 Hz, 2 H), 2.41 (s, 3 H), 2.21 (s, 3 H); MS (EI/CI) *m/z*: 303 $[M+H]^+$.

6-((1R,2S)-2-Aminocyclohexylamino)-4-(3,5-dimethylphenylamino)pyridazine-3-carboxamide [**26**]

Step 1

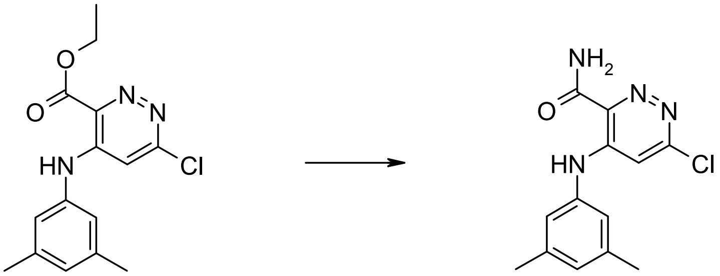
Ethyl 6-chloro-4-(3,5-dimethylphenylamino)pyridazine-3-carboxylate



To a solution of ethyl 4,6-dichloropyridazine-3-carboxylate (700 mg, 3.17 mmol) in acetonitrile (10.6 mL) was added ethyl 4,6-dichloropyridazine-3-carboxylate (700 mg, 3.17 mmol) and the mixture heated at 140°C in a sealed vial for 48 h. The mixture was cooled and then concentrated *in vacuo*, then purified by chromatography (silica, 0 to 30% acetone in dichloromethane) to give ethyl 6-chloro-4-(3,5-dimethylphenylamino)pyridazine-3-carboxylate (104 mg, 340 µmol, 11 %) as a light brown solid. ^{1}H NMR (400 MHz, CHLOROFORM-d) δ ppm 9.70 (s, 1 H), 7.03 (s, 1 H), 6.99 (s, 1 H), 6.88 (s, 2 H), 4.57 (q, J=7.6 Hz, 2 H), 2.38 (s, 6 H), 1.52 (t, J=7.6 Hz, 3 H); MS (EI/CI) *m/z*: 305.9 [M + H].

Step 2

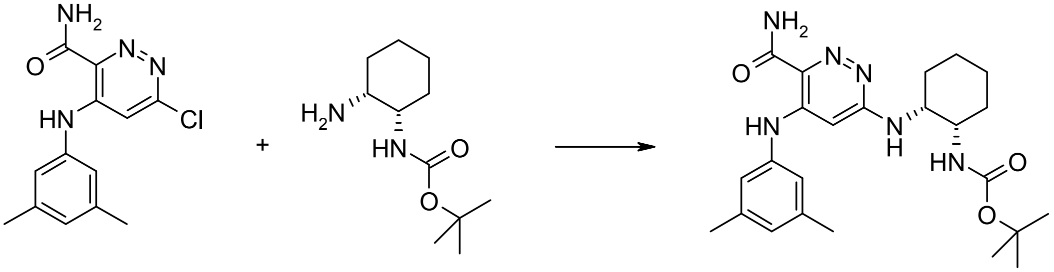
6-Chloro-4-(3,5-dimethylphenylamino)pyridazine-3-carboxamide



To a solution of ethyl 6-chloro-4-(3,5-dimethylphenylamino)pyridazine-3-carboxylate (104 mg, 340 μmol) in methanol was added ammonia in methanol (4.86 mL, 34.0 mmol) and stirred at 50 °C for 16 h. The mixture was concentrated to give 6-chloro-4-(3,5-dimethylphenylamino)pyridazine-3-carboxamide (90 mg, 325 μmol, 96 %) as a brown solid, which was used directly without purification. ^{1}H NMR (400 MHz, DMSO-*d*) δ ppm 10.84 (s, 1 H), 8.72 (s, 1 H), 8.08 (s, 1 H), 7.12 (s, 1 H), 6.98 (s, 2 H), 6.98 (s, 1 H), 6.93 (s, 1 H), 2.30 (s, 6 H); MS (EI/CI) *m/z*: 276.9 [M + H].

Step 3

tert-Butyl (1S,2R)-2-(6-carbamoyl-5-(3,5-dimethylphenylamino)pyridazin-3-ylamino) cyclohexylcarbamate

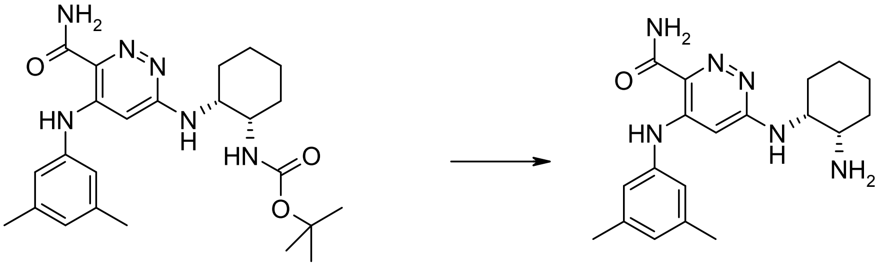


To a solution of 6-chloro-4-(3,5-dimethylphenylamino)pyridazine-3-carboxamide (44 mg, 159 μmol) in NMP (530 μL) was added *tert*-butyl (1S,2R)-2-aminocyclohexylcarbamate (68.2 mg, 318 μmol) and the mixture heated to 140 °C for 20 h. Additional *tert*-butyl

(1S,2R)-2-aminocyclohexylcarbamate (68.2 mg, 318 µmol) was then added and heating continued for a further 7 h. Finally, another portion of *tert*-butyl (1S,2R)-2-aminocyclohexylcarbamate (34.1 mg, 159 µmol) was added and the mixture heated for 16 h. The reaction mixture was cooled, and then diluted with ethyl acetate. The phases were separated and the organic phase then washed with water and brine. The organic phase was collected, concentrated *in vacuo*, and the residue obtained was purified by chromatography (silica, 30 to 70% ethyl acetate in hexanes) to give *tert*-butyl (1S,2R)-2-(6-carbamoyl-5-(3,5-dimethylphenylamino)pyridazin-3-ylamino)cyclohexylcarbamate (30 mg, 66.0 µmol, 42 %) as a brown oil. MS (EI/CI) *m/z*: 455.2 [M + H].

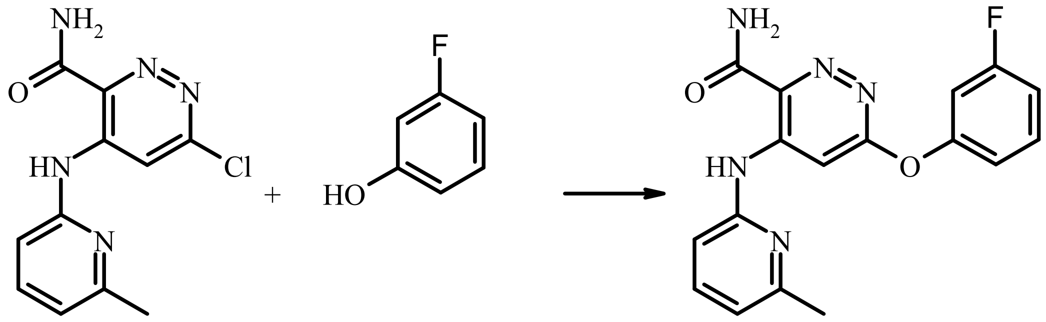
Step 4

<u>6-((1R,2S)-2-Aminocyclohexylamino)-4-(3,5-dimethylphenylamino)pyridazine-3-carboxamide</u>



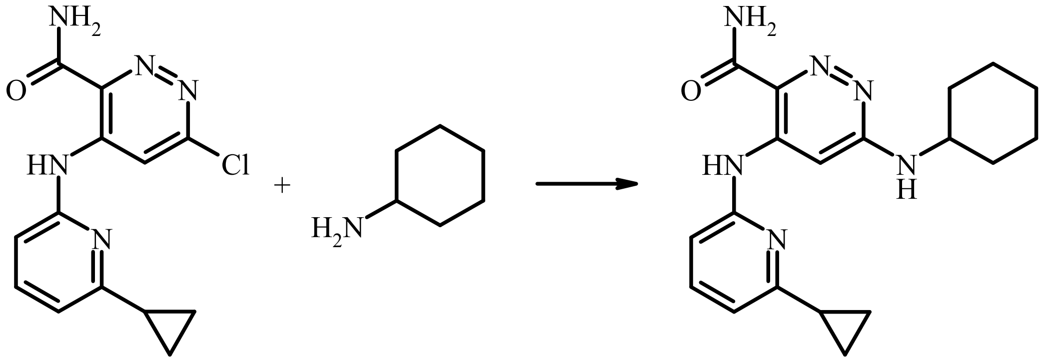
To a solution of *tert*-butyl (1S,2R)-2-(6-carbamoyl-5-(3,5-dimethylphenylamino)pyridazin-3-ylamino)cyclohexylcarbamate (30 mg, 66.0 µmol) in dichloromethane (1 mL) was added trifluoroacetic acid (151 mg, 102 µL, 1.32 mmol) and the mixture stirred at room temperature for 16 h. The mixture was concentrated *in vacuo* then diluted with dichloromethane and drops of 25 % NH_4OH added until the pH was measured to be ~8. The weakly basic solution was washed with water, then the organic phase was concentrated *in vacuo* and purified by chromatography (silica, 3 to 10% methanol in dichloromethane), to give 6-((1R,2S)-2-aminocyclohexylamino)-4-(3,5-dimethylphenylamino)pyridazine-3-carboxamide (17 mg, 48.0 µmol, 73 %) as a light brown solid. ^{1}H NMR (400 MHz, METHANOL-d_6) δ ppm 6.90 (s, 2 H), 6.88 (s, 1 H), 6.50 (s, 1 H), 4.32 (s, 1 H), 1.63 - 1.84 (m, 6 H), 1.51 (m, 2 H); MS (EI/CI) *m/z*: 355.1 [M + H].

6-[3-(3-Fluoro-phenoxy)-4-(6-methyl-pyridin-2-ylamino)-pyridazine-3-carboxamide [**27**]



A solution of 3-fluorophenol (0.021 mL, 0.23 mmol) in 1 mL of *N*,*N*-dimethylformamide was treated with 60% sodium hydride in mineral oil (9 mg, 0.2 mmol). The yellow solution was stirred for 5 min., then treated with 6-chloro-4-(6-methylpyridin-2-ylamino)pyridazine-3-carboxamide (21.1 mg, 0.0800 mmol) and the yellow solution was stirred at 90 °C for 2 d. The solution was concentrated to a yellow solid. Purification by chromatography (12 g silica gel column from Thompson, eluting from hexanes to 50% ethyl acetate/hexanes), afforded **27** (22.2 mg, 82 %) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.28 (s, 3 H) 6.86 (dd, *J*=15.49, 7.55 Hz, 2 H) 7.11 - 7.22 (m, 2 H) 7.28 (d, *J*=10.20 Hz, 1 H) 7.53 (q, *J*=7.93 Hz, 1 H) 7.64 (t, *J*=7.74 Hz, 1 H) 8.04 (br. s., 1 H) 8.67 (s, 1 H) 8.71 (br. s., 1 H) 11.84 - 11.92 (m, 1 H). MS (EI/CI) *m/z*: 340.1 [M + H].

6-(Cyclohexylamino)-4-(6-cyclopropylpyridin-2-ylamino)pyridazine-3-carboxamide [**28**]

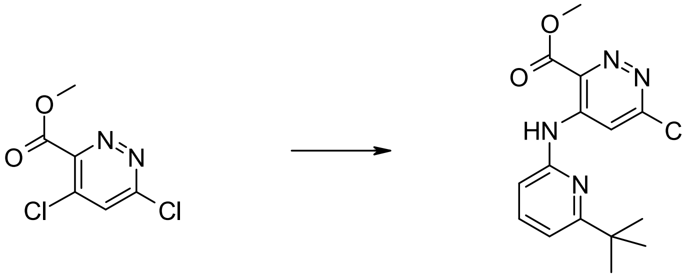


A suspension of 6-chloro-4-(6-cyclopropylpyridin-2-ylamino)pyridazine-3-carboxamide (100 mg, 345 µmol, prepared as described above) and cyclohexylamine (342 mg, 395 µL, 3.45 mmol) in N-methyl-2-pyrrolidinone (0.5 mL) was heated at 130 °C for 14 h. The mixture was cooled and concentrated under high vacuum at 120 °C to remove all residual solvent and amine starting material. The brown solid obtained was purified by chromatography (silica, 11 g spherical, 0-20 % acetone in dichloromethane, 20 min) to give **28** (81 mg, 230 µmol, 67 %) as a white solid. ^{1}H NMR (DMSO-d_6) δ: 11.72 (br. s., 1H), 8.37 (br. s., 1H), 7.83 (s, 1H), 7.62 (br. s., 1H), 7.56 (t, J = 7.7 Hz, 1H), 7.10 (d, J = 7.8 Hz, 1H), 6.88 (d, J = 7.3 Hz, 1H), 6.66 (d, J = 8.1 Hz, 1H), 3.52 - 3.69 (m, 1H), 2.04 - 2.18 (m, 1H), 1.90 - 2.00 (m, 2H), 1.72 - 1.82 (m, 2H), 1.63 (d, J = 12.6 Hz, 1H), 1.24 - 1.45 (m, 4H), 1.09 - 1.23 (m, 1H), 0.95 - 1.03 (m, 4H); MS (EI/CI) *m/z*: 353.1 [M + H].

6-((1R,2S)-2-aminocyclohexylamino)-4-(6-tert-butylpyridin-2-ylamino)pyridazine-3-carboxamide [**29**]

Step 1

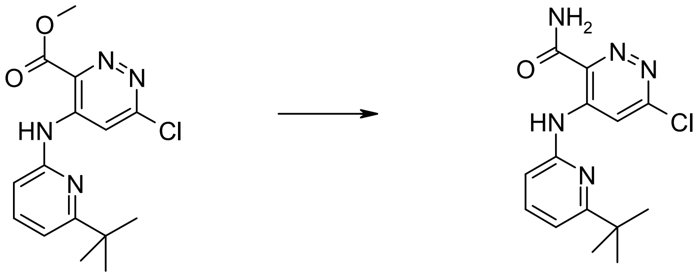
Methyl 4-(6-tert-butylpyridin-2-ylamino)-6-chloropyridazine-3-carboxylate



A mixture of methyl 4,6-dichloropyridazine-3-carboxylate (0.69 g, 3.33 mmol) and 6-tert-butylpyridin-2-amine (1.00 g, 6.67 mmol) was dissolved in acetonitrile (3 mL) and heated at 130°C for 14 h. The dark brown mixture was cooled, concentrated onto silica, and purified by chromatography (silica, 80 g, 0 % to 20 % acetone in dichloromethane, 40 min) to give methyl 4-(6-tert-butylpyridin-2-ylamino)-6-chloropyridazine-3-carboxylate (372 mg, 1.16 mmol, 35 %) as a yellow solid. MS (EI/CI) *m/z*: 321.0 [M + H].

Step 2

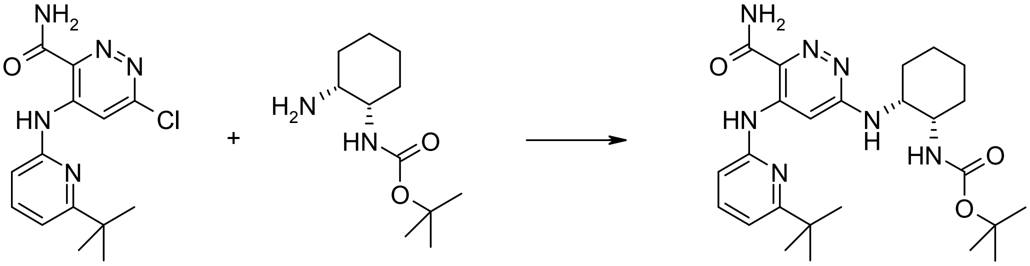
4-(6-*tert*-Butylpyridin-2-ylamino)-6-chloropyridazine-3-carboxamide



Methyl 4-(6-*tert*-butylpyridin-2-ylamino)-6-chloropyridazine-3-carboxylate (360 mg, 1.12 mmol) was suspended in 7N ammonia in methanol (12 mL, 84.0 mmol) and stirred at room temperature for 16 h. The mixture was concentrated *in vacuo* to give 4-(6-*tert*-butylpyridin-2-ylamino)-6-chloropyridazine-3-carboxamide (317 mg, 1.03 mmol, 92 %) as a yellow powder. MS (EI/CI) *m/z*: 306.0 [M + H]. This material was used directly in the next step without further purification.

Step 3

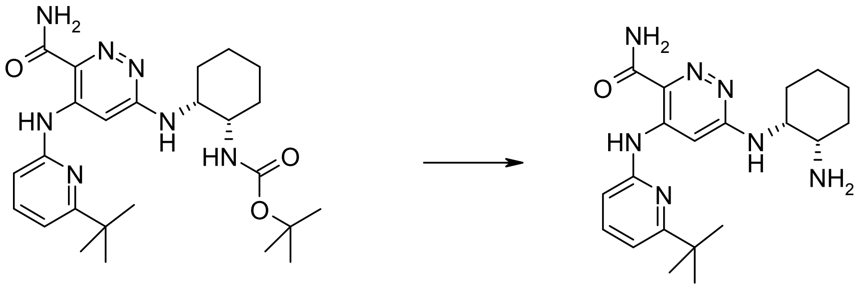
<u>*tert*-Butyl (1S,2R)-2-(5-(6-*tert*-butylpyridin-2-ylamino)-6-carbamoylpyridazin-3-ylamino)cyclohexylcarbamate</u>



A stirrred solution of 4-(6-*tert*-butylpyridin-2-ylamino)-6-chloropyridazine-3-carboxamide (317 mg, 1.04 mmol) and *tert*-butyl (1S,2R)-2-aminocyclohexylcarbamate (444 mg, 2.07 mmol) in NMP (4 mL) was heated at 140°C for 24 h in a sealed tube. The mixture was concentrated *in vacuo* (Kuglerohr, high vacuum) to a brown solid. Purification by chromatography (silica, 40g, 0-10 % of a 9:1 MeOH solution in dichloromethane, 30 min) then a second purification by chromatography (silica, 24 g, 10 % acetone in dichloromethane, 10 min, then 9:1:90 MeOH:NH_4OH:DCM, 10 min) gave *tert*-butyl (1S,2R)-2-(5-(6-*tert*-butylpyridin-2-ylamino)-6-carbamoylpyridazin-3-ylamino)cyclohexylcarbamate (197 mg, 407 µmol, 39 %) as a brown viscous gum. MS (EI/CI) *m/z*: 484.2 [M + H]. This material contained small amounts of residual NMP and was used directly in the next step without further purification.

Step 4

6-((1R,2S)-2-Aminocyclohexylamino)-4-(6-*tert*-butylpyridin-2-ylamino)pyridazine-3-carboxamide

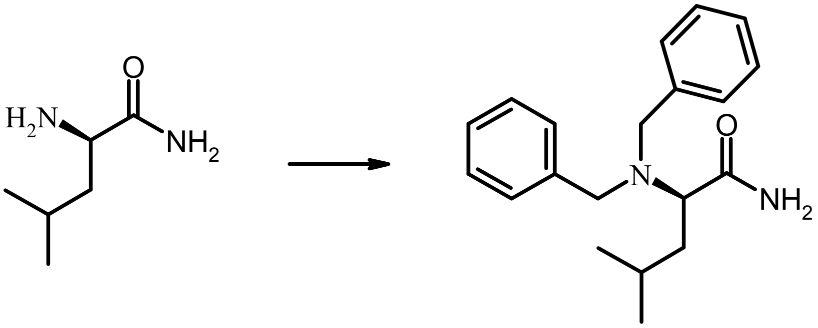


To a solution of *tert*-butyl (1S,2R)-2-(5-(6-*tert*-butylpyridin-2-ylamino)-6-carbamoylpyridazin-3-ylamino)cyclohexylcarbamate (192 mg, 397 µmol) in dichloromethane (2 mL) was added TFA (747 mg, 505 µL, 6.55 mmol). After 14 h, the mixture was concentrated *in vacuo* then purified by chromatography (spherical silica, 50 g, 0-20 % of a 9:1 MeOH:NH_4OH solution in dichloromethane, 30 min) to give a pale brown gum. This was triturated with ethanol and concentrated to give a yellow solid. Trituration again with hot ethanol, followed by decanting of the supernatant, gave **29** (57 mg, 149 µmol, 37 %) as a cream powder after drying under high vacuum. ^1H NMR (DMSO-d_6) δ: 11.80 (s, 1H), 8.39 (d, J = 2.0 Hz, 1H), 7.98 (s, 1H), 7.55 - 7.76 (m, 2H), 6.96 - 7.09 (m, 1H), 6.77 (dd, J = 8.1, 0.5 Hz, 1H), 6.70 (d, J = 8.1 Hz, 1H), 3.67 (br. s., 1H), 3.03 - 3.12 (m, 1H), 1.45 - 1.72 (m, 8H), 1.22 - 1.41 (m, 11H); MS (EI/CI) *m/z*: 384.2 [M + H].

(*R*)-6-(1-Amino-4-methylpentan-2-ylamino)-4-(6-*tert*.-butylpyridin-2-ylamino)pyridazine-3-carboxamide [**30**]

Step 1

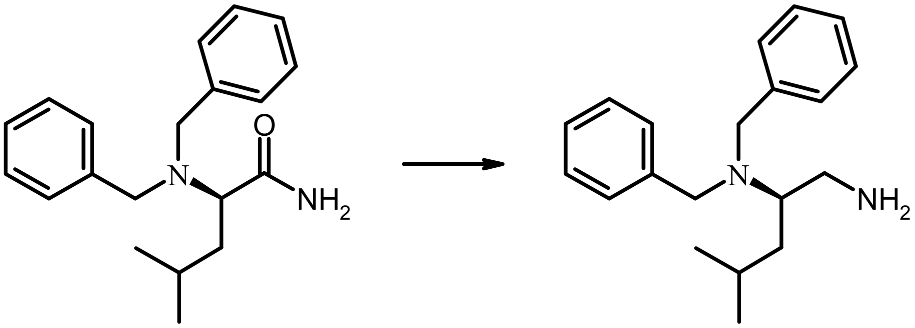
(R)-2-(dibenzylamino)-4-methylpentanamide



H-D-LEU-NH_2 (2.0 g, 15.4 mmol) and benzaldehyde (1.79 g, 16.9 mmol) were dissolved in dry DCM (90 mL) then sodium triacetoxyborohydride (3.91 g, 18.4 mmol) was added. The suspension was stirred at room temperature for 12 h. A second portion of benzaldehyde (1.79 g, 16.9 mmol) and sodium triacetoxyborohydride (3.91 g, 18.4 mmol) was added. After 24 h, saturated ammonium chloride was added, and the phases were separated. The organic phase was washed with saturated aqueous sodium bicarbonate then brine, dried, filtered and concentrated in vacuo to a colorless oil. Purification by chromatography (silica, 80 g, 0-5 % acetone in dichloromethane, 30 min) gave (R)-2-(dibenzylamino)-4-methylpentanamide (3.42 g, 11.0 mmol, 72 %) as a colorless oil. MS (EI/CI) *m/z*: 311.2 $[M+H]^+$. This contained ~32 % benzyl alcohol, and was reacted directly in the next step without further purification.

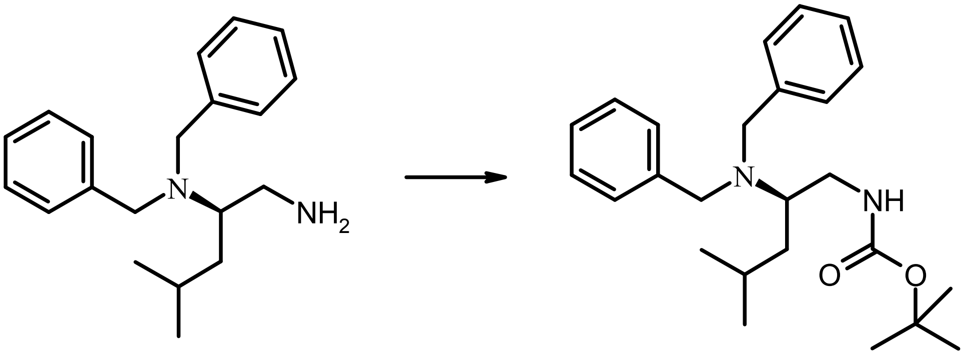
Step 2

(R)-N2,N2-Dibenzyl-4-methylpentane-1,2-diamine



(R)-2-(dibenzylamino)-4-methylpentanamide (3.42 g, 11.0 mmol) was dissolved in THF (30 mL) then $LiAlH_4$ (2 M in THF, 5.51 mL, 11.0 mmol) was added dropwise under nitrogen. The mixture was stirred at room temperature for 18 h. $LiAlH_4$ (2 M in THF, 2.5 mL, 5.0 mmol) was added and the mixture heated to 70 °C. After 2 h, the red, cloudy solution was cooled and then quenched with sodium sulfate decahydrate (~2g). The color was lost, becoming clear. After stirring for 1 h, the mixture was filtered and concentrated *in vacuo* to a clear yellow oil (2.8 g). The crude material was purified by chromatography (silica, 80 g, 0 % to 10 % of 1:9 NH_4OH:MeOH solution in DCM) to give (R)-N2,N2-dibenzyl-4-methylpentane-1,2-diamine (1.59 g, 5.36 mmol, 49 %) as a colorless oil. MS (EI/CI) *m/z*: 297.2 $[M+H]^+$.

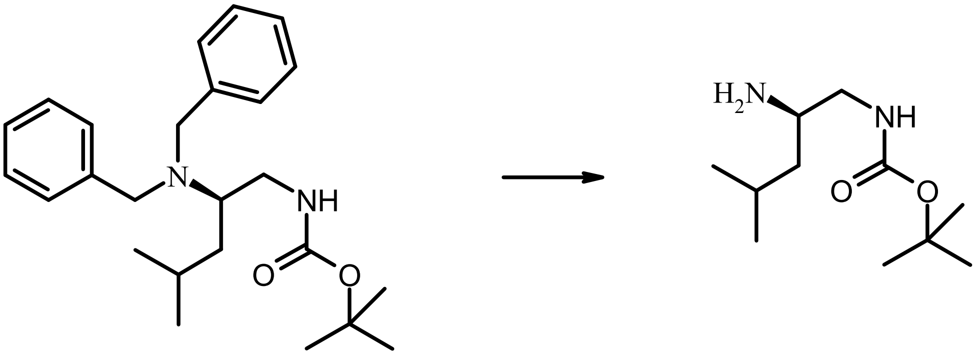
Step 3



(R)-N2,N2-dibenzyl-4-methylpentane-1,2-diamine (1.593 g, 5.37 mmol) was dissolved in THF (10 mL). To this solution was added Boc_2O (1.23 g, 5.64 mmol) and DMAP (67.0 mg, 537 µmol). The solution was stirred for 18h at room temperature, then the mixture was concentrated *in vacuo* to afford (R)-*tert*-butyl 2-(dibenzylamino)-4-methylpentylcarbamate (2.1 g) as a yellow viscous oil which was used in the next step without any further purification.

Step 4

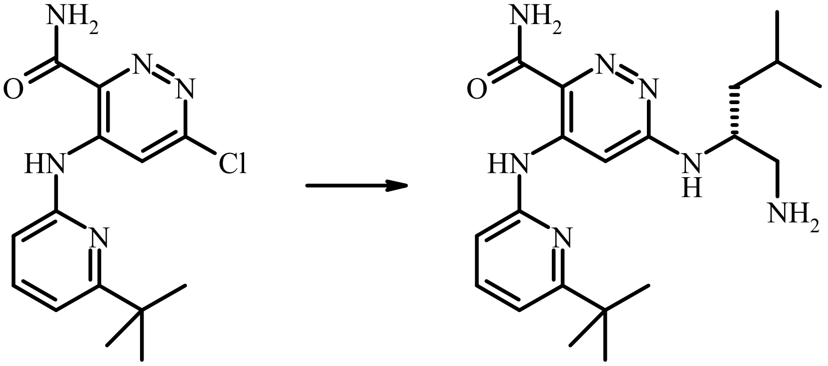
(*R*)-*tert*-Butyl 2-amino-4-methylpentylcarbamate



A suspension of 10 % Pd/C (500 mg, 4.7 mmol) in MeOH (30 mL) was added to a solution of (R)-*tert*-butyl 2-(dibenzylamino)-4-methylpentylcarbamate (2.1 g, 5.3 mmol) in MeOH (30 mL). The reaction mixture was shaken in a Parr apparatus for 1.5 h under a hydrogen atmosphere (40 psi). Additional 10 % Pd/C (500 mg, 4.7 mmol) was added and the reaction was reacted in the Parr apparatus for a further 3h. The reaction mixture was filtered over celite and evaporated affording 1.20g viscous oil. Purification by chromatography (silica, 11g, Versaflash from Supelco, eluting with 0 % to 60 % of a solution comprising 2400 mL DCM, 240 mL MeOH and 40mL NH_4OH in dichloromethane) gave (*R*)-*tert*-butyl 2-amino-4-methylpentylcarbamate (315 mg, 28 %) as a colorless oil. MS (EI/CI) *m/z*: 217.2 $[M+H]^+$.

Step 5

(*R*)-6-(1-Amino-4-methylpentan-2-ylamino)-4-(6-*tert*-butylpyridin-2-ylamino)pyridazine-3-carboxamide [30]



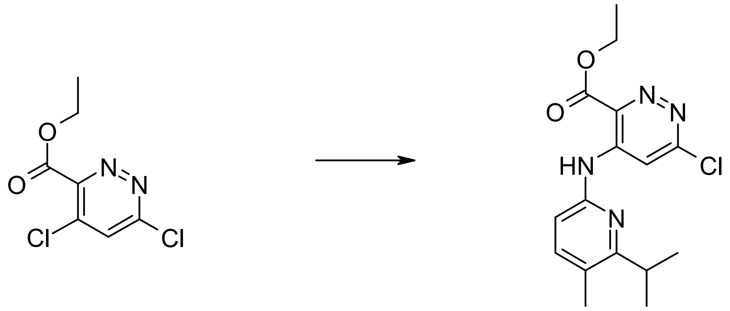
A pressure tube was charged with 4-(6-*tert*-butylpyridin-2-ylamino)-6-chloropyridazine-3-carboxamide (100 mg, 327 µmol), (*R*)-*tert*-butyl 2-amino-4-methylpentylcarbamate (141 mg, 654 µmol) and NMP (3 mL). The reaction mixture was stirred at 140 °C for 22 h, then was cooled and concentrated using a Kugelrohr apparatus under high vacuum and at 120 °C to afford a light brown solid. Purification by chromatography (spherical silica 20-45 µm, 11g, Versaflash from Supelco, eluting with 100% dichloromethane to 90:9.5:0.5 dichloromethane:MeOH:NH_4OH over 40 min) gave (*R*)-*tert*.-butyl 2-(5-(6-*tert*-butylpyridin-2-ylamino)-6-carbamoylpyridazin-3-ylamino)-4-methylpentylcarbamate as a white solid (69 mg, 55 %). This product was dissolved in dichloromethane (2 mL) and TFA (740 mg, 500 µL, 6.49 mmol). The mixture was stirred at room temperature for 1.5 h, then concentrated in vacuo and purified chromatography (spherical silica 20-45 µm, 11g, Versaflash from Supelco, eluting with 100% dichloromethane to 90:9.5:0.5 dichloromethane:MeOH:NH_4OH over 40 min) to give **30** (22 mg, 18 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.72 (br. s., 1 H), 8.37 (br. s., 1 H), 7.92 (br. s., 1 H), 7.56 - 7.76 (m, 3 H), 7.00 (d, *J*=7.33 Hz, 1

H), 6.87 (br. s., 1 H), 6.76 (d, *J*=7.83 Hz, 1 H), 2.66 (br. s., 2 H), 1.21 - 1.78 (m, 4 H), 1.35 (br. s., 9 H), 0.88 (dd, *J*=19.83, 5.68 Hz, 6 H); MS (EI/CI) *m/z*: 386 $[M+H]^+$.

6-((1R,2S)-2-Aminocyclohexylamino)-4-(6-isopropyl-5-methylpyridin-2-ylamino)pyridazine-3-carboxamide [**31**]

Step 1

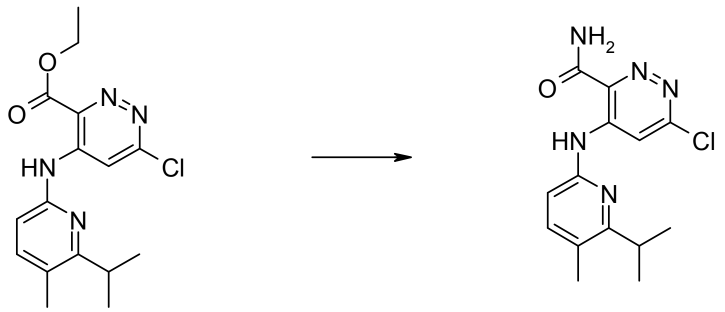
Ethyl 6-chloro-4-(6-isopropyl-5-methylpyridin-2-ylamino) pyridazine-3-carboxylate



A heavy walled sealable tube was charged with ethyl 4,6-dichloropyridazine-3-carboxylate (0.985 g, 4.46 mmol) and 6-isopropyl-5-methylpyridin-2-amine (1.005 g, 6.69 mmol). To the mixture was added acetonitrile (5 mL) and the yellow solution was heated (oilbath/hotplate) with stirring at 130 °C for 20 h to give a brown solution. After cooling to room temperature, the acetonitrile was removed *in vacuo* to obtain a dark brown solid. The residue was dissolved in dichloromethane, adsorbed on silica gel and purified by flash column (spherical silica 20-45 µm, 50 g, Versaflash from Supelco, eluting with 0 % to 5 % acetone in dichloromethane over 20 min, holding for 5 min. and then from 5 % to 20 % over 20 min, and holding for 5 min) to give ethyl 6-chloro-4-(6-isopropyl-5-methylpyridin-2-ylamino) pyridazine-3-carboxylate as yellow crystals **(**848 mg, 57 %) . ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 10.60 (br. s., 1 H), 9.36 (s, 1 H), 7.44 (d, *J*=8.08 Hz, 1 H), 6.70 (d, *J*=8.08 Hz, 1 H), 4.58 (q, *J*=7.07 Hz, 2 H), 3.30 (spt, *J*=6.70 Hz, 1 H), 2.34 (s, 3 H), 1.52 (t, *J*=7.20 Hz, 3 H), 1.33 (d, *J*=6.82 Hz, 6 H). LC-MS 335 $[M+H]^+$.

Step 2

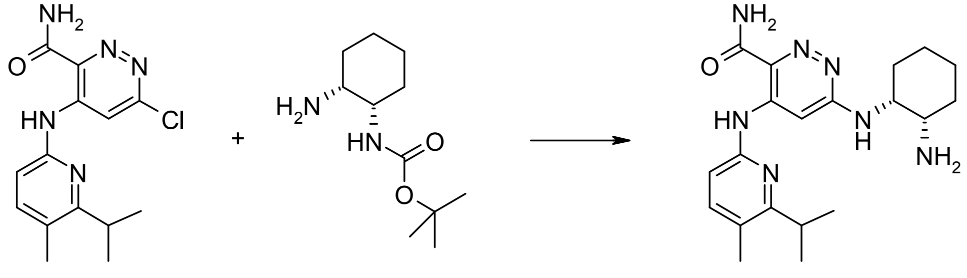
6-Chloro-4-(6-isopropyl-5-methylpyridin-2-ylamino)pyridazine-3-carboxamide



A pressure tube was charged with ethyl 6-chloro-4-(6-isopropyl-5-methylpyridin-2-ylamino) pyridazine-3-carboxylate (850 mg, 2.54 mmol) and an ammonia solution in methanol (7M, 20 mL, 140 mmol). The light yellow suspension was stirred at 50 °C for 1.5 h. After that, the reaction mixture was concentrated *in vacuo* to give 6-chloro-4-(6-isopropyl-5-methylpyridin-2-ylamino) pyridazine-3-carboxamide (693 mg, 89 %) as an orange solid. ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 11.48 (br. s., 1 H), 9.39 (s, 1 H), 8.18 (br. s., 1 H), 7.42 (d, *J*=8.08 Hz, 1 H), 6.71 (d, *J*=8.08 Hz, 1 H), 5.67 (br. s., 1 H), 3.29 (dt, *J*=13.52, 6.63 Hz, 1 H), 2.33 (s, 3 H), 2.26 - 2.26 (m, 1 H), 1.33 (d, *J*=6.82 Hz, 2 H). LC-MS 306 [M+H]$^+$.

Step 3

6-((1R,2S)-2-Aminocyclohexylamino)-4-(6-isopropyl-5-methylpyridin-2-ylamino)pyridazine-3-carboxamide [**31**]

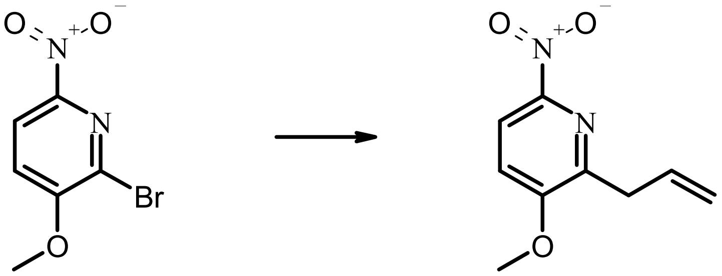


A pressure tube was charged with 6-chloro-4-(6-isopropyl-5-methylpyridin-2-ylamino) pyridazine-3-carboxamide (230 mg, 752 μmol), *tert*-butyl (1S,2R)-2-aminocyclohexylcarbamate (242 mg, 1.13 mmol) and NMP (4 mL). The yellow solution was stirred at 130 °C for 2.5 days. After that, the NMP was distilled off using a Kugelrohr apparatus under high vacuum to afford a light brown solid. The crude solid was dissolved in dichloromethane and MeOH and adsorbed on silica gel, then purified by flash column (spherical silica 20-45 μm, 11g, Versaflash from Supelco, eluting with 100 % dichloromethane to 88:11.4:0.6 dichloromethane:methanol:NH_4OH over 40 min) to give a light brown solid. The crude product was dissolved in dichloromethane (2 mL) and TFA (740 mg, 500 μL, 6.49 mmol) was added. The mixture was stirred at room temperature for 4 h. After that, the TFA and the dichloromethane were removed *in vacuo*, the brown solid obtained dissolved in dichloromethane, adsorbed on silica gel and then purified by flash column (spherical silica 20-45 μm, 11g, Versaflash from Supelco, eluting with 100 % dichloromethane to 88:11.4:0.6 dichloromethane:methanol:NH_4OH over 40 min) to give **31** (70 mg, 24 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.65 (br. s., 1 H), 8.37 (br. s., 1 H), 7.92 (s, 1 H), 7.61 (br. s., 1 H), 7.48 (d, *J*=8.34 Hz, 1 H), 6.71 (d, *J*=8.08 Hz, 1 H), 6.66 (d, *J*=8.08 Hz, 1 H), 3.24 (dt, *J*=13.39, 6.70 Hz, 1 H), 3.08 (d, *J*=2.53 Hz, 1 H), 2.26 (s, 3 H), 1.30 - 1.75 (m, 11 H), 1.26 (dd, *J*=6.70, 1.39 Hz, 6 H)**.** LC-MS 384 $[M+H]^+$.

6-((1R,2S)-2-aminocyclohexylamino)-4-(5-methoxy-6-propylpyridin-2-ylamino)pyridazine-3-carboxamide [**32**]

Step 1

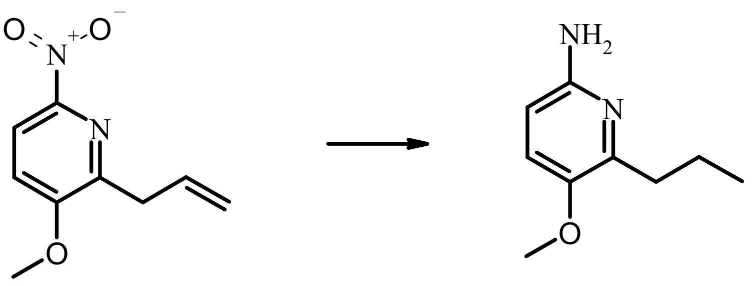
2-Allyl-3-methoxy-6-nitropyridine



2-Bromo-3-methoxy-6-nitropyridine (3.22 g, 13.8 mmol), cesium fluoride (6.3 g, 41.5 mmol) and tetrakis(triphenylphosphine)palladium (0) (1.6 g, 1.38 mmol) were combined with 2-allyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (2.55 g, 2.85 mL, 15.2 mmol) in THF (27 mL) and heated at 66 °C for 20 h. The mixture was cooled then diluted with water and ethyl acetate. The phases were separated then the organic phase was washed with water (2x) and brine, concentrated *in vacuo* and then purified by chromatography (silica, 10 to 50 % ethyl acetate in hexanes) to give 2-allyl-3-methoxy-6-nitropyridine (2.0 g, 10.3 mmol, 75 %) as a blue solid. MS (EI/CI) *m/z*: 194.8 [M + H].

Step 2

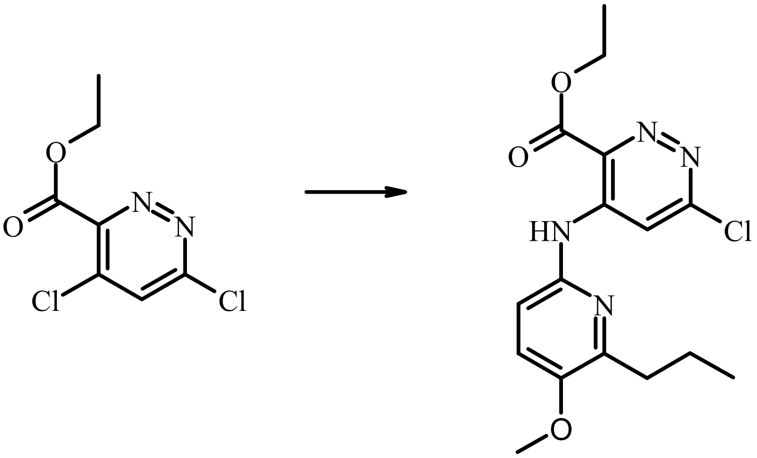
5-Methoxy-6-propylpyridin-2-amine



To a solution of 2-allyl-3-methoxy-6-nitropyridine (2.0 g, 10.3 mmol) in ethanol (34 mL) was added 10 % palladium on carbon (219 mg, 2.06 mmol). The reaction was evacuated and back filled with hydrogen. This was repeated two more times. The reaction mixture was stirred under hydrogen at 1 atm for 16 h, then filtered through a pad of celite and the filter cake washed thoroughly with ethyl acetate. The filtrates were concentrated *in vacuo* and purified by chromatography (silica, 25 to 90 % ethyl acetate in hexanes) to give 5-methoxy-6-propylpyridin-2-amine (1.41 g, 8.48 mmol, 82 %) as an off white solid. ^{1}H NMR (400 MHz, CHLOROFORM-d_6) δ ppm 7.09 (d, *J*=8.8 Hz, 1 H), 6.38 (d, *J*=8.8 Hz, 1 H), 4.28 (br. s, 2 H), 3.77 (s, 3 H), 2.68 (t, *J*=7.9 Hz, 2 H), 1.69 (m, 2 H), 0.99 (t, *J*=7.4 Hz, 3 H); MS (EI/CI) *m/z*: 166.8 [M + H].

Step 3

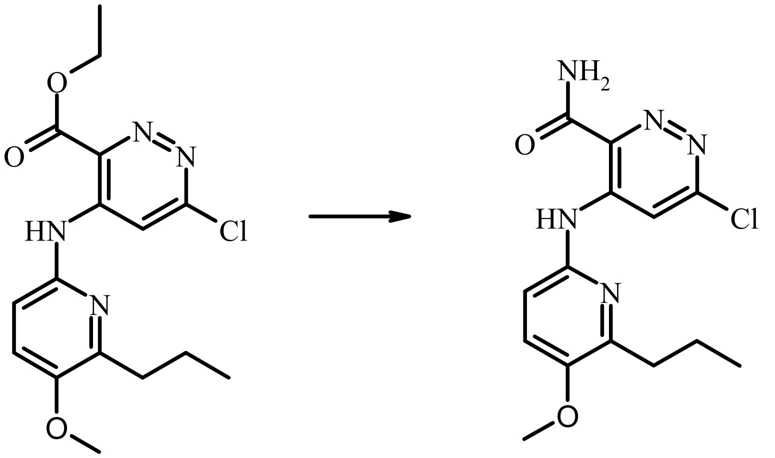
Ethyl 6-chloro-4-(5-methoxy-6-propylpyridin-2-ylamino)pyridazine-3-carboxylate



To a solution of ethyl 4,6-dichloropyridazine-3-carboxylate (1.9 g, 8.6 mmol) in acetonitrile (28.7 mL) was added 5-methoxy-6-propylpyridin-2-amine (1.43 g, 8.6 mmol) and the mixture heated at 70 °C for 72 h. The mixture was concentrated *in vacuo* then purified by chromatography (silica, 10 to 60% ethyl acetate in hexanes) to give ethyl 6-chloro-4-(5-methoxy-6-propylpyridin-2-ylamino)pyridazine-3-carboxylate (1.22 g, 3.48 mmol, 41 %) as a yellow solid. ^{1}H NMR (400 MHz, CHLOROFORM-d_6) δ ppm 10.58 (s, 1 H), 8.84 (s, 1 H), 7.25 (d, *J*=8.7 Hz, 1 H), 6.88 (d, *J*=8.5 Hz, 1 H), 4.58 (q, *J*=7.3 Hz, 2 H), 3.88 (s, 3 H), 2.87 (t, *J*=7.5 Hz, 2 H), 1.84 (m, 2 H), 1.52 (t, *J*=7.3 Hz, 3 H), 1.05 (t, *J*=7.1 Hz, 3 H); MS (EI/CI) *m/z*: 351.0 [M + H].

Step 4

6-Chloro-4-(5-methoxy-6-propylpyridin-2-ylamino)pyridazine-3-carboxamide

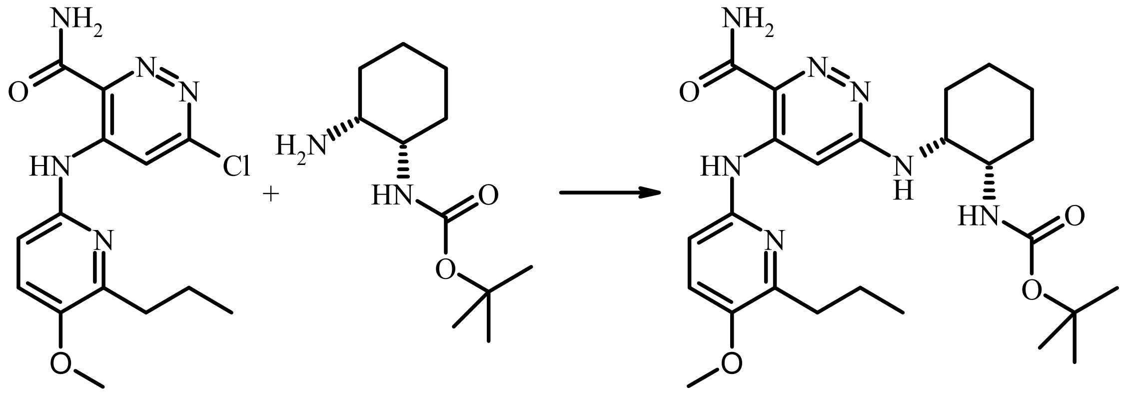


To a mixture of ethyl 6-chloro-4-(5-methoxy-6-propylpyridin-2-ylamino)pyridazine-3-carboxylate (1.22 g, 3.48 mmol) in methanol (10 mL) was added 7 N ammonia in methanol (23.6 g, 30 mL, 210 mmol) and the mixture stirred at 50 °C in a sealed tube for 16 h. The mixture was concentrated *in vacuo* to give 6-chloro-4-(5-methoxy-6-propylpyridin-2-ylamino)pyridazine-3-carboxamide (1.113 g, 3.46 mmol, 100 %) as a yellow solid. ^{1}H NMR

(400 MHz, DMSO-d_6) δ ppm 11.72 (s, 1 H), 8.89 (s, 1 H), 8.77 (s, 1 H), 8.13 (s, 1 H), 7.47 (d, *J*=8.6 Hz, 1 H), 7.01 (d, *J*=8.6 Hz, 1 H), 3.81 (s, 3 H), 2.74 (t, *J*=7.3 Hz, 2 H), 1.75 (m, 2 H), 0.97 (t, *J*=7.3 Hz, 3 H); MS (EI/CI) *m/z*: 321.9 [M + H].

Step 5

tert-Butyl (1S,2R)-2-(6-carbamoyl-5-(5-methoxy-6-propylpyridin-2-ylamino)pyridazin-3-ylamino)cyclohexylcarbamate

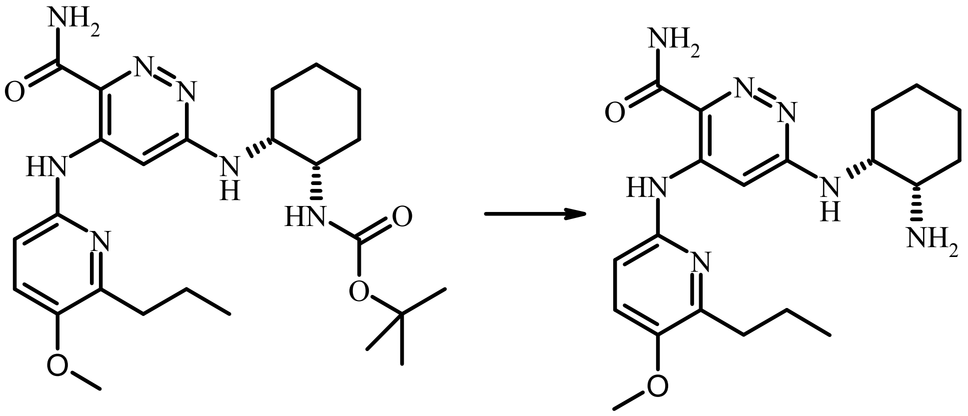


To a solution of 6-chloro-4-(5-methoxy-6-propylpyridin-2-ylamino)pyridazine-3-carboxamide (200 mg, 622 µmol) in NMP (2.07 mL) was added *tert*-butyl (1S,2R)-2-aminocyclohexylcarbamate (133 mg, 622 µmol) and the mixture heated at 140 °C for 20 h. A second portion of *tert*-butyl (1S,2R)-2-aminocyclohexylcarbamate (133 mg, 622 µmol) was added and the mixture stirred for a further 7 h. A final portion of *tert*-butyl (1S,2R)-2-aminocyclohexylcarbamate (133 mg, 622 µmol) was added and the mixture heated for 16 h, then cooled, diluted with ethyl acetate, and washed with brine (4x). The organic layer was collected, concentrated *in vacuo*, and the residue obtained was purified by chromatography (silica, 1 to 5 % methanol in dichloromethane) to give *tert*-butyl (1S,2R)-2-(6-carbamoyl-5-

(5-methoxy-6-propylpyridin-2-ylamino)pyridazin-3-ylamino)cyclohexylcarbamate (96 mg, 192 μmol, 31 %) as a light brown solid. MS (EI/CI) *m/z*: 500.2 [M + H].

Step 6

6-((1R,2S)-2-aminocyclohexylamino)-4-(5-methoxy-6-propylpyridin-2-ylamino)pyridazine-3-carboxamide [**32**]

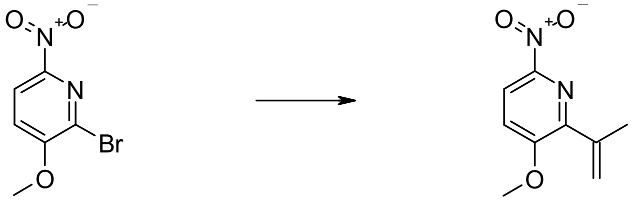


To a solution of *tert*-butyl (1S,2R)-2-(6-carbamoyl-5-(5-methoxy-6-propylpyridin-2-ylamino)pyridazin-3-ylamino)cyclohexylcarbamate (96 mg, 192 μmol) in dichloromethane (3 mL) was added trifluoroacetic acid (438 mg, 296 μL, 3.84 mmol) and the mixture stirred at room temperature overnight. The mixture was concentrated *in vacuo* then diluted with 25 % aqueous NH_4OH and dichloromethane. The phases were separated and the organic phase washed with water. The organic layer was concentrated *in vacuo* then purified by chromatography (silica, 3 to 20% methanol in dichloromethane) to give **32** (59 mg, 148 μmol, 77 %) as a light brown solid. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 11.36 (s, 1 H), 8.30 (s, 1 H), 7.72 (s, 1 H), 7.60 (s, 1 H), 7.42 (d, *J*=8.8 Hz, 1 H), 6.87 (d, *J*=8.8 Hz, 1 H), 6.72 (d, *J*=7.6 Hz, 1 H), 3.96 (s, 1 H), 3.79 (s, 3 H), 3.28 (s, 1 H), 2.72 (t, *J*=7.3 Hz, 2 H), 1.54-1.74 (m, 8 H), 1.35 (m, 2 H), 0.94 (t, *J*=7.4 Hz, 3 H); MS (EI/CI) *m/z*: 400.2 [M + H].

6-((1R,2S)-2-aminocyclohexylamino)-4-(5-methoxy-6-propylpyridin-2-ylamino)pyridazine-3-carboxamide [**33**]

Step 1

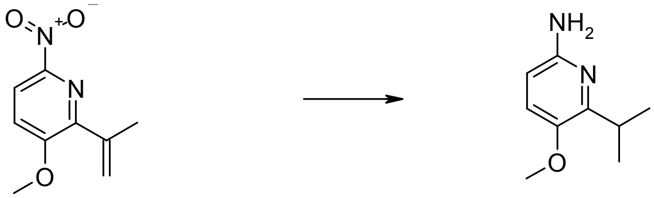
3-Methoxy-6-nitro-2-(prop-1-en-2-yl)pyridine



In a microwave vial was added a mixture of 2-bromo-3-methoxy-6-nitropyridine (1.5 g, 6.44 mmol), 4,4,5,5-tetramethyl-2-(prop-1-en-2-yl)-1,3,2-dioxaborolane (1.41 g, 8.37 mmol), tetrakis(triphenylphosphine)palladium (0) (744 mg, 644 µmol), potassium phosphate tribasic (2.73 g, 12.9 mmol), DMA (16.1 mL) and water (5.36 mL). The vial was sealed and heated in the microwave for 20 min at 150°C, then cooled and diluted with ethyl acetate and brine. The organic phase was separated and washed with brine (3x), then concentrated *in vacuo* and purified by chromatography (silica, 5 to 35 % ethyl acetate in hexanes) to give 3-methoxy-6-nitro-2-(prop-1-en-2-yl)pyridine (824 mg, 4.24 mmol, 66 %) as a brown solid. MS (EI/CI) *m/z*: 194.8 [M + H].

Step 2

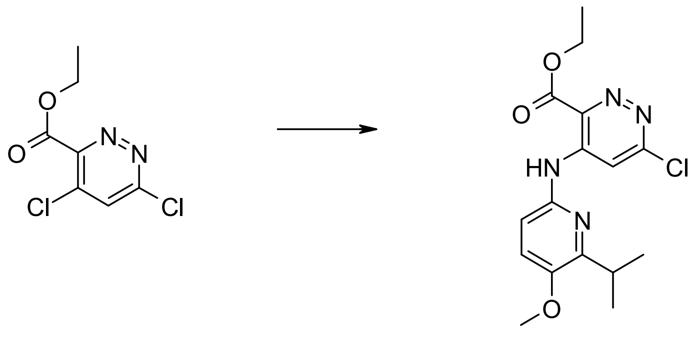
6-Isopropyl-5-methoxypyridin-2-amine



To a solution of 3-methoxy-6-nitro-2-(prop-1-en-2-yl)pyridine (824 mg, 4.24 mmol) in ethanol (14.1 mL) was added 10% palladium on carbon (45.2 mg, 424 μmol). The reaction mixture was evacuated and back filled with hydrogen. This was repeated two more times. The reaction was stirred under hydrogen at 1 atm for 16 h. The mixture was then filtered reaction through a pad of celite, the filtrate concentrated *in vacuo*, and then purified by chromatography (silica, 10 to 60 % ethyl acetate in hexanes) to give 6-isopropyl-5-methoxypyridin-2-amine (562 mg, 3.38 mmol, 80 %) as a yellow solid. ^{1}H NMR (400 MHz, CHLOROFORM-d_6) δ ppm 7.04 (d, *J*=8.7 Hz, 1 H), 6.33 (d, *J*=8.7 Hz, 1 H), 4.12 (br. s, 2 H), 3.78 (s, 3 H), 3.36 (m, 1 H), 1.22 (d, *J*=7.0 Hz, 6 H); MS (EI/CI) *m/z*: 166.8 [M + H].

Step 3

<u>6-Chloro-4-(6-isopropyl-5-methoxypyridin-2-ylamino)pyridazine-3-carboxylate</u>

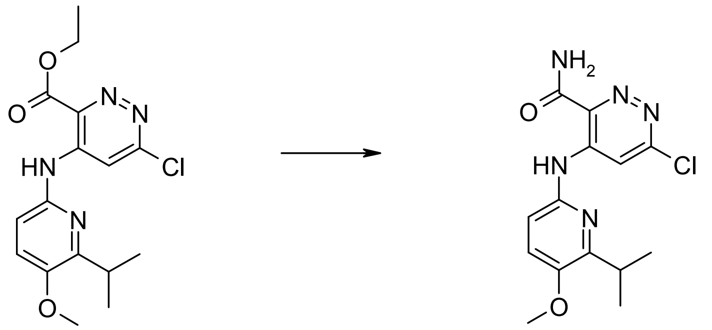


To a solution of ethyl 4,6-dichloropyridazine-3-carboxylate (747 mg, 3.38 mmol) in acetonitrile (11.3 mL) was added 6-isopropyl-5-methoxypyridin-2-amine (562 mg, 3.38 mmol) and heated to 80°C for 20 h. The mixture was cooled and concentrated *in vacuo*. Purification by chromatography (silica, 10 to 50 % ethyl acetate in hexanes) gave ethyl 6-chloro-4-(6-isopropyl-5-methoxypyridin-2-ylamino)pyridazine-3-carboxylate (438 mg, 1.25 mmol, 37 %) as a yellow solid. ^{1}H NMR (400 MHz, CHLOROFORM-d_6) δ ppm 10.61 (s, 1

H), 9.15 (s, 1 H), 7.22 (d, *J*=8.7 Hz, 1 H), 6.61 (d, *J*=8.7 Hz, 1 H), 4.57 (q, *J*=7.6 Hz, 2 H), 3.88 (s, 3 H), 3.53 (m, 1 H), 1.53 (t, *J*=7.0 Hz, 3 H), 1.31 (d, *J*=6.7 Hz, 6 H); MS (EI/CI) *m/z*: 351.0 [M + H].

Step 4

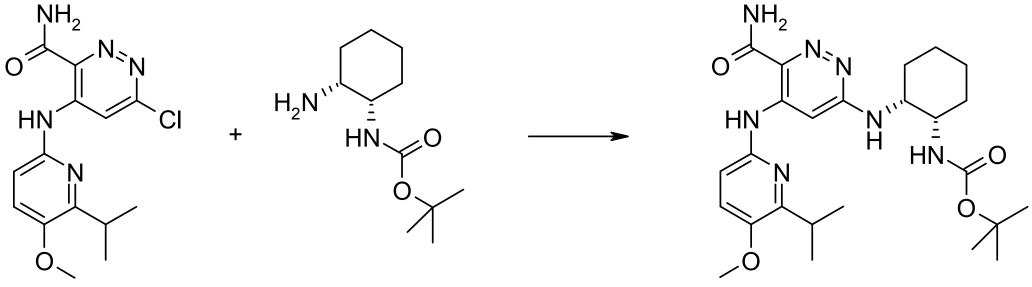
Ethyl 6-chloro-4-(6-isopropyl-5-methoxypyridin-2-ylamino)pyridazine-3-carboxylate



A mixture of ethyl 6-chloro-4-(6-isopropyl-5-methoxypyridin-2-ylamino)pyridazine-3-carboxylate (438 mg, 1.25 mmol) and ammonia in methanol (7 N, 8.92 mL, 62.4 mmol) in methanol (1 mL) was warmed at 40 °C for 16 h. The mixture was then concentrated *in vacuo* to give ethyl 6-chloro-4-(6-isopropyl-5-methoxypyridin-2-ylamino)pyridazine-3-carboxylate (438 mg, 1.25 mmol, 100 %) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.75 (s, 1 H), 9.03 (s, 1 H), 8.78 (s, 1 H), 8.13 (s, 1 H), 7.48 (d, *J*=8.5 Hz, 1 H), 7.00 (d, *J*=8.5 Hz, 1 H), 3.82 (s, 3 H), 3.44 (m, 1 H), 1.22 (d, *J*=6.6 Hz, 6 H); MS (EI/CI) *m/z*: 321.9 [M + H].

Step 5

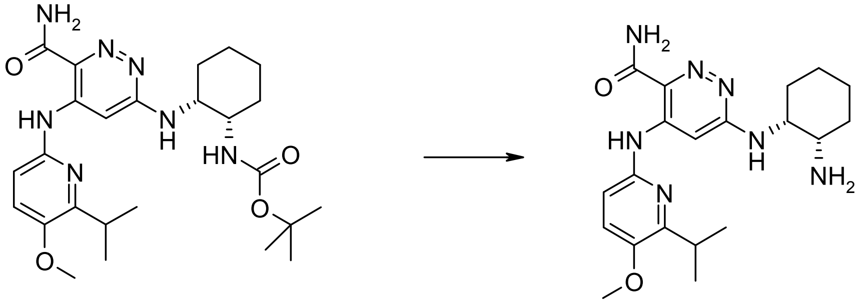
tert-Butyl (1S,2R)-2-(6-carbamoyl-5-(6-isopropyl-5-methoxypyridin-2-ylamino)pyridazin-3-ylamino)cyclohexylcarbamate



To a solution of 6-chloro-4-(6-isopropyl-5-methoxypyridin-2-ylamino)pyridazine-3-carboxamide (199 mg, 618 µmol) in NMP (2.06 mL) was added *tert*-butyl (1S,2R)-2-aminocyclohexylcarbamate (532 mg, 2.47 mmol) in four portions approximately every 12 h while heating at 140 °C. After a total heating time of 48 h, the mixture was cooled, diluted with ethyl acetate and brine, then the phases were separated and the organic phase washed twice more with brine. The organic phase was concentrated *in vacuo* then purified by chromatography (silica, 1 to 5 % methanol in dichloromethane) to give *tert*-butyl (1S,2R)-2-(6-carbamoyl-5-(6-isopropyl-5-methoxypyridin-2-ylamino)pyridazin-3-ylamino) cyclohexylcarbamate (100 mg, 200 µmol, 32 %) as a brown solid. MS (EI/CI) *m/z*: 500.4 [M + H].

Step 6

6-((1R,2S)-2-Aminocyclohexylamino)-4-(6-isopropyl-5-methoxypyridin-2-ylamino)pyridazine-3-carboxamide [**33**]

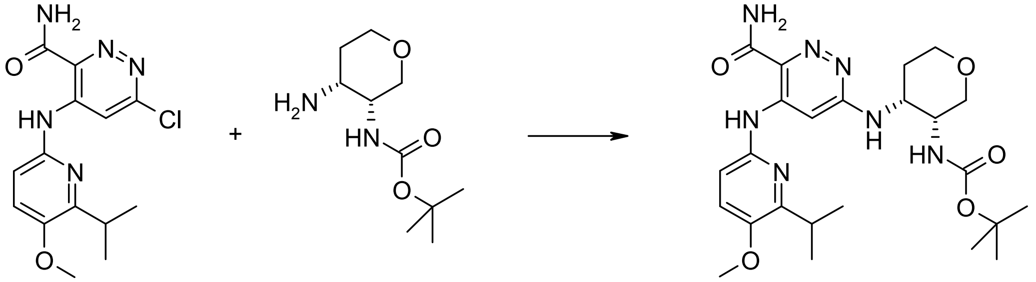


To a solution of *tert*-butyl (1S,2R)-2-(6-carbamoyl-5-(6-isopropyl-5-methoxypyridin-2-ylamino)pyridazin-3-ylamino)cyclohexylcarbamate (100 mg, 200 µmol) in dichloromethane (3 mL) was added trifluoroacetic acid (456 mg, 308 µL, 4.00 mmol) and the mixture stirred at room temperature for 16 h. The mixture was concentrated *in vacuo* then 25% aqueous NH_4OH solution added. This was then diluted with dichloromethane and water, the phases separated, and the organic phase washed twice with water. The organic layer was dried then purified by chromatography (silica, 0 to 15 % methanol in dichloromethane) to give **33** (43 mg, 108 µmol, 54 %) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.45 (s, 1 H), 8.31 (s, 1 H), 7.69 (s, 1 H), 7.58 (s, 1 H), 7.42 (d, *J*=8.7 Hz, 1 H), 6.85 (d, *J*=8.7 Hz, 1 H), 6.63 (d, *J*=8.0 Hz, 1 H), 3.80 (s, 3 H), 3.42 (m, 1 H), 3.15 (s, 1 H), 1.52-1.69 (m, 8 H), 1.32 (m, 2 H), 1.24 (d, *J*=6.4 Hz, 6 H); MS (EI/CI) *m/z*: 400.3 [M + H].

6-((3R,4R)-3-Aminotetrahydro-2H-pyran-4-ylamino)-4-(6-isopropyl-5-methoxypyridin-2-ylamino)pyridazine-3-carboxamide [**34**]

Step 1

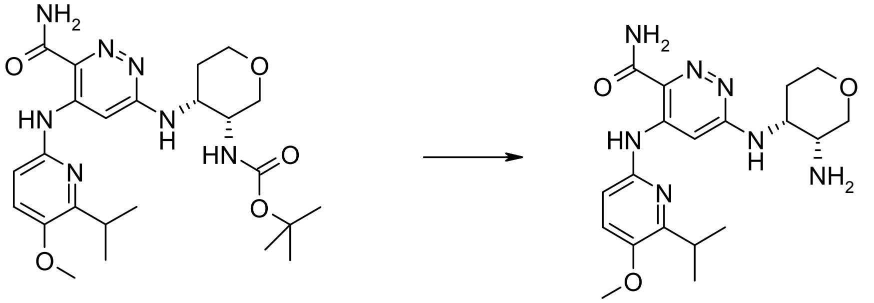
tert-Butyl (3R,4R)-4-(6-carbamoyl-5-(6-isopropyl-5-methoxypyridin-2-ylamino)pyridazin-3-ylamino)tetrahydro-2H-pyran-3-ylcarbamate



To a solution of 6-chloro-4-(6-isopropyl-5-methoxypyridin-2-ylamino)pyridazine-3-carboxamide (199 mg, 618 μmol) in NMP (2.06 mL) was added *tert*-butyl (3R,4R)-4-aminotetrahydro-2H-pyran-3-ylcarbamate (536 mg, 2.47 mmol) in four portions approximately every 12 h and the mixture heated at 140 °C. After 48 h total heating time the mixture was cooled and then diluted with ethyl acetate and brine. The phases were separated then the organic phase was washed with brine (2x), then concentrated *in vacuo* and the residue obtained was purified by chromatography (silica, 10 to 50 % methanol in dichloromethane) to give 6-chloro-4-(6-isopropyl-5-methoxypyridin-2-ylamino)pyridazine-3-carboxamide (199 mg, 618 μmol, quantitative) as a light brown solid. MS (EI/CI) *m/z*: 502.2 [M + H].

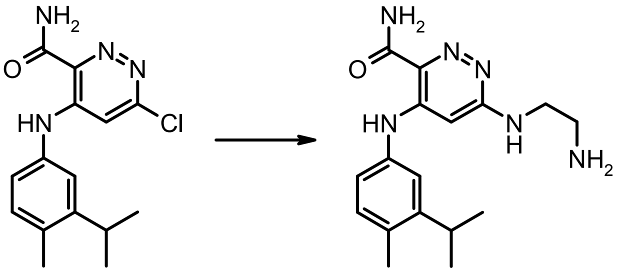
Step 2

6-((3R,4R)-3-Aminotetrahydro-2H-pyran-4-ylamino)-4-(6-isopropyl-5-methoxypyridin-2-ylamino)pyridazine-3-carboxamide [**34**]



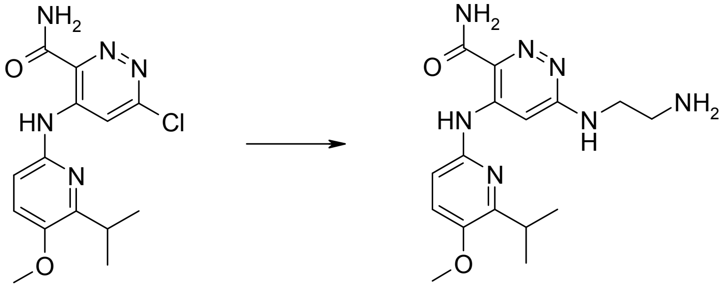
To a solution of *tert*-butyl (3R,4R)-4-(6-carbamoyl-5-(6-isopropyl-5-methoxypyridin-2-ylamino)pyridazin-3-ylamino)tetrahydro-2H-pyran-3-ylcarbamate (86 mg, 171 µmol) in dichloromethane (2.7 mL) was added trifluoroacetic acid (391 mg, 264 µL, 3.43 mmol) and stirred at room temperature for 16 h. The reaction mixture was then diluted with dichloromethane, concentrated *in vacuo*, and purified by chromatography (silica, 0 to 12 % of a 99.5:0.5 methanol:NH_4OH solution in dichloromethane) to give **34** (51 mg, 127 µmol, 74 %) as a yellow solid. ^1H NMR (400 MHz, METHANOL-d_6) δ ppm 8.08 (s, 1 H), 7.42 (d, *J*=8.6 Hz, 1 H), 6.91 (d, *J*=8.9 Hz, 1 H), 4.35 (m, 1 H), 4.12 (dd, *J*=12.1, 3.4 Hz, 1 H), 4.07 (d, *J*=12.6 Hz, 1 H), 3.89 (s, 1 H), 3.88 (s, 3 H), 3.80 (d, *J*=12.8 Hz, 1 H), 3.65 (m, 1 H), 3.52 (m, 1 H), 2.12 (m, 1 H), 1.96 (d, *J*=13.1 Hz, 1 H), 1.32 (d, *J*=5.8 Hz, 6 H); MS (EI/CI) *m/z*: 402.3 [M + H].

6-(2-aminoethylamino)-4-(6-isopropyl-5-methylpyridin-2-ylamino)pyridazine-3-carboxamide [**35**]



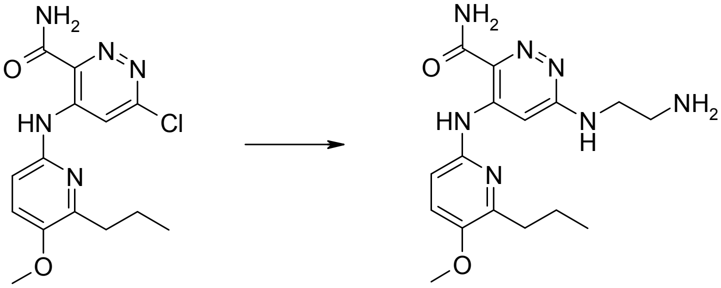
A flask was charged with 6-chloro-4-(6-isopropyl-5-methylpyridin-2-ylamino)pyridazine-3-carboxamide (100 mg, 327 µmol) and DMSO (2 mL). To this solution was added ethylenediamine (197 mg, 221 µL, 3.27 mmol) and the reaction mixture was heated with stirring at 120 °C for 1.5 h. The solvents were concentrated under high vacuum and the residue then purified by chromatography (spherical silica 20-45 µM, 23g, Versaflash Supelco, eluting with 0 to 10 % of a 9:1 MeOH:NH_4OH solution in dichloromethane, 20 min) to give **35** (70 mg, 65 %) as an off-white solid. ^{1}H NMR (DMSO-d6) δ: 11.61 (s, 1H), 8.35 (br. s., 1H), 7.93 (s, 1H), 7.60 (br. s., 1H), 7.45 (d, J = 8.3 Hz, 1H), 7.07 - 7.21 (m, 1H), 6.67 (d, J = 8.3 Hz, 1H), 3.14 - 3.27 (m, 2H), 3.03 - 3.14 (m, 1H), 2.75 (t, J = 6.2 Hz, 2H), 2.23 (s, 3H), 1.55 (br. s., 2H), 1.23 (d, J = 6.8 Hz, 6H); MS (EI/CI) *m/z*: 330.2 $[M+H]^+$.

6-(2-aminoethylamino)-4-(6-isopropyl-5-methoxypyridin-2-ylamino)pyridazine-3-carboxamide [**36**]



To a solution of 6-chloro-4-(6-isopropyl-5-methoxypyridin-2-ylamino)pyridazine-3-carboxamide (170 mg, 528 µmol) in NMP (1.76 mL) was added ethane-1,2-diamine (127 mg, 142 µl, 2.11 mmol, Eq: 4) and heated to 100°C for 16 h. The mixture was concentrated and diluted with methanol. Precipitate was filtered to give **36** (81 mg, 235 µmol, 44.4 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.48 (s, 1 H), 8.31 (s, 1 H), 7.75 (s, 1 H), 7.57 (s, 1 H), 7.41 (d, *J*=9.0 Hz, 1 H), 7.08 (t, *J*=5.6 Hz, 1 H), 6.83 (d, *J*=8.7 Hz, 1 H), 3.79 (s, 3 H), 3.40 (m, 1 H), 2.78 (t, J=5.9 Hz, 2 H), 1.22 (d, *J*=6.8 Hz, 6 H); MS (EI/CI) *m/z*: 346.3 [M + H].

6-(2-aminoethylamino)-4-(5-methoxy-6-propylpyridin-2-ylamino)pyridazine-3-carboxamide [**37**]

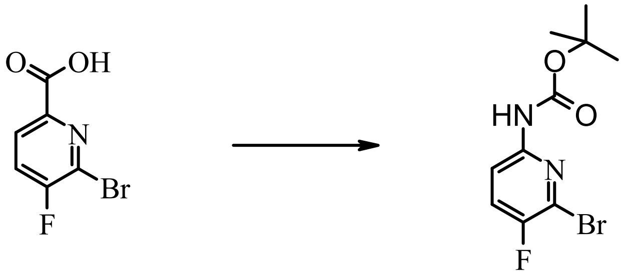


To a solution of 6-chloro-4-(5-methoxy-6-propylpyridin-2-ylamino)pyridazine-3-carboxamide (200 mg, 622 µmol) in NMP (2.07 mL) was added ethane-1,2-diamine (149 mg, 167 µl, 2.49 mmol, Eq: 4) and heated to 100°C for 16 h. Purified by reverse phase chromatography (c-18; 10-10% water/ ACN gradient), followed by HPLC (water/ methanol gradient with NH_4OAc modifier). Recrystallized from water and filtered to give **37** (80 mg, 232 µmol, 37.3 %) as a light yellow solid. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 11.40 (s, 1 H), 8.30 (s, 1 H), 7.77 (s, 1 H), 7.56 (s, 1 H), 7.41 (d, *J*=8.5 Hz, 1 H), 7.09 (t, *J*=5.4 Hz, 1 H), 6.83 (d, *J*=8.5 Hz, 1 H), 3.78 (s, 3 H), 2.77 (t, J=6.2 Hz, 2 H), 2.71 (t, *J*=7.5 Hz, 2 H), 1.70 (m, 2 H), 0.94 (t, *J*=7.4 Hz, 3 H); MS (EI/CI) *m/z*: 346.2 [M + H].

6-((3R,4R)-3-Aminotetrahydro-2H-pyran-4-ylamino)-4-(5-fluoro-6-isopropylpyridin-2-ylamino)pyridazine-3-carboxamide [**38**]

Step 1

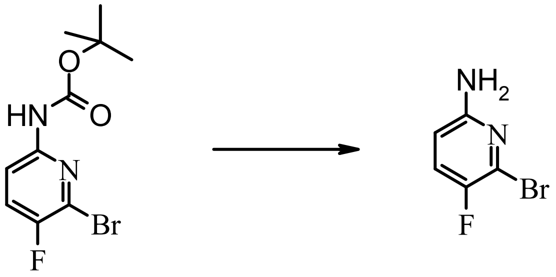
(6-Bromo-5-fluoro-pyridin-2-yl)-carbamic acid tert-butyl ester



To a solution of 6-bromo-5-fluoro-2-picolinic acid (2 g, 9.09 mmol) in tert-butanol (46 mL) and triethylamine (1.27 mL, 9.09 mmol, Eq: 1.00) was added DPPA (1.97 mL, 9.09 mmol). The slurry was stirred at room temperature until all solids dissolved (~15 min), after which it was heated to 85°C for 2 h. Upon cooling the mixture was concentrated onto silica gel and chromatographed (silica, 5% to 30 % EtOAc in hexanes) to give slightly impure (6-bromo-5-fluoro-pyridin-2-yl)-carbamic acid tert-butyl ester (1.55 g, 59 %). ^{1}H NMR (400 MHz, CHLOROFORM-d_6) δ ppm 7.97 (d, *J*=8.6 Hz, 1 H), 7.43 (d, *J*=8.6 Hz, 1 H), 1.53 (s, 9 H).

Step 2

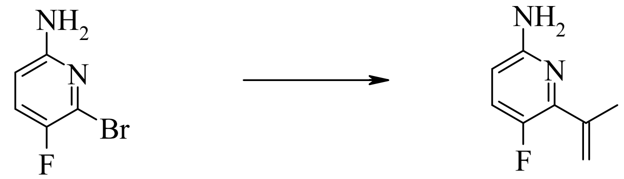
6-Bromo-5-fluoro-pyridin-2-ylamine



To a solution of (6-bromo-5-fluoro-pyridin-2-yl)-carbamic acid tert-butyl ester (1.43 g, 4.91 mmol,) in DCM (25 mL) was added TFA (3.78 mL, 49.1 mmol, Eq: 10.0). The mixture was stirred at room temperature for 2 h, after which it was concentrated *in vacuo*, and redissolved in EtOAc. The organic layer was washed with sat. aq. $NaHCO_3$ followed by water and brine. The resulting organic layer was concentrated on to silica gel and chromatographed (10 % to 40 % EtOAc / hexanes) to give 6-bromo-5-fluoro-pyridin-2-ylamine (850 mg, 91 %). ^1H NMR (400 MHz, CHLOROFORM-*d*$_6$) δ ppm 7.23 (dd, *J*=8.6, 7.5 Hz, 1 H), 6.41 (dd, *J*=8.6, 2.6 Hz, 1 H), 4.40 (br. s, 2 H).

Step 3

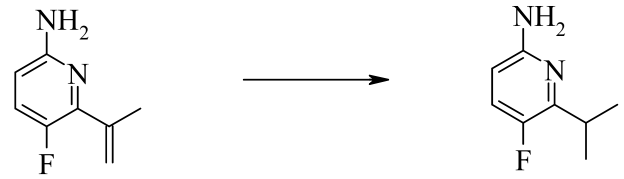
5-Fluoro-6-isopropenyl-pyridin-2-ylamine



To a solution 6-bromo-5-fluoro-pyridin-2-ylamine (850 mg, 4.45 mmol) in dimethylacetamide (13.5 mL) was added 4,4,5,5-tetramethyl-2-(prop-1-en-2-yl)-1,3,2-dioxaborolane (1.09 mL, 5.79 mmol), palladium tetrakis(triphenylphosphine) (514 mg, 445 µmol) and tribasic potassium phosphate (1.89 g, 8.9 mmol) in water (4 mL, 223 mmol,). The mixture was sealed in a microwave vial and heated at 150 °C in a microwave for 15 min. Upon cooling, the mixture was diluted with EtOAc, washed with water and brine, concentrated on to silica gel, and chromatographed (20 % to 100 % EtOAc in hexanes) to give 5-fluoro-6-isopropenyl-pyridin-2-ylamine contaminated with catalyst-derived impurities (~800 mg) that was used directly in the next step without further purification.

Step 4

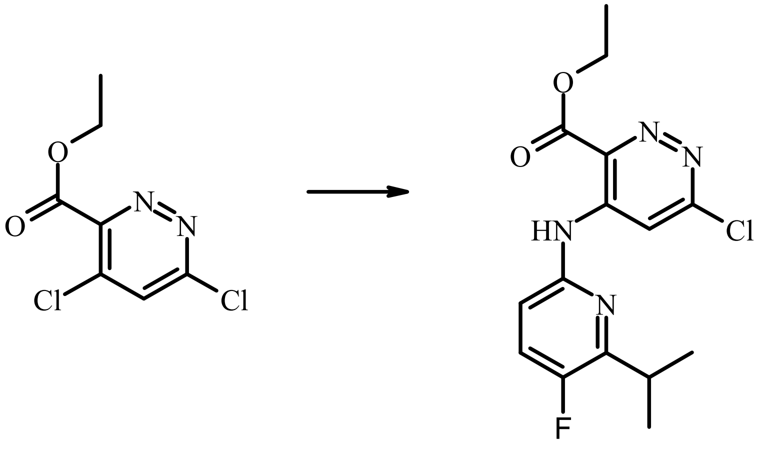
5-Fluoro-6-isopropylpyridin-2-amine



To a solution of 5-fluoro-6-isopropenyl-pyridin-2-ylamine (crude from last step, 4.45 mmol) in methanol (13.5 mL) was added 10% palladium on carbon (95 mg) at room temperature. A hydrogen balloon (1 atm) was attached and the mixture was stirred overnight. After 18 h, the mixture was filtered over Celite, concentrated on to silica gel, and chromatographed (10 % to 40 % EtOAc in hexanes) to give 5-fluoro-6-isopropylpyridin-2-amine (470 mg, 69 % over two steps). ^{1}H NMR (400 MHz, CHLOROFORM-d_6) δ ppm 7.12 (t, *J*=9.2 Hz, 1 H), 6.30 (dd, *J*=8.5, 3.0 Hz, 1 H), 4.31 (br. s, 2 H), 4.15 (m, 1 H), 1.26 (d, *J*=7.2 Hz, 6 H).

Step 5

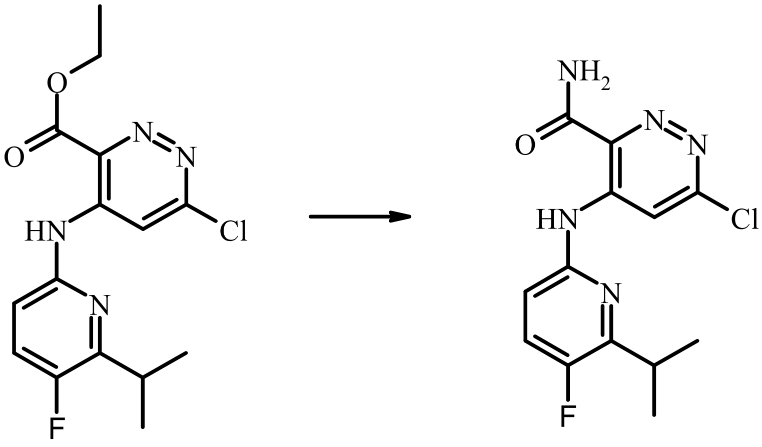
Ethyl 6-chloro-4-(5-fluoro-6-isopropylpyridin-2-ylamino)pyridazine-3-carboxylate



To a solution of ethyl 4,6-dichloropyridazine-3-carboxylate (674 mg, 3.05 mmol) in acetonitrile (10 mL) was added 5-fluoro-6-isopropylpyridin-2-amine (470 mg, 3.05 mmol) and heated at 130°C in a sealed tube for 18 h. Upon completion, the mixture was concentrated on to silica gel and purified by chromatography (silica. 10% to 33% EtOAc in hexanes) to give recovered aniline (300 mg) and ethyl 6-chloro-4-(5-fluoro-6-isopropylpyridin-2-ylamino)pyridazine-3-carboxylate (150 mg, 22 %). ^{1}H NMR (400 MHz, CHLOROFORM-*d*$_6$) δ ppm 10.72 (s, 1 H), 9.23 (s, 1 H), 7.38 (t, *J*=8.4 Hz, 1 H), 6.79 (dd, *J*=8.5, 2.8 Hz, 1 H), 4.57 (m, 2 H), 3.45 (m, 1 H), 1.53 (m, 3 H), 1.36 (d, *J*=6.9 Hz, 6 H).

Step 6

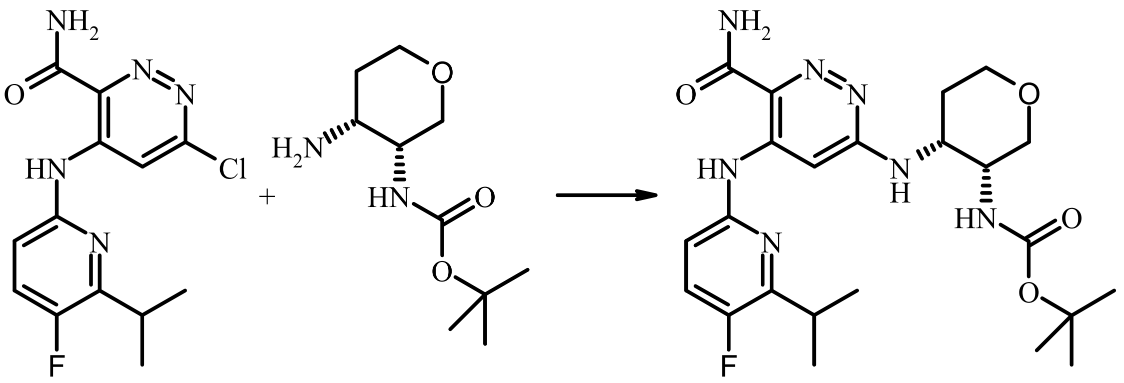
6-Chloro-4-(5-fluoro-6-isopropylpyridin-2-ylamino)pyridazine-3-carboxamide



To a solution of ethyl 6-chloro-4-(5-fluoro-6-isopropylpyridin-2-ylamino)pyridazine-3-carboxylate (290 mg, 856 μmol) was added 7N ammonia in MeOH (12.2 mL, 85.6 mmol). The mixture was stirred at 40°C for 18 h, after which the solvent was removed to give 6-chloro-4-(5-fluoro-6-isopropylpyridin-2-ylamino)pyridazine-3-carboxamide (250 mg, 94 %) as an off-white solid. ^{1}H NMR (400 MHz, CHLOROFORM-*d*$_6$) δ ppm 11.86 (s, 1 H), 9.26 (s, 1 H), 8.19 (br. s, 1 H), 7.36 (t, *J*=8.8 Hz, 1 H), 6.79 (dd, *J*=8.5, 2.8 Hz, 1 H), 5.70 (br. s, 1 H), 3.45 (m, 1 H), 1.37 (d, *J*=6.7 Hz, 6 H).

Step 7

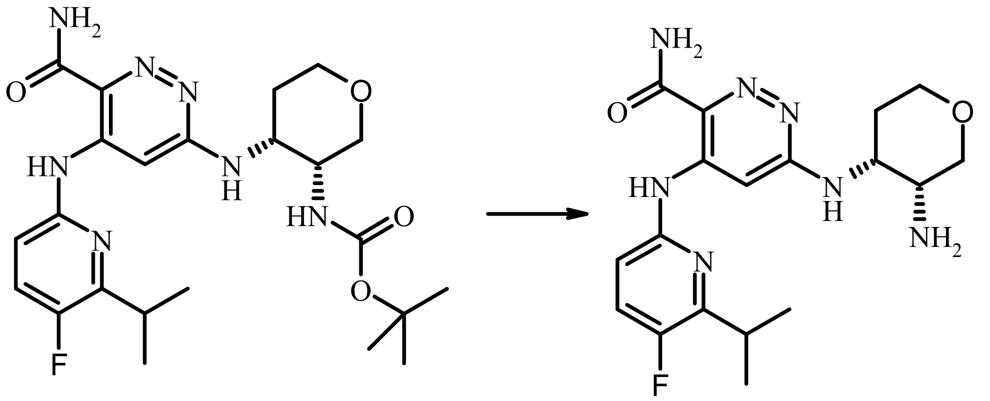
tert-Butyl (3R,4R)-4-(6-carbamoyl-5-(5-fluoro-6-isopropylpyridin-2-ylamino)pyridazin-3-ylamino)tetrahydro-2H-pyran-3-ylcarbamate



To a solution of 6-chloro-4-(5-fluoro-6-isopropylpyridin-2-ylamino)pyridazine-3-carboxamide (250 mg, 807 μmol) in NMP (3.2 mL) was added tert-butyl (3R,4R)-4-aminotetrahydro-2H-pyran-3-ylcarbamate (174 mg, 807 μmol) and the mixture heated to 140°C. Over the next 36 h three additional portions of tert-butyl (3R,4R)-4-aminotetrahydro-2H-pyran-3-ylcarbamate (174 mg, 807 μmol) were added at 12 h intervals. At 48 h the mixture was cooled, diluted with EtOAc, and washed with water and brine (2x). The organic layer was concentrated onto silica and purified by chromatography (70% to 100% EtOAc in hexanes) to give tert-butyl (3R,4R)-4-(6-carbamoyl-5-(5-fluoro-6-isopropylpyridin-2-ylamino)pyridazin-3-ylamino)tetrahydro-2H-pyran-3-ylcarbamate (100 mg, 25 %). ^{1}H NMR (400 MHz, CHLOROFORM-d_6) δ ppm 11.49 (s, 1 H), 8.22 (s, 1 H), 8.06 (br. s, 1 H), 7.29 (t, *J*=9.4 Hz, 1 H), 6.70 (dd, *J*=8.9, 3.0 Hz, 1 H), 6.07 (br. s, 1 H), 5.50 (br. s, 1 H), 5.35 (br. s, 1 H), 4.26 (br. s, 1 H), 4.03 (m, 2 H), 3.92 (d, *J*=11.4 Hz, 1 H), 3.68 (d, *J*=11.5 Hz, 1 H), 3.61 (t, *J*=11.8 Hz, 1 H), 3.41 (m, 1 H), 2.24 (d, *J*=11.2 Hz, 1 H), 1.81 (m, 1 H), 1.49 (s, 9 H), 1.35 (d, *J*=6.9 Hz, 6 H).

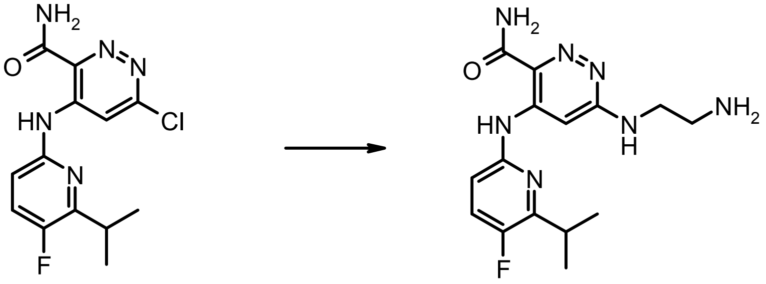
Step 8

6-((3R,4R)-3-aminotetrahydro-2H-pyran-4-ylamino)-4-(5-fluoro-6-isopropylpyridin-2-ylamino)pyridazine-3-carboxamide [**38**]



To a solution of *tert*-butyl (3R,4R)-4-(6-carbamoyl-5-(5-fluoro-6-isopropylpyridin-2-ylamino)pyridazin-3-ylamino)tetrahydro-2H-pyran-3-ylcarbamate (100 mg, 204 µmol) in dichloromethane (3 mL) was added trifluoroacetic acid (466 mg, 315 µL, 4.1 mmol) and the mixture stirred at room temperature for 16 h. The mixture was diluted with 25 % aqueous NH_4OH, dichloromethane, and water. The organic phase was separated and washed with water (2x), then concentrated *in vacuo* and purified by chromatography (silica, 3 to 10 % methanol in dichloromethane) to give **38** (39 mg, 100 µmol, 49 %) as a white solid. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 11.76 (s, 1 H), 8.39 (s, 1 H), 7.85 (s, 1 H), 7.65 (s, 1 H), 7.60 (t, *J*=9.2 Hz, 1 H), 6.88 (dd, *J*=8.9, 2.9 Hz, 1 H), 6.78 (d, *J*=7.7 Hz, 1 H), 3.96 (br. s, 1 H), 3.80 (m, 1 H), 3.70 (dd, *J*=11.4, 2.8 Hz, 1 H), 3.49 (dd, *J*=11.4. 1.7 Hz, 1 H), 3.37 (m, 2 H), 2.96 (s, 1 H), 1.80 (m, 1 H), 1.69 (m, 2 H), 1.29 (d, *J*=6.8 Hz, 6 H); MS (EI/CI) *m/z*: 390.2 [M + H].

6-(2-Aminoethylamino)-4-(5-fluoro-6-isopropylpyridin-2-ylamino)pyridazine-3-carboxamide [**39**]



To a solution of 6-chloro-4-(5-fluoro-6-isopropylpyridin-2-ylamino)pyridazine-3-carboxamide (145 mg, 468 µmol, prepared as described for **38**) in NMP (1.6 mL) was added ethane-1,2-diamine (113 mg, 125 µL, 1.87 mmol) and the mixture heated to 100°C for 24 h. The mixture was cooled and concentrated *in vacuo* then purified by HPLC (C-18, 10-100% water in acetonitrile gradient containing acetic acid modifier). The product obtained was neutralized with NH_4OH and dried *in vacuo* to give **39** (21 mg, 63 µmol, 14 %) as a white solid. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 11.76 (s, 1 H), 8.38 (s, 1 H), 7.88 (s, 1 H), 7.64 (s, 1 H), 7.59 (t, *J*=8.9 Hz, 1 H), 7.21 (t, *J*=5.5 Hz, 1 H), 6.85 (dd, *J*=9.0, 2.8 Hz, 1 H), 3.35 (m, 2 H), 2.82 (t, *J*=6.2 Hz, 2 H), 1.29 (d, *J*=6.8 Hz, 6 H); MS (EI/CI) *m/z*: 334.3 [M + H].

SYK Assay Information

Determination of IC50 of Spleen Tyrosine Kinase (SYK) inhibition:

SYK kinase assay is a standard kinase assay adapted to a 96 well plate format. This assay is performed in 96-well format for IC_{50} determination with 8 samples which represented 10 half log dilutions and a 40 μL reaction volume. The assay measures the incorporation of radiolabeled ^{33}P -ATP into an *N*-terminally biotinylated peptide substrate, derived from naturally occurring phosphoacceptor consensus sequence (Biotin-11aa DY*E). Phosphorylated products were detected upon termination of reactions with EDTA and the addition of Streptavidin coated beads. Representative results are in Table 1 and 2 above.

Assay plates: 96-well MultiScreen 0.65um filter plates (Millipore Cat. No.: MADVNOB10)

Streptavidin coated beads: Streptavidin Sepharose TM, suspension 5.0mL, in 50mM EDTA/PBS diluted (1:100), (Amersham, Cat. No.: 17-5113-01)

Compounds: 10 mM in 100% dimethylsulfoxide (DMSO), final conc.: compound 0.003-100uM in 10% DMSO

Enzyme: SYK RPA purified, truncated construct of Spleen Tyrosine Kinase aa 360-635, stock solution 1 mg/mL, MW: 31.2 KDa, final conc.:0.0005 μM.

Peptide 1: biotinylated peptide is derived from a naturally occurring phosphor-acceptor consensus sequence (Biotin-EPEGDYEEVLE), special order from QCB, stock solution 20mM, final conc.: 5.0 μM.

ATP: Adenosine-5'-triphosphate 20 mM, (ROCHE Cat. No.: 93202720), final concentration: 20μM

Buffer: HEPES: 2-Hydroxyethyl piperazine-2-ethanesulfonic acid (Sigma , Cat. No.: H-3375) final concentration: 50mM HEPES pH7.5

BSA: Bovine Serum Albumin Fraction V, fatty acid free (Roche Diagnostics GmbH, Cat. No. 9100221) diluted to a final concentration of 0.1%

EDTA: EDTA stock solution 500 mM, (GIBCO, Cat. No.: 15575-038) final concentration: 0.1mM

DTT: 1,4-Dithiothreitol (Roche Diagnostics GmbH, Cat. No.: 197777), final conc.: 1mM

$MgCl_2$ x $6H_2O$: MERCK, Cat. No.: 105833.1000, final concentration: 10mM

Assay Dilution Buffer (ADB): 50 mM HEPES, 0.1mM EGTA, 0.1mM Na Vanadate, 0.1mM β-glycerophosphate, 10 mM $MgCl_2$, 1 mM DTT, 0,1% BSA, pH 7.5

Bead wash buffer: 10 g/L PBS (Phosphate buffered saline) with 2M NaCl+ 1% phosphoric acid.

Experimental Method:

In 40μL volume, 26μL of ADB diluted, purified recombinant human SYK360-635 [0.5 nM] was mixed with 4 μL of 10X concentrations of the test compounds, [usually 100μM-0.003μM] in [10%] DMSO and the mixture was incubated for 10 min at RT.

The kinase reaction was initiated by the addition of 10μL 4x substrate cocktail containing the DYE peptide substrate [0 or 5 μM], ATP [20 μM] and ^{33} PγATP [2μCi/rxn]. After incubation at 30° C for 15 min, the reaction was terminated by the transfer of 25μL pf the reaction sample to a 96 well 0.65μm Millipore MADVNOB membrane/plate containing 200μL 5mM EDTA and 20% Streptavidine coated beads in PBS.

The unbound radionucleotides were washed under vacuum with 3 x 250μL 2M NaCl; 2 x 250 μL 2M NaCl+1% phosphoric acid; 1 x 250μL H_2O. After the last wash membrane/ plates were transferred to an adaptor plate, heat dried for 15 min at 60° C, and 50 μL scintillation cocktail was added to each well and 4 h later the amount of radioactivity was counted in a top counter.

The percent inhibition was calculated based on the uninhibited enzyme rate:

$$\% \text{ Inhibition} = 100 / (1 + (IC_{50}/\text{Inhibitor conc})^n)$$

The IC_{50} was calculated using a non-linear curve fit with XLfit software (ID Business Solution Ltd., Guilford, Surrey, UK).

Ramos/Jurkat calcium influx FLIPR assay

Human B cell lymphoma cell line Ramos or human T cell lymphoma cell line Jurkat was cultivated in RPMI-1640 with 10% Fetal Bovine Serum (Invitrogen). Cells were seeded into assay plate and loaded with calcium dye by adding 30 μl of dye loading buffer according to manufacturer's instruction (Beckton Dickinson Calcium Kit, Cat# 80500). The cells were then treated with serial diluted compounds for 30 min before stimulation. Baseline fluorescence was recorded for about 20 seconds followed by stimulation with mouse anti-human IgM (10 μg/mL, clone M2E6, Antibody Solutions Inc.) for Ramos cells or mouse anti-human CD3 (10 μg/mL, BD Biosciences) for Jurkat cells, and record of the maximal fluorescent counts in each well. Maximal counts divided by baseline counts, defined as fold change from baseline for each well, was used for IC_{50} calculation.

B cell CD69 up-regulation assay in human whole blood

Human Blood was collected from healthy volunteers into Vacutainers (BD Biosciences, San Jose, CA) containing sodium heparin. Test compound was suspended in DMSO and nine half-log serial dilutions were made. The concentration of compound in the assay was 0.5%. 100 µL whole blood was pre-incubated with compound for 30 min and then stimulated with goat F(ab')$_2$ anti-human IgM (50µg/mL, Southern Biotech) for 20 h. At the end of the 20 hour incubation, samples were incubated with fluorochrome-conjugated antibodies, PE mouse anti-human CD20 and APC Mouse anti-human CD69 (BD Biosciences), for 30 minutes. Samples were then lysed with Lyse solution (BD) and washed with PBS containing 2% fetal bovine serum (FBS). Fluorescent signals were acquired on flow cytometer LSR II (BD) and data were analyzed by Flow Jo. The percentage of activated (CD69hi) B-cell lymphocytes (CD20+) were determined using un-stimulated (negative control) and stimulated (positive control) wells as reference guidelines. The percentage inhibition was calculated and an IC$_{50}$ curve was constructed using GraphPad Prism software with sigmoidal curve fitting.

hERG channel inhibition

The hERG current measurement was performed at the automated patch clamp system PatchXpress 7000A (Molecular Devices Inc., USA). Electrophysiologcal recordings of K$^+$ currents (IK$_{\mathrm{hERG}}$) were conducted at room temperature (22-25°C) using Aviva Bioscience SealChip16™ (USA). CHO cells stably expressing hERG K$^+$ channels (Roche, USA) were stimulated by a voltage pattern to activate hERG channels. The IK$_{\mathrm{hERG}}$ currents were recorded under control conditions and after compound application in the extracellular buffer (NaCl 150 mM, KCl 4 mM, CaCl$_2$ 1.2 mM, MgCl$_2$ 1 mM, HEPES 10 mM, pH 7.4 with NaOH, 300-310 mOsm). Offline analysis of the peak tail current was performed using DataXpress2 software (Molecular Devices Inc., USA). Concentration-response curves were fitted by non-linear regression analysis and the IC_{20}/IC_{50} values were reported.

Cytochrome P450 Inhibition Assays

CYP450 inhibition profiling was evaluated by quantification of drug substrate metabolism to specific products known to be catalyzed by CYP450 isozymes present in pooled human liver microsomal fractions; diclofenac-4′-hydroxylation catalyzed by CYP2C9; dextromethorphan O-demethylase catalyzed by CYP2D6 and midazolam–1′–hydroxylation catalyzed by CYP3A4. Test compounds were delivered from Roche Compound Repository in 100% DMSO at 10 mM in 384-well microtiter plates. Serial dilution of test compounds in 100% DMSO was carried out on a TECAN automated liquid handling system.

Incubations were also performed using a TECAN automated liquid handling system. In brief, this required a 1.1x concentrated stock solution of human liver microsomes in 100mM potassium phosphate buffer, pH7.4 combined with relevant substrate, stock solutions of the control inhibitor or test compound in DMSO, NADPH regenerating system (mixture of 3mM glucose-6-phosphate, 1mM NADP and 3mM $MgCl_2$ made up in 100 mM potassium phosphate buffer, pH7.4) and the quench reagent, acetonitrile (containing internal standards). Human liver microsomal protein content was fixed at 0.2 mg/mL. Incubation times, substrate concentrations at CYP isoform selective concentrations and internal standards are shown below.

The automated liquid handling system made a serial dilution of the test and control inhibitors in DMSO. The inhibition potential of test compounds was evaluated at six concentrations (0–50μM). For each incubation, HLM/substrate solution was transferred to an incubation plate, spiked with inhibitor/DMSO solution, mixed and warmed for 10 minutes to 37°C. The reactions were started by the addition of NADPH regenerating system (1mM final concentration) and stopped after between 5 and 20 minutes (dependent upon substrate used) by addition of cold acetonitrile (quench reagent containing internal standards). Quenched samples were centrifuged at 2500 g for 15 min (4°C) and then stored overnight at 4°C prior to analysis.

Enzyme	**Substrate (Reaction)**	**Substrate Conc (µM)**	**Incubation Time (mins)**	**Internal Standard**	**Control Inhibitor**
CYP2C9	Diclofenac (4'-hydroxylase)	5	5	$^{13}C_6$-4'-OH-Diclofenac	sulfaphenazole
CYP2D6	Dextromethorphan (O-demethylase)	5	20	D_3-Dextrorphan	quinidine
CYP3A	Midazolam (1'-hydroxylase)	5	5	D_4-1'-OH-Midazolam	ketoconazole

RF-MS System Setup

The RapidFire RF200 system (Agilent Technologies [Biocius], Woburn, MA) was interfaced with an API 4000 mass spectrometer. The main components include a Zymark Twister robotic arm that can handle standard microtiter plates and the RapidFire in-line purification SPE system with injection valves and a barcode reader. The entire system is operated using the RapidFire software that is interfaced with Analyst software for the mass spectrometer. Samples were aspirated directly from 384-well plates into a 10-µL sample loop and passed through a C4 cartridge (Biocius) with solvent A at a flow rate of 1.5 mL/min for 3s. After the desalting step, analyte retained on the cartridge was eluted with solvent B to the mass spectrometer at a flow rate of 0.4 mL/min for 5 s. Adding another 0.5 s from the reequilibration step, the entire sampling cycle was 9 s per well, which reduced the sample analysis time by at least 20-fold compared with the standard LC-MS analysis.

Data Processing IC_{50} Determinations

Metabolite peak areas were divided by the internal standard peak areas to give metabolite peak area ratios. After background subtraction, the metabolite peak area ratios were normalised with the mean of the zero inhibition controls set to 100%. Where enzyme inhibition was observed, IC_{50} values were estimated using the non-linear curve fitting procedure of xlfit v4 (IDBS, Guildford, UK). Within the fitting function (Eqn 1), the maximum activity asymptote was set at 100% and the minimum was set to 0%.

The program then fitted an inhibition effect curve to the data and generated the IC_{50} values and 95% confidence intervals.

$$\text{Percent Control Activity} = \frac{a}{1 + \left(\frac{\text{Inhibitor Conc}}{IC_{50}}\right)^{s}} + b$$

Eqn 1