Inhibition of Virulence-promoting Disulfide Bond Formation Enzyme DsbB is blocked by Mutating Residues in two distinct regions

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Running title: DsbB mutations resistant to pyridazinone-related molecules

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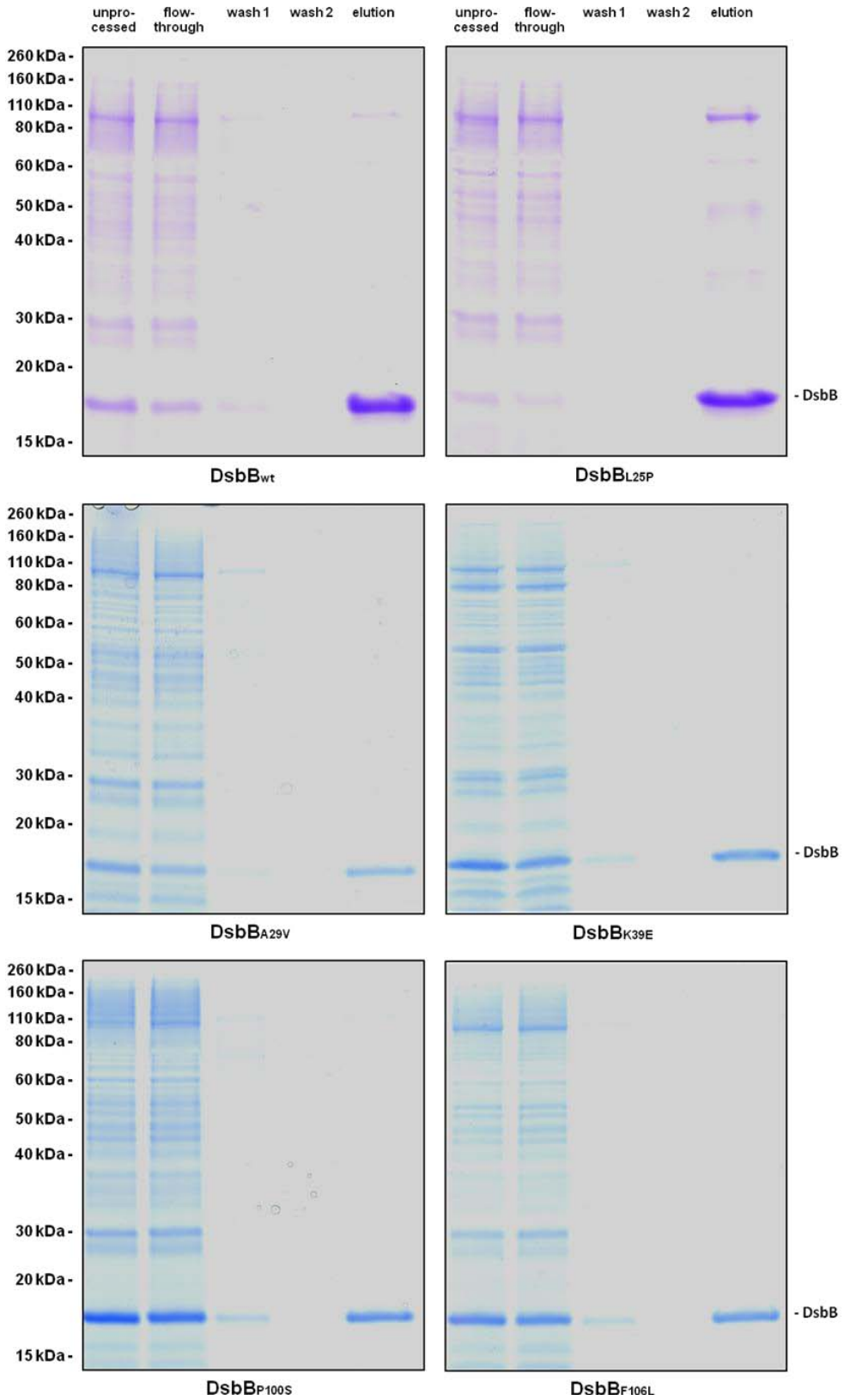
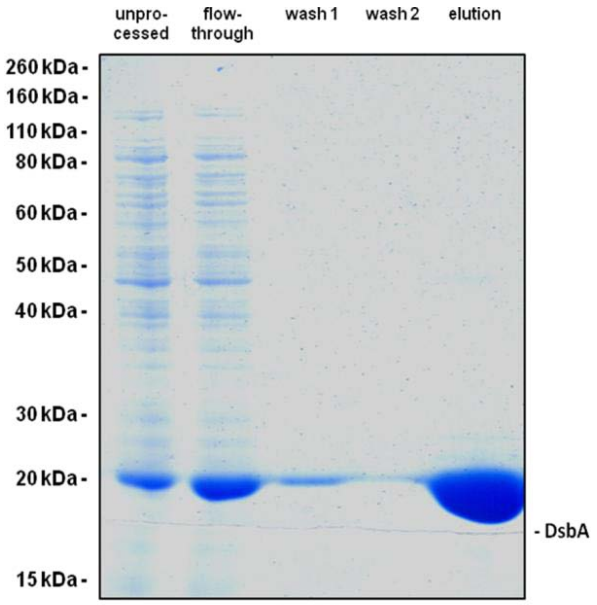
Supplementary Figure 1. Purity of purified proteins used in this study.




unpro-
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160 kDa -
110 kDa -
80 kDa -
60 kDa -
50 kDa -
40 kDa -
30 kDa -
20 kDa -
15 kDa -
- DsbA

DsbA

Final Report - Sundia_Harvard_20140604_20Targets

August 25, 2014

Name and address of CRO: SundiaMediTech Company Ltd.
Bldg. 8, 388 Jialilue Rd.
Zhangjiang Hi-tech Park
Shanghai 201203
China

Sundia Project Leader: Fangjian Zhang

Names of all experimenter: Zhihua Fang
Lei Fan
Weiwei Zhang

Name of client: Harvard

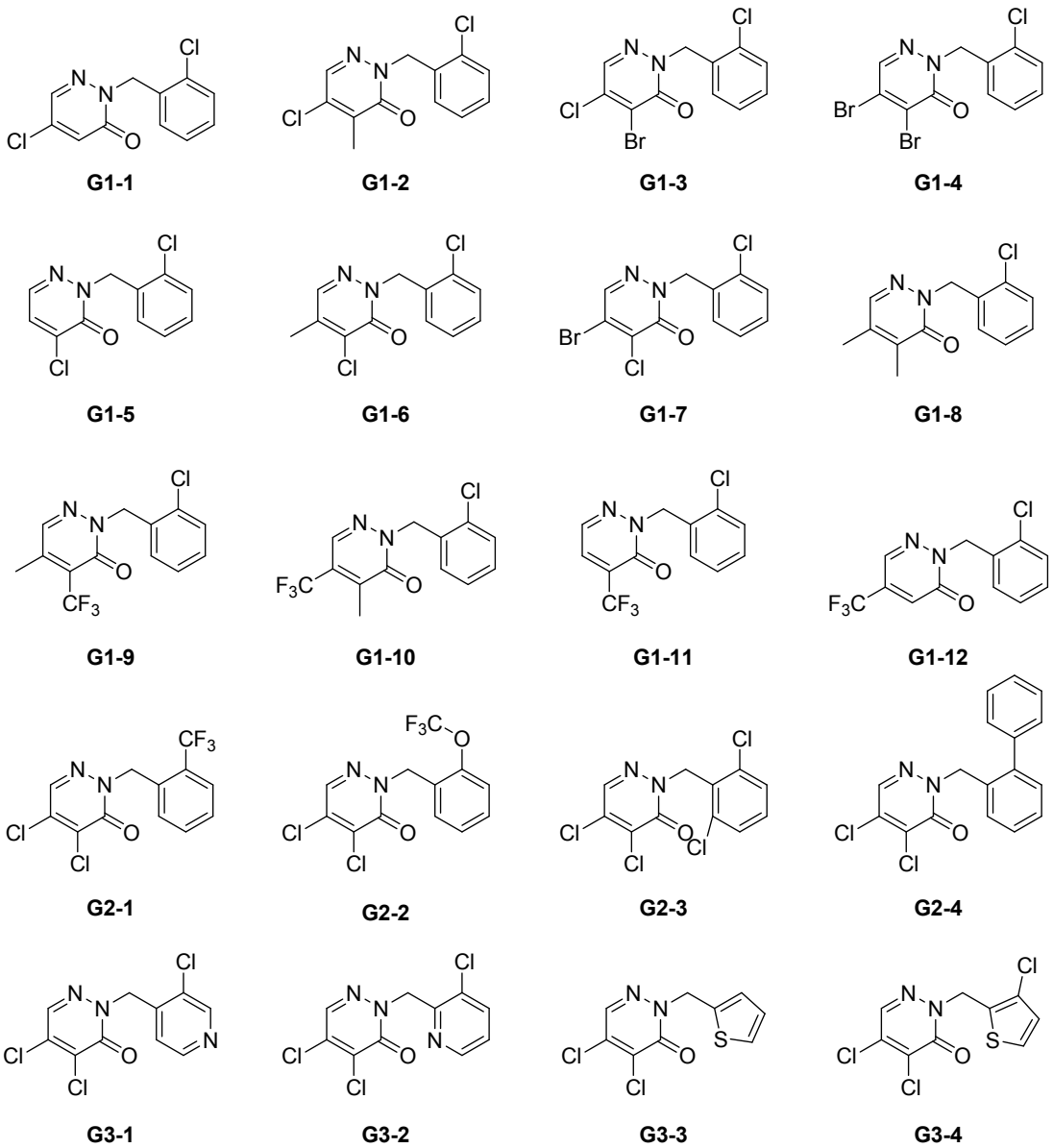
Working period: 2014.06.11-2014.07.29

Table of Contents

1. Objective

-- To synthesize twenty compounds shown in the diagram 1, each amount is >50 mg each with >95% purity (by NMR and LCMS).

Diagram 1. **Structure of Target compound**



2. Summary

Project ID: Sundia_Harvard_20140604_20Targets

PO Number: /

Project Leader: Fangjian Zhang

Names of Chemist: Zhihua Fang, Lei Fan and Weiwei Zhang

Notebook number: NB07589, NB07231 and NB07588

Date Started: 2014.06.11

Date Completed: 2014.07.29

Quantity of Delivery and Date of Delivery: 18 compounds (**G1-1**, **G1-2**, **G1-4**, **G1-5**, **G1-6**, **G1-8**, **G1-9**, **G1-10**, **G1-11**, **G1-12**, **G2-1**, **G2-2**, **G2-3**, **G2-4**, **G3-1**, **G3-2**, **G3-3** and **G3-4**) were delivered on 2014.07.01; 2 compounds (**G1-3** and **G1-7**) were delivered on 2014.07.29.

3. Experimental part

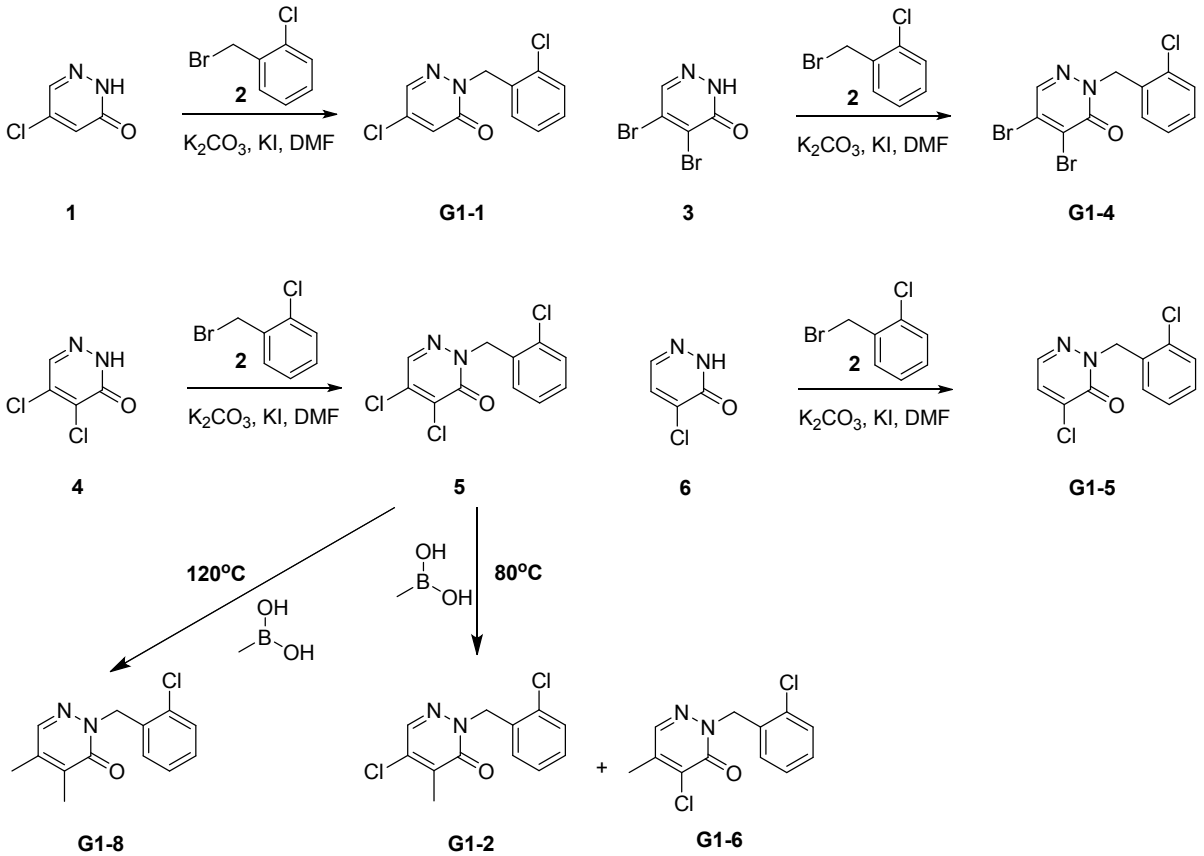
3.1 General experimental methods

^{1}H NMR spectra were recorded on Bruker Avance III 400 MHz and Varian Mercury plus 300 MHz and TMS was used as an internal standard.

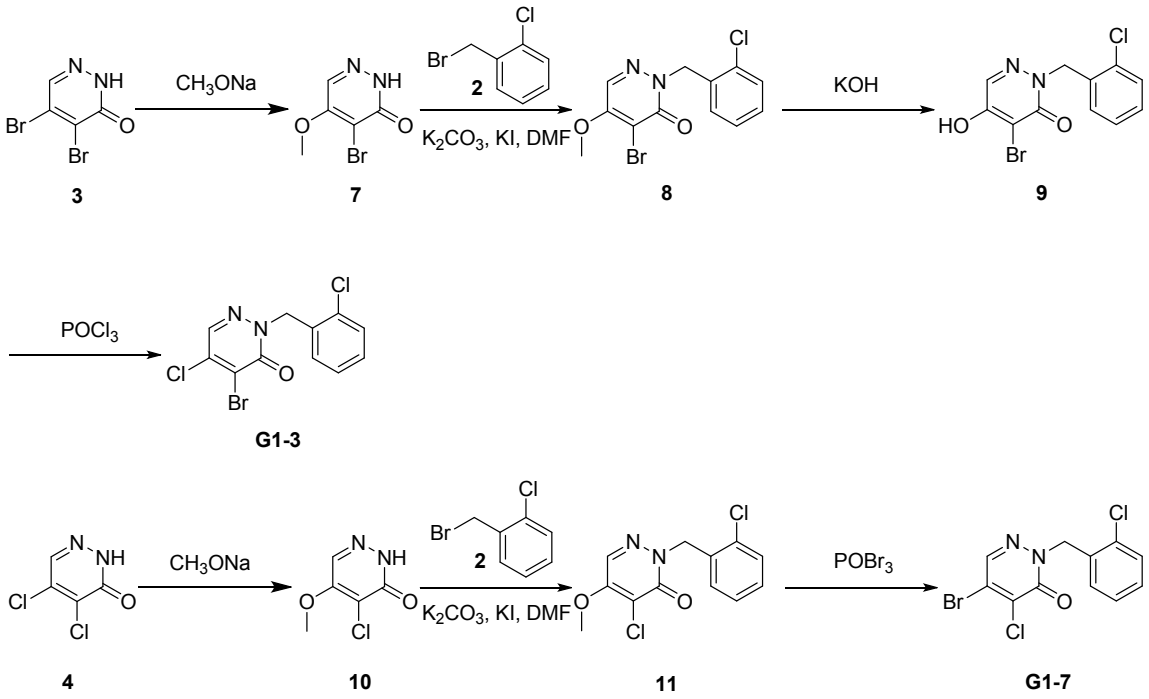
LCMS was taken on a quadrupole Mass Spectrometer on Agilent LC/MSD 1200 Series (Column: C18 (50 × 4.6 mm, 5 μm) operating in ES (+) or (-) ionization mode; T = 30 ^{o}C; flow rate = 1.5 mL/min; detected wavelength: 214 nm.

3.2 Experimental procedures

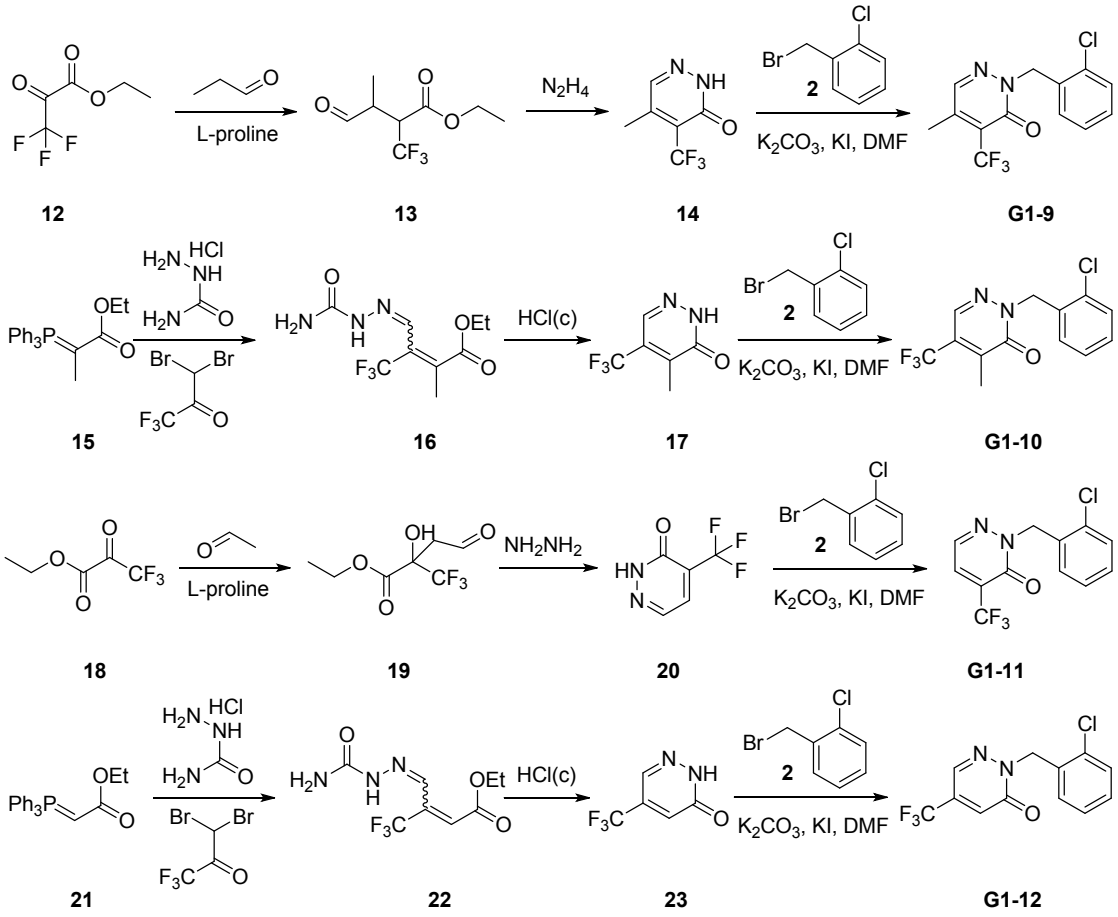
Scheme 1.



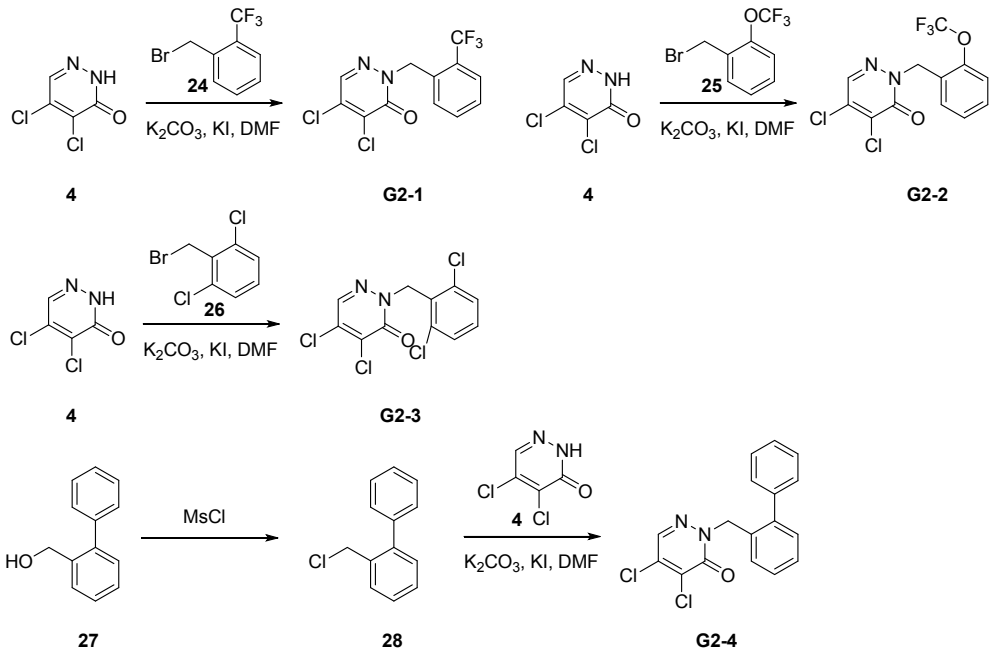
Scheme 2.



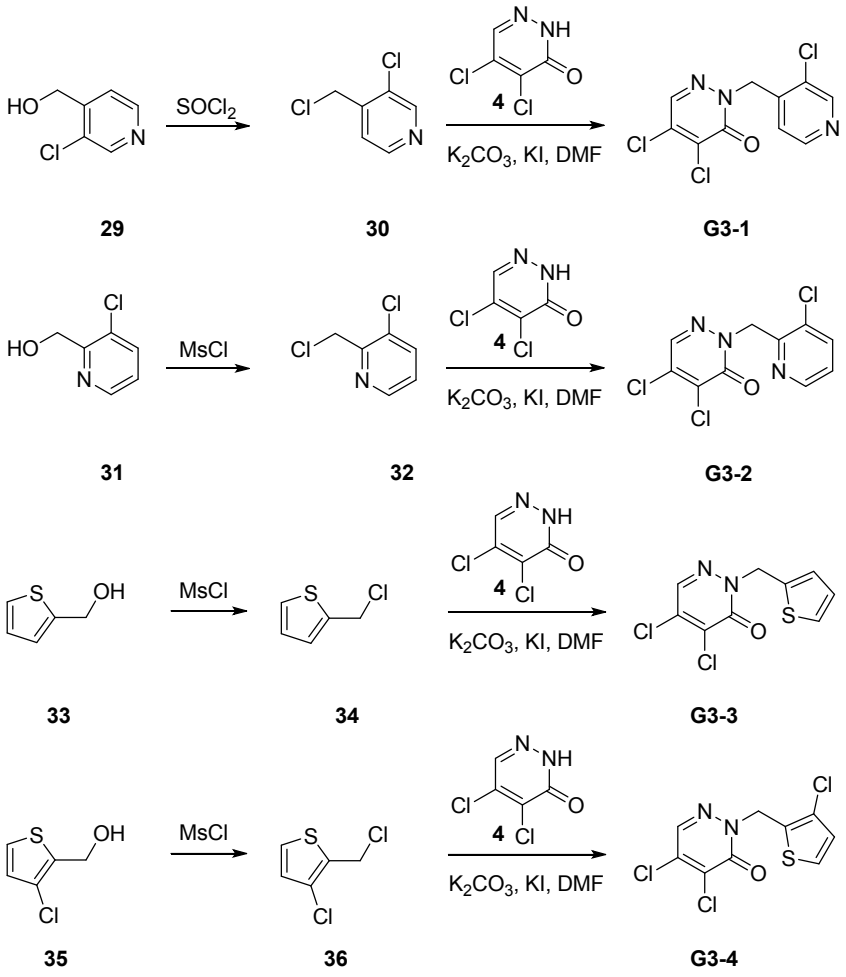
Scheme 3.



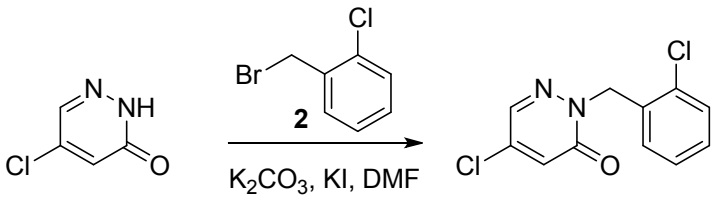
Scheme 4.



Scheme 5.



3.2.1 Synthesis of 5-chloro-2-(2-chlorobenzyl)pyridazin-3(2H)-one (**G1-1**)

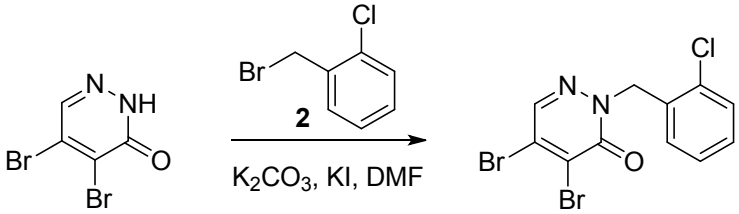


1 **G1-1**

To a solution of compound **1** (200 mg, 1.53 mmol), compound **2** (378 mg, 1.84 mmol) and K_2CO_3 (423 mg, 3.06 mmol) in DMF (3 mL) was added KI (25 mg, 0.15 mmol). The solution was stirred at 90 °C for 2h. The mixture was cooled to room temperature and quenched with water, extracted with EtOAc for 3 times, combined the organic layer, washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, purified by column chromatography [eluting with PE to PE/ EtOAc (4:1)] to give compound **G1-1** (210 mg, 54 %) as a yellow solid.

^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.31 (s, 2H), 7.14 (d, J = 7.2 Hz, 1H), 7.27-7.37 (m, 3H), 7.49 (d, J = 7.8 Hz, 1H), 8.10 (d, J = 2.1 Hz, 1H); LCMS [mobile phase: 10-95% Acetonitrile +0.02% NH_4OAc] purity is >95%, Rt = 4.239 min; MS Calcd.: 254; MS Found: 255 $(M+1)^+$.

3.2.2 Synthesis of 4,5-dibromo-2-(2-chlorobenzyl)pyridazin-3(2H)-one (**G1-4**)

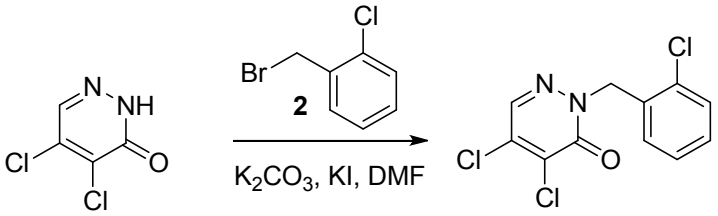


3 **G1-4**

To a solution of compound **3** (150 mg, 0.59 mmol), compound **2** (121 mg, 0.59 mmol) and K_2CO_3 (163 mg, 1.18 mmol) in DMF (3 mL) was added KI (10 mg, 0.06 mmol). The solution was stirred at 90 °C for 2h. The mixture was cooled to room temperature and quenched with water, extracted with EtOAc for 3 times, combined the organic layer, washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure to give the crude product and washed with CH_3OH to give compound **G1-4** (80 mg, 36 %) as a gray solid.

^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.36 (s, 2H), 7.19 (d, J = 11.1 Hz, 1H), 7.27-7.38 (m, 2H), 7.50 (d, J = 8.1 Hz, 1H), 8.20 (s, 1H); LCMS [mobile phase: 30-95% Acetonitrile +0.02% NH_4OAc] purity is >95%, Rt = 3.849 min; MS Calcd.: 377; MS Found: 378 $(M+1)^+$.

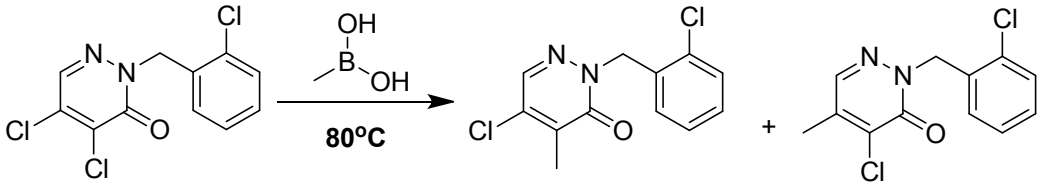
3.2.3 Synthesis of 4,5-dichloro-2-(2-chlorobenzyl)pyridazin-3(2H)-one **(5)**



4 **5**

To a solution of compound **4** (3 g, 18.2 mmol), compound **2** (4.5 g, 21.8 mmol) and K_2CO_3 (5 g, 36.4 mmol) in DMF (30 mL) was added KI (0.3 g, 1.8 mmol). The solution was stirred at 90 °C for 2h. The mixture was cooled to room temperature and quenched with water, extracted with EtOAc for 3 times, combined the organic layer, washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, purified by column chromatography [eluting with PE to PE/EtOAc (10:1)] to give compound **5** (5 g, 96 %) as a white solid.

3.2.4 Synthesis of 5-chloro-2-(2-chlorobenzyl)-4-methylpyridazin-3(2H)-one (**G1-2**) and 4-chloro-2-(2-chlorobenzyl)-5-methylpyridazin-3(2H)-one (**G1-6**)



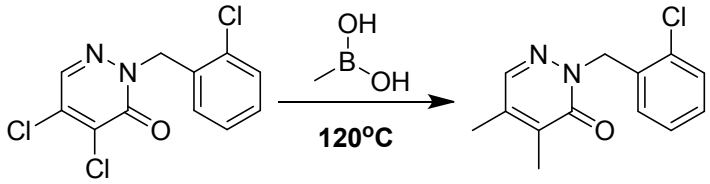
5 **G1-2** **G1-6**

To a solution of compound **5** (900 mg, 3.1 mmol), Methylboronic acid (187 mg, 3.1 mmol), TBAB (100 mg, 0.3 mmol) and K_2CO_3 (1074 mg, 7.8 mmol) in Dioxane/H_2O (10 mL/ 3 mL) was added $Pd(PPh_3)_2Cl_2$ (219 mg, 0.3 mmol). The solution was stirred at 80 °C overnight. The mixture was cooled to room temperature and quenched with water, extracted with EtOAc for 3 times, combined the organic layer, washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, purified by HPLC to give **G1-2** (115 mg) and **G1-6** (130 mg) as a white solid.

G1-2: ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.19 (s, 3H), 5.33 (s, 2H), 7.12 (d, J = 7.2 Hz, 1H), 7.28-7.36 (m, 2H), 7.49 (d, J = 7.8 Hz, 1H), 8.07 (s, 1H); LCMS [mobile phase: 30-95% Acetonitrile +0.02% NH_4OAc] purity is >95%, Rt = 3.718 min; MS Calcd.: 269; MS Found: 270 $(M+1)^+$.

G1-6: ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.29 (s, 3H), 5.37 (s, 2H), 7.11 (d, J = 7.5 Hz, 1H), 7.29-7.37 (m, 2H), 7.49 (d, J = 7.8 Hz, 1H), 7.98 (s, 1H); LCMS [mobile phase: 30-95% Acetonitrile +0.02% NH_4OAc] purity is >95%, Rt = 3.237 min; MS Calcd.: 269; MS Found: 270 $(M+1)^+$.

3.2.5 Synthesis of 2-(2-chlorobenzyl)-4,5-dimethylpyridazin-3(2H)-one **(G1-8)**

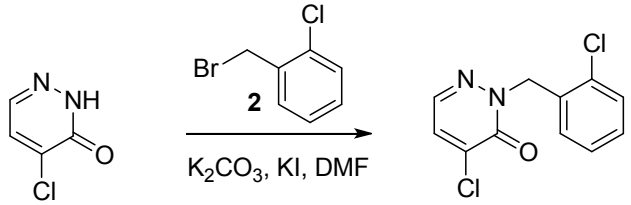


5 **G1-8**

To a solution of compound **5** (500 mg, 1.7 mmol), Methylboronic acid (208 mg, 3.5 mmol), TBAB (56 mg, 0.2 mmol) and K_2CO_3 (597 mg, 4.3 mmol) in Dioxane/H_2O (6 mL / 2 mL) was added $Pd(PPh_3)_2Cl_2$ (121 mg, 0.2 mmol). The solution was stirred at 120 °C overnight. The mixture was cooled to room temperature and quenched with water, extracted with EtOAc for 3 times, combined the organic layer, washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, purified by HPLC to give **G1-8** (207 mg, 48%) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.05 (s, 3H), 2.16 (s, 3H), 5.31 (s, 2H), 7.00 (d, J = 7.5 Hz, 1H), 7.26-7.34 (m, 2H), 7.48 (d, J = 7.5 Hz, 1H), 7.79 (s, 1H); LCMS [mobile phase: 30-95% Acetonitrile +0.02% NH_4OAc] purity is >95%, Rt = 3.019 min; MS Calcd.: 249; MS Found: 250 $(M+1)^+$.

3.2.6 Synthesis of 4-chloro-2-(2-chlorobenzyl)pyridazin-3(2H)-one **(G1-5)**



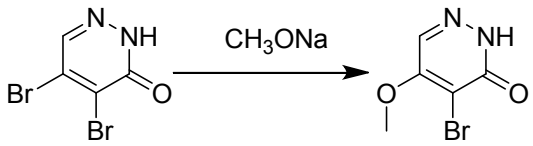
6 **G1-5**

To a solution of compound **6** (200 mg, 1.5 mmol), compound **2** (378 mg, 1.8 mmol) and K_2CO_3

(422 mg, 3.1 mmol) in DMF (3 mL) was added KI (25 mg, 0.15 mmol). The solution was stirred at 90 °C for 2h. The mixture was cooled to room temperature and quenched with water, extracted with EtOAc for 3 times, combined the organic layer, washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, purified by HPLC to give **G1-5** (180 mg, 46%) as a white solid.

^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.52 (s, 2H), 7.20-7.26 (m, 3H), 7.38-7.41 (m, 2H), 7.71 (d, J = 1.5 Hz, 1H); LCMS [mobile phase: 10-95% Acetonitrile +0.02% NH_4OAc] purity is >95%, Rt = 4.050 min; MS Calcd.: 255; MS Found: 256 $(M+1)^+$.

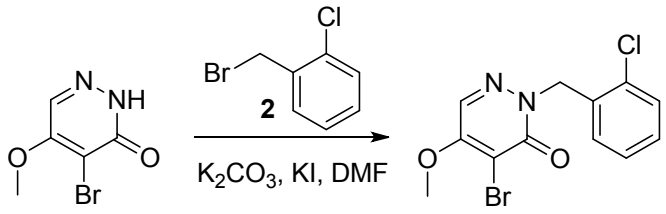
3.2.7 Synthesis of 4-bromo-5-methoxypyridazin-3(2H)-one **(7)**



3 **7**

To a solution of compound **3** (4 g, 15.7 mmol) in CH_3OH (50 mL) was added CH_3ONa (2.6 g, 47.2 mmol). The solution was stirred at 80 °C overnight. The mixture was concentrated under reduced pressure, purified by column chromatography [eluting with PE to PE/DCM/MeOH (10:1:1)] to give compound **7** (1.1 g, 35%) as a white solid.

3.2.8 Synthesis of 4-bromo-2-(2-chlorobenzyl)-5-methoxypyridazin-3(2H)-one **(8)**

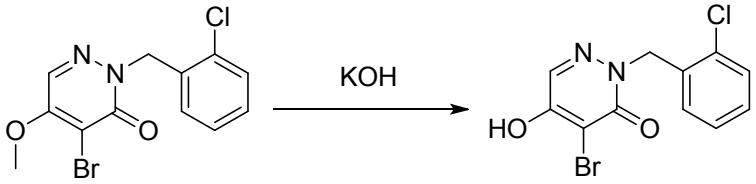


7 **8**

To a solution of compound **7** (1 g, 4.88 mmol), compound **2** (1.1 g, 5.37 mmol) and K_2CO_3 (1.3 g, 9.76 mmol) in DMF (15 mL) was added KI (81 mg, 0.49 mmol). The solution was stirred at 90 °C for 2h. The mixture was cooled to room temperature and quenched with water, extracted with EtOAc for 3 times, combined the organic layer, washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, purified by column chromatography [eluting with PE to

PE/EtOAc (5:1)] to give compound **8** (350 mg, 22 %) as a white solid.

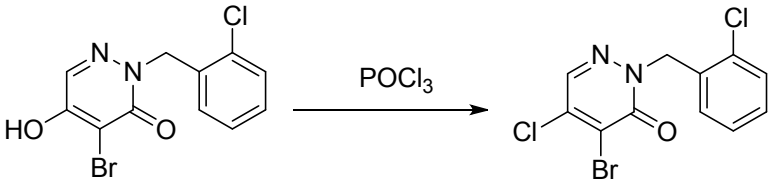
3.2.9 Synthesis of 4-bromo-2-(2-chlorobenzyl)-5-hydroxypyridazin-3(2H)-one **(9)**



8 **9**

To a solution of compound **8** (350 mg, 1.06 mmol) in H_2O (3 mL) was added KOH (119 mg, 2.12 mmol). The solution was stirred at reflux overnight. The mixture was cooled to room temperature and neutralized with concentrated HCl and extracted with EtOAc, washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure to give compound **9** (322 mg, 91%) as a white solid.

3.2.10 Synthesis of 4-bromo-5-chloro-2-(2-chlorobenzyl)pyridazin-3(2H)-one **(G1-3)**

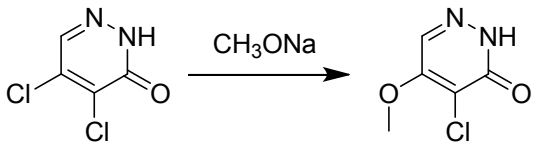


9 **G1-3**

A solution of compound **9** (322 mg, 1.02 mmol) in $POCl_3$ (3 mL) was stirred at 100 °C overnight. The mixture was cooled to room temperature and quenched with water and sat. NaOH, extracted with EtOAc, washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, purified by HPLC to give compound **G1-3** (122 mg, 36%) as a white solid.

^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.52 (s, 2H), 7.12-7.28 (m, 3H), 7.38-7.41 (m, 1H), 7.71 (d, J = 4.5 Hz, 1H); LCMS [mobile phase: 10-95% Acetonitrile +0.02% NH_4OAc] purity is >95%, Rt = 4.050 min; MS Calcd.: 333; MS Found: 334 $(M+1)^+$.

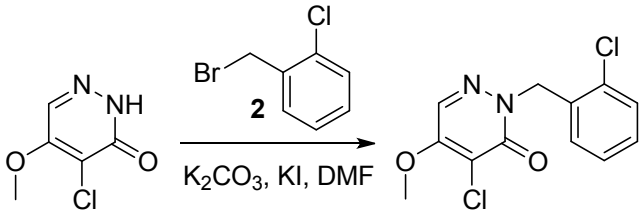
3.2.11 Synthesis of 4-chloro-5-methoxypyridazin-3(2H)-one **(10)**



4 **10**

To a solution of compound **4** (4.1 g, 24.8 mmol) in CH_3OH (50 mL) was added CH_3ONa (2.6 g, 74.5 mmol). The solution was stirred at 80 °C overnight. The mixture was concentrated under reduced pressure, purified by column chromatography [eluting with PE to PE/DCM/MeOH (10:1:1)] to give **10** (1.2 g, 30%) as a white solid.

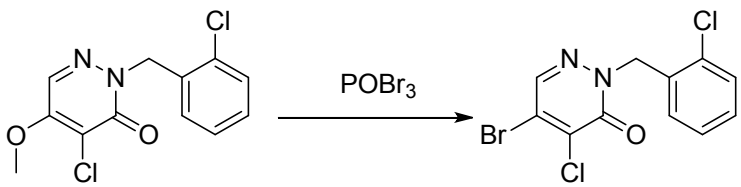
3.2.12 Synthesis of 4-chloro-2-(2-chlorobenzyl)-5-methoxypyridazin-3(2H)-one **(11)**



10 **11**

To a solution of compound **10** (1 g, 6.25 mmol), compound **2** (1.5 g, 7.50 mmol) and K_2CO_3 (1.7 g, 12.5 mmol) in DMF (15 mL) was added KI (104 mg, 0.63 mmol). The solution was stirred at 90 °C for 2h. The mixture was cooled to room temperature and quenched with water, extracted with EtOAc for 3 times, combined the organic layer, washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, purified by column chromatography [eluting with PE to PE/EtOAc (4:1)] to give compound **11** (700 mg, 44 %) as a white solid.

3.2.13 Synthesis of 5-bromo-4-chloro-2-(2-chlorobenzyl)pyridazin-3(2H)-one **(G1-7)**

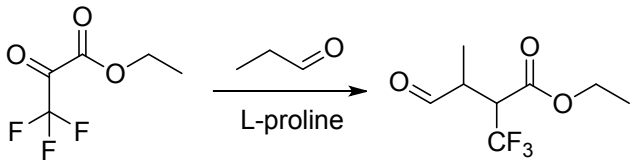


11 **G1-7**

A solution of compound **11** (322 mg, 1.02 mmol) and $POBr_3$ (4.2 g, 14.7 mmol) was stirred at 100

°C overnight. The mixture was cooled to room temperature and quenched with water and sat. NaOH, extracted with EtOAc, washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, purified by HPLC to give compound **G1-7** (35 mg, 4.3%) as a white solid.
^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.47 (s, 2H), 7.23-7.29 (d, *J* = 15.9 Hz, 3H), 7.39-7.41 (d, *J* = 7.2 Hz, 1H), 7.88 (s, 1H); LCMS [mobile phase: 30-95% Acetonitrile +0.02% NH_4OAc] purity is >95%, Rt = 4.050 min; MS Calcd.: 333; MS Found: 334 $(M+1)^+$.

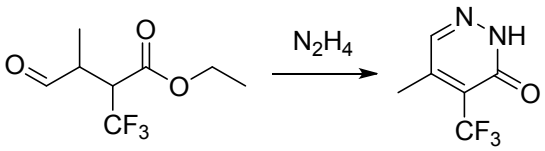
3.2.14 Synthesis of ethyl 3-methyl-4-oxo-2-(trifluoromethyl)butanoate **(13)**



12 **13**

To a solution of compound **12** (10 g, 58.8 mmol), Propionaldehyde (3.4 g, 58.8 mmol) in DCM (100 mL) was added L-proline (3.4 g, 29.4 mmol). The solution was stirred at room temperature for 2h. The mixture was quenched with water, extracted with DCM, washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure to give compound **13** (12 g, 97 %) as an orange liquid.

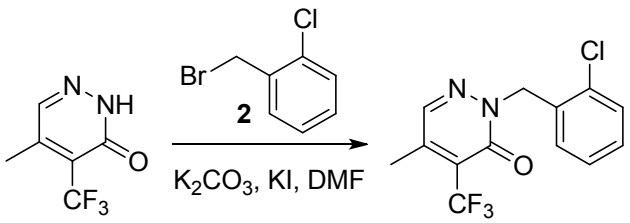
3.2.15 Synthesis of 5-methyl-4-(trifluoromethyl)pyridazin-3(2H)-one **(14)**



13 **14**

To a solution of compound **13** (3 g, 14.3 mmol) in EtOH (30 mL) was added 85% $N_2H_4.H_20$ (1.3 g, 21.5 mmol). The solution was stirred at room temperature for 1h, and then heated at reflux for 2h. The mixture was extracted with EtOAc, washed with water and brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, purified by column chromatography [eluting with PE to PE/EtOAc (5:1)] to give compound **14** (1.3 g, 52 %) as a white solid.

3.2.16 Synthesis of 2-(2-chlorobenzyl)-5-methyl-4-(trifluoromethyl)pyridazin-3(2H)-one **(G1-9)**

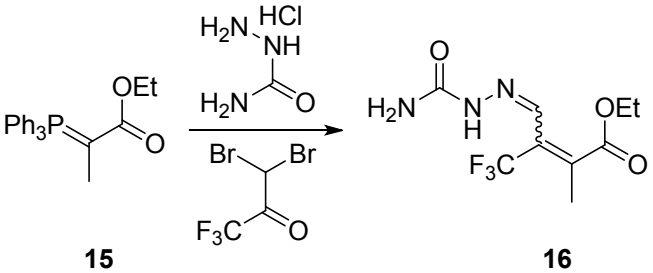


14 **G1-9**

To a solution of compound **14** (300 mg, 1.68 mmol), compound **2** (380 mg, 1.85 mmol) and K_2CO_3 (464 mg, 3.36 mmol) in DMF (3 mL) was added KI (28 mg, 0.17 mmol). The solution was stirred at 90 °C for 2h. The mixture was cooled to room temperature and quenched with water, extracted with EtOAc for 3 times, combined the organic layer, washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, purified by HPLC to give **G1-9** (190 mg, 38%) as a white solid.

^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.37-2.40 (m, 3H), 5.35 (s, 2H), 7.17 (d, J = 9.3 Hz, 1H), 7.29-7.37 (m, 2H), 7.49 (d, J = 9.0 Hz, 1H), 8.01 (s, 1H); LCMS [mobile phase: 20-95% Acetonitrile +0.02% NH_4OAc] purity is >95%, Rt = 4.010 min; MS Calcd.: 303; MS Found: 304 $(M+1)^+$.

3.2.17 Synthesis of ethyl 3-((2-carbamoylhydrazono)methyl)-4,4,4-trifluoro-2-methylbut-2-enoate **(16)**

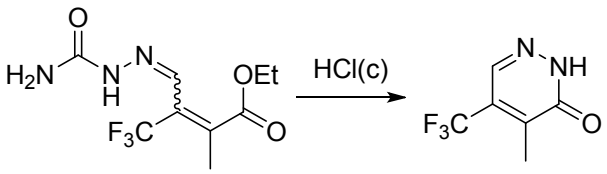


15 **16**

Sodium acetate (12 g, 148 mmol) was added to 3,3-dibromo-1,1,1-trifluoroacetone (10 g, 37 mmol) in water (120 mL) and heated to 80 °C for 90 minutes. The reaction mixture was cooled to room temperature, Semicarbazide hydrochloride (5 g, 44 mmol) was added and stirred at room temperature for 5h. A white precipitate was formed, which was filtered and dried in vacuo. The dried solid was combined with compound **15** (13.4 g, 37 mmol) in THF (100 mL) and stirred overnight. The reaction mixture was then concentrated, water was added, and the mixture was

extracted with EtOAc, washed with water and brine, dried over Na_2SO_4, concentrated in vacuo to give **16** (3.2 g, 32%) as a yellow solid.

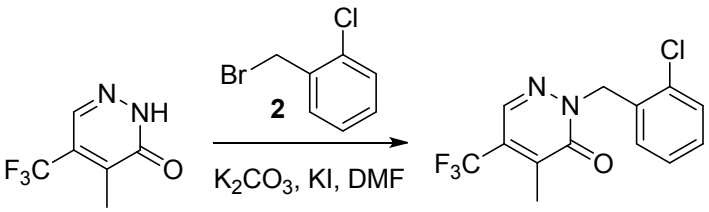
3.2.18 Synthesis of 4-methyl-5-(trifluoromethyl)pyridazin-3(2H)-one **(17)**



16 **17**

A solution of compound **16** (3.2 g, 12 mmol) in concentrated HCl (35 mL) was stirred at reflux for 4h, the reaction mixture was allowed to cool to room temperature overnight. Water was added, the mixture was extracted with DCM, washed with water and brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, purified by column chromatography [eluting with PE to PE/EtOAc (5:1)] to give compound **17** (1.3 g, 62 %) as a white solid.

3.2.19 Synthesis of 2-(2-chlorobenzyl)-4-methyl-5-(trifluoromethyl)pyridazin-3(2H)-one **(G1-10)**

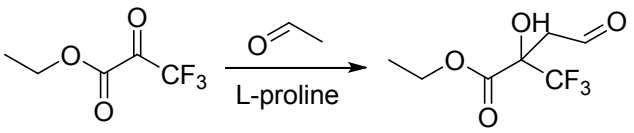


17 **G1-10**

To a solution of compound **17** (300 mg, 1.68 mmol), compound **2** (380 mg, 1.85 mmol) and K_2CO_3 (464 mg, 3.36 mmol) in DMF (3 mL) was added KI (28 mg, 0.17 mmol). The solution was stirred at 90 °C for 2h. The mixture was cooled to room temperature and quenched with water, extracted with EtOAc for 3 times, combined the organic layer, washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, purified by HPLC to give **G1-10** (150 mg, 30%) as a white solid.

^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.27-2.29 (m, 3H), 5.39 (s, 2H), 7.17 (d, J = 7.5 Hz, 1H), 7.28-7.38 (m, 2H), 7.49 (d, J = 7.5 Hz, 1H), 8.18 (s, 1H); LCMS [mobile phase: 30-95% Acetonitrile +0.02% NH_4OAc] purity is >95%, Rt = 3.957 min; MS Calcd.: 303; MS Found: 304 $(M+1)^+$.

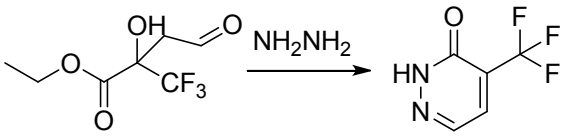
3.2.20 Synthesis of (S)-ethyl 2-hydroxy-4-oxo-2-(trifluoromethyl)butanoate **(19)**



18 **19**

To a solution of compound **18** (5 g, 29.4 mmol), Acetaldehyde (1.3 g, 29.4 mmol) in DCM (50 mL) was added L-proline (1.7 g, 14.7 mmol). The solution was stirred at room temperature for 2h. The mixture was quenched with water, extracted with DCM, washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure to give compound **19** (5 g, 87 %) as an orange oil.

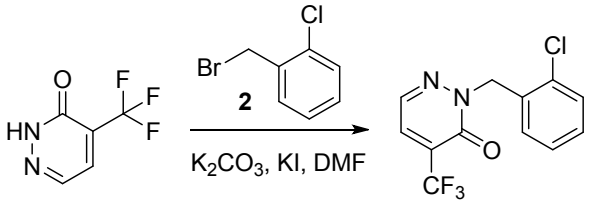
3.2.21 Synthesis of 4-(trifluoromethyl)pyridazin-3(2H)-one **(20)**



19 **20**

To a solution of compound **19** (5 g, 25.6 mmol) in EtOH (50 mL) was added 85% $N_2H_4 \cdot H_20$ (2.3 g, 38.5 mmol). The solution was stirred at room temperature for 1h, and then heated at reflux for 2h. The mixture was extracted with EtOAc, washed with water and brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, purified by column chromatography [eluting with PE to PE/EtOAc (10:1)] to give compound **20** (1.2 g, 28 %) as a white solid.

3.2.22 Synthesis of 2-(2-chlorobenzyl)-4-(trifluoromethyl)pyridazin-3(2H)-one **(G1-11)**

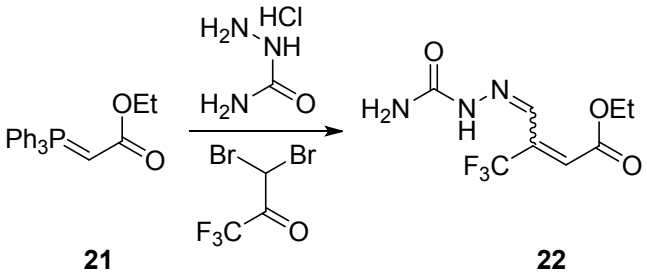


20 **G1-11**

To a solution of compound **20** (200 mg, 1.22 mmol), compound **2** (301 mg, 1.46 mmol) and K_2CO_3 (337 mg, 2.44 mmol) in DMF (3 mL) was added KI (20 mg, 0.12 mmol). The solution was stirred at 100 °C for 2h. The mixture was cooled to room temperature and quenched with water, extracted with EtOAc for 3 times, combined the organic layer, washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, purified by HPLC to give **G1-11** (160 mg, 46%) as a white solid.

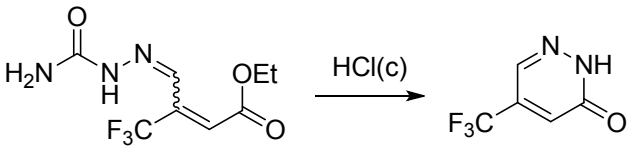
^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.42 (s, 2H), 7.20 (d, J = 6.3 Hz, 1H), 7.03-7.39 (m, 2H), 7.50 (d, J = 7.2 Hz, 1H), 7.96 (d, J = 2.4 Hz, 1H), 8.15 (d, J = 3.6 Hz, 1H); LCMS [mobile phase: 20-95% Acetonitrile +0.02% NH_4OAc] purity is >95%, Rt = 3.873 min; MS Calcd.: 289; MS Found: 290 $(M+1)^+$.

3.2.23 Synthesis of ethyl 3-((2-carbamoylhydrazono)methyl)-4,4,4-trifluorobut-2-enoate **(22)**



Sodium acetate (12.1 g, 148 mmol) was added to 3,3-dibromo-1,1,1-trifluoroacetone (10 g, 37 mmol) in water (120 mL) and heated to 80 °C for 90 minutes. The reaction mixture was cooled to room temperature, Semicarbazide hydrochloride (5 g, 44 mmol) was added and stirred at room temperature for 5h. A white precipitate was formed, which was filtered and dried in vacuo. The dried solid was combined with compound **21** (12.9 g, 37 mmol) in THF (100 mL) and stirred overnight. The reaction mixture was then concentrated, water was added, and the mixture was extracted with EtOAc, washed with water and brine, dried over Na_2SO_4, concentrated in vacuo to give **22** (3 g, 32%) as a yellow solid.

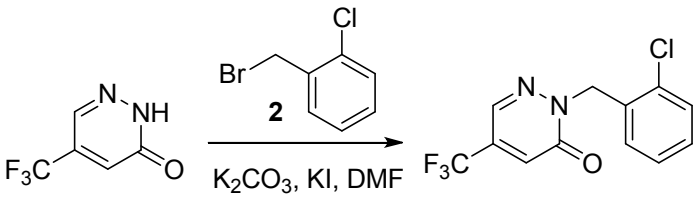
3.2.24 Synthesis of 5-(trifluoromethyl)pyridazin-3(2H)-one **(23)**



22 **23**

A solution of compound **22** (3 g, 11.9 mmol) in concentrated HCl (40 mL) was stirred at reflux for 4h, the reaction mixture was allowed to cool to room temperature overnight. Water was added, the mixture was extracted with DCM, washed with water and brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, purified by column chromatography [eluting with PE to PE/EtOAc (5:1)] to give compound **23** (1.3 g, 76 %) as a white solid.

3.2.25 Synthesis of 2-(2-chlorobenzyl)-5-(trifluoromethyl)pyridazin-3(2H)-one **(G1-12)**

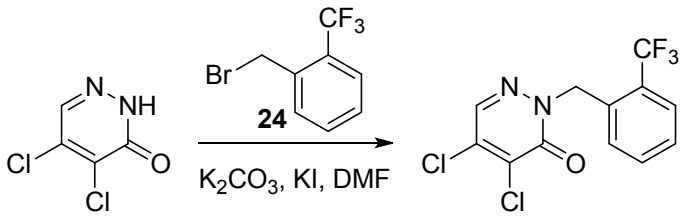


23 **G1-12**

To a solution of compound **23** (300 mg, 1.83 mmol), compound **2** (451 mg, 2.20 mmol) and K_2CO_3 (505 mg, 3.66 mmol) in DMF (3 mL) was added KI (30 mg, 0.18 mmol). The solution was stirred at 90 °C for 2h. The mixture was cooled to room temperature and quenched with water, extracted with EtOAc for 3 times, combined the organic layer, washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, purified by HPLC to give **G1-12** (80 mg, 15%) as a white solid.

^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.39 (s, 2H), 7.19 (d, J = 7.5 Hz, 1H), 7.29-7.39 (m, 2H), 7.50 (d, J = 7.5 Hz, 1H), 7.58 (s, 1H), 8.35 (d, J = 2.4 Hz, 1H); LCMS [mobile phase: 20-95% Acetonitrile +0.02% NH_4OAc] purity is >95%, Rt = 3.977 min; MS Calcd.: 289; MS Found: 290 $(M+1)^+$.

3.2.26 Synthesis of 4,5-dichloro-2-(2-(trifluoromethyl)benzyl)pyridazin-3(2H)-one **(G2-1)**

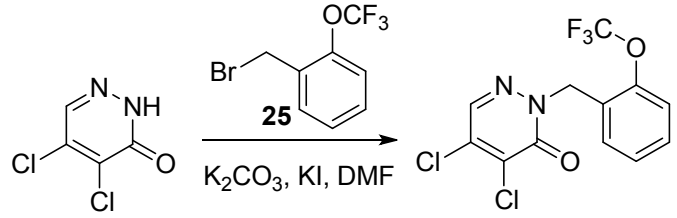


4 **G2-1**

To a solution of compound **4** (200 mg, 1.21 mmol), compound **24** (348 mg, 1.45 mmol) and K_2CO_3 (335 mg, 2.42 mmol) in DMF (3 mL) was added KI (20 mg, 0.12 mmol). The solution was stirred at 90 °C for 2h. The mixture was cooled to room temperature and quenched with water, extracted with EtOAc for 3 times, combined the organic layer, washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, purified by HPLC to give **G2-1** (230 mg, 59 %) as a white solid.

^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.46 (s, 2H), 7.23 (d, J = 7.5 Hz, 1H), 7.51-7.66 (m, 2H), 7.79 (d, J = 7.8 Hz, 1H), 8.29 (s, 1H); LCMS [mobile phase: 30-95% Acetonitrile +0.02% NH_4OAc] purity is >95%, Rt = 3.901 min; MS Calcd.: 323; MS Found: 324 $(M+1)^+$.

3.2.27 Synthesis of 4,5-dichloro-2-(2-(trifluoromethoxy)benzyl)pyridazin-3(2H)-one **(G2-2)**

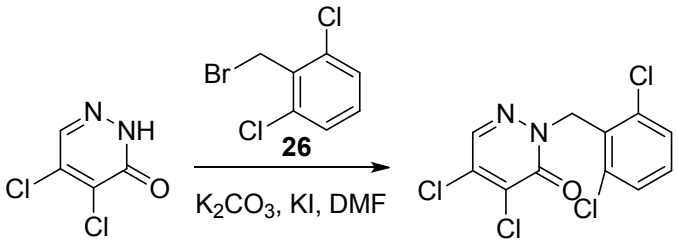


4 **G2-2**

To a solution of compound **4** (200 mg, 1.21 mmol), compound **25** (371 mg, 1.45 mmol) and K_2CO_3 (335 mg, 2.42 mmol) in DMF (3 mL) was added KI (20 mg, 0.12 mmol). The solution was stirred at 90 °C for 2h. The mixture was cooled to room temperature and quenched with water, extracted with EtOAc for 3 times, combined the organic layer, washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, purified by HPLC to give **G2-2** (220 mg, 54%) as a white solid.

^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.36 (s, 2H), 7.36-7.41 (m, 3H), 7.44-7.51 (m, 1H), 8.25 (s, 1H); LCMS [mobile phase: 30-95% Acetonitrile +0.02% NH_4OAc] purity is >95%, Rt = 3.989 min; MS Calcd.: 339; MS Found: 340 $(M+1)^+$.

3.2.28 Synthesis of 4,5-dichloro-2-(2,6-dichlorobenzyl)pyridazin-3(2H)-one **(G2-3)**

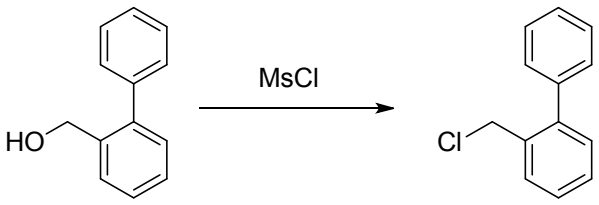


4 **G2-3**

To a solution of compound **4** (200 mg, 1.21 mmol), compound **26** (349 mg, 1.45 mmol) and K_2CO_3 (335 mg, 2.42 mmol) in DMF (3 mL) was added KI (20 mg, 0.12 mmol). The solution was stirred at 90 °C for 2h. The mixture was cooled to room temperature and quenched with water, extracted with EtOAc for 3 times, combined the organic layer, washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure to give the crude product and washed with CH_3OH to give compound **G2-3** (220 mg, 56 %) as a brown solid.

^1H NMR ($CDCl_3$, 300 MHz): δ 5.61 (s, 2H), 7.23-7.28 (m, 1H), 7.35-7.38 (m, 2H), 7.67 (s, 1H); LCMS [mobile phase: 30-95% Acetonitrile +0.02% NH_4OAc] purity is >95%, Rt = 3.953 min; MS Calcd.: 324; MS Found: 325 $(M+1)^+$.

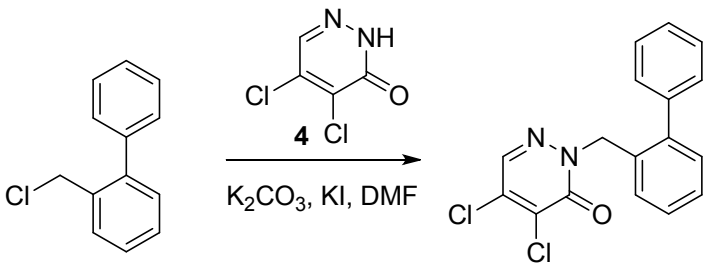
3.2.29 Synthesis of 2-(chloromethyl)-1,1'-biphenyl **(28)**



27 **28**

To a solution of compound **27** (400 mg, 2.17 mmol) in DCM (4 mL) was added DIEA (840 mg, 6.51 mmol) at 0 °C, then MsCl (273 mg, 2.39 mmol) was added dropwise at 0 °C. The mixture was stirred at room temperature for 3h. Water was added, and mixture was extracted with DCM, washed with sat. $NaHCO_3$ and brine, dried over Na_2SO_4,, filtered, concentrated under reduced pressure to give compound **28** (350 mg, 80 %) as a colorless oil.

3.2.30 Synthesis of 2-([1,1'-biphenyl]-2-ylmethyl)-4,5-dichloropyridazin-3(2H)-one **(G2-4)**

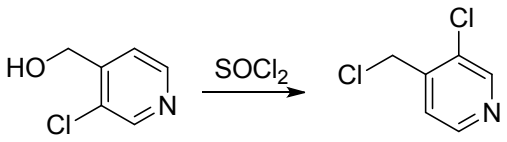


28 **G2-4**

To a solution of compound **4** (200 mg, 1.21 mmol), compound **28** (295 mg, 1.45 mmol) and K_2CO_3 (335 mg, 2.42 mmol) in DMF (3 mL) was added KI (20 mg, 0.12 mmol). The solution was stirred at 90 °C for 2h. The mixture was cooled to room temperature and quenched with water, extracted with EtOAc for 3 times, combined the organic layer, washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, purified by HPLC to give **G2-4** (200 mg, 50%) as a white solid.

^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.26 (s, 2H), 7.16-7.19 (m, 1H), 7.24-7.26 (m, 1H), 7.31-7.46 (m, 7H), 8.16 (s, 1H); LCMS [mobile phase: 30-95% Acetonitrile +0.02% NH_4OAc] purity is >95%, Rt = 4.218 min; MS Calcd.: 331; MS Found: 332 $(M+1)^+$.

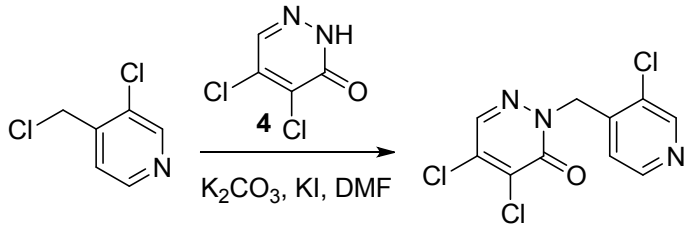
3.2.31 Synthesis of 3-chloro-4-(chloromethyl)pyridine **(30)**



29 **30**

To a solution of compound **29** (250 mg, 1.74 mmol) in DCM (5 mL) was added $SOCl_2$ (249 mg, 2.09 mmol) and DMF (cat) at 0 °C. The mixture was stirred at room temperature for 3h. Water was added, and the mixture was extracted with DCM, washed with sat. $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure to give compound **30** (240 mg, 85 %) as a yellow oil.

3.2.32 Synthesis of 4,5-dichloro-2-((3-chloropyridin-4-yl)methyl)pyridazin-3(2H)-one **(G3-1)**

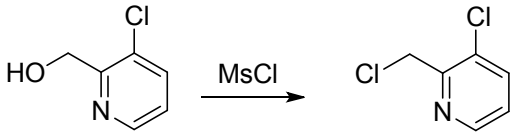


30 **G3-1**

To a solution of compound **4** (200 mg, 1.21 mmol), compound **30** (235 mg, 1.45 mmol) and K_2CO_3 (335 mg, 2.42 mmol) in DMF (3 mL) was added KI (20 mg, 0.12 mmol). The solution was stirred at 90 °C for 2h. The mixture was cooled to room temperature and quenched with water, extracted with EtOAc for 3 times, combined the organic layer, washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, purified by HPLC to give **G3-1** (135 mg, 47%) as a brown solid.

^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.40 (s, 2H), 7.23 (d, J = 5.1 Hz, 1H), 8.31 (s, 1H), 8.47 (d, J = 4.8 Hz, 1H), 8.67 (s, 1H); LCMS [mobile phase: 20-95% Acetonitrile +0.02% NH_4OAc] purity is >95%, Rt = 3.163 min; MS Calcd.: 291; MS Found: 292 $(M+1)^+$.

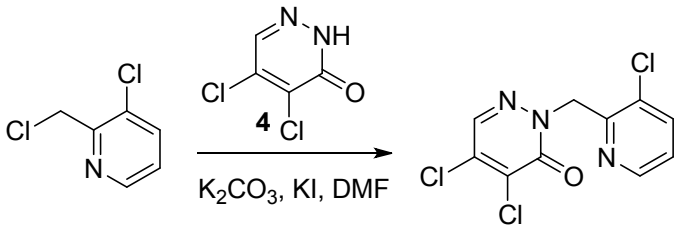
3.2.33 Synthesis of 3-chloro-2-(chloromethyl)pyridine **(32)**



31 **32**

To a solution of compound **31** (300 mg, 2.1 mmol) in DCM (4 mL) was added DIEA (539 mg, 4.2 mmol) at 0 °C, then MsCl (263 mg, 2.3 mmol) was added dropwise at 0 °C. The mixture was stirred at room temperature for 3h. Water was added, and the mixture was extracted with DCM, washed with sat. $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure to give compound **32** (240 mg, 69 %) as a yellow oil.

3.2.34 Synthesis of 4,5-dichloro-2-((3-chloropyridin-2-yl)methyl)pyridazin-3(2H)-one **(G3-2)**

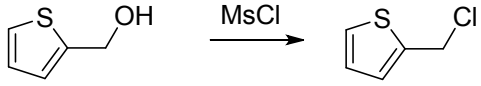


32 **G3-2**

To a solution of compound **4** (200 mg, 1.21 mmol), compound **32** (235 mg, 1.45 mmol) and K_2CO_3 (335 mg, 2.42 mmol) in DMF (3 mL) was added KI (20 mg, 0.12 mmol). The solution was stirred at 90 °C for 2h. The mixture was cooled to room temperature and quenched with water, extracted with EtOAc for 3 times, combined the organic layer, washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, purified by HPLC to give **G3-2** (126 mg, 42%) as a yellow solid.

^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.53 (s, 2H), 7.38-7.42 (m, 1H), 7.99 (d, J = 8.1 Hz, 1H), 8.27 (s, 1H), 8.41 (d, J = 4.5 Hz, 1H); LCMS [mobile phase: 20-95% Acetonitrile +0.02% NH_4OAc] purity is >95%, Rt = 3.365 min; MS Calcd.: 290; MS Found: 291 $(M+1)^+$.

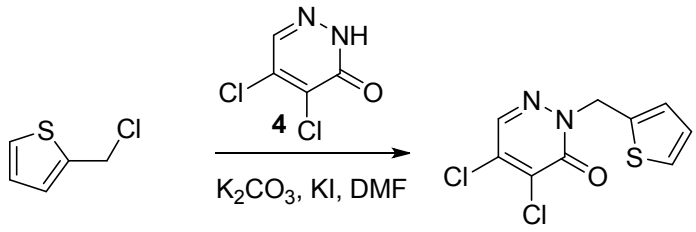
3.2.35 Synthesis of 2-(chloromethyl)thiophene **(34)**



33 **34**

To a solution of compound **33** (300 mg, 2.62 mmol) in DCM (4 mL) was added DIEA (1.02 g, 7.88 mmol) at 0 °C, then MsCl (330 mg, 2.89 mmol) was added by dropwise at 0 °C. The mixture was stirred at room temperature for 3h. Water was added, and the mixture was extracted with DCM, washed with sat. $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure to give compound **34** (220 mg, 63 %) as a colorless oil.

3.2.36 Synthesis of 4,5-dichloro-2-(thiophen-2-ylmethyl)pyridazin-3(2H)-one **(G3-3)**

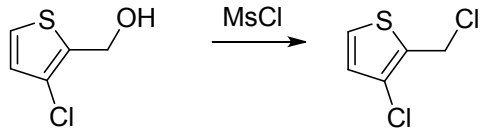


34 **G3-3**

To a solution of compound **4** (200 mg, 1.21 mmol), compound **34** (193 mg, 1.45 mmol) and K_2CO_3 (335 mg, 2.42 mmol) in DMF (3 mL) was added KI (20 mg, 0.12 mmol). The solution was stirred at 90 °C for 2h. The mixture was cooled to room temperature and quenched with water, extracted with EtOAc for 3 times, combined the organic layer, washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, purified by HPLC to give **G3-3** (210 mg, 66%) as a yellow solid.

^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.44 (s, 2H), 6.98-7.00 (m, 1H), 7.16-7.17 (m, 1H), 7.50 (d, J = 11.7 Hz, 1H), 8.26 (s, 1H); LCMS [mobile phase: 10-95% Acetonitrile +0.02% NH_4Ac] purity is >95%, Rt = 4.210 min; MS Calcd.: 261; MS Found: 262 $(M+1)^+$.

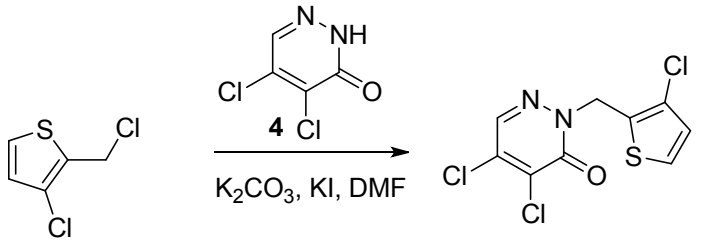
3.2.37 Synthesis of 3-chloro-2-(chloromethyl)thiophene **(36)**



35 **36**

To a solution of compound **35** (300 mg, 2.0 mmol) in DCM (4 mL) was added DIEA (521 mg, 4.0 mmol) at 0 °C, then MsCl (254 mg, 2.2 mmol) was added by dropwise at 0 °C. The mixture was stirred at room temperature for 3h. Water was added, and the mixture was extracted with DCM, washed with sat. $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure to give compound **36** (250 mg, 74 %) as a yellow oil.

3.2.38 Synthesis of 4,5-dichloro-2-((3-chlorothiophen-2-yl)methyl)pyridazin-3(2H)-one **(G3-4)**



36 **G3-4**

To a solution of compound **4** (200 mg, 1.21 mmol), compound **36** (243 mg, 1.45 mmol) and K_2CO_3 (335 mg, 2.42 mmol) in DMF (3 mL) was added KI (20 mg, 0.12 mmol). The solution was stirred at 90 °C for 2h. The mixture was cooled to room temperature and quenched with water, extracted with EtOAc for 3 times, combined the organic layer, washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, purified by HPLC to give **G3-4** (125 mg, 41%) as a white solid.

^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.43 (s, 2H), 7.07 (d, J = 5.7 Hz, 1H), 7.06-7.08 (d, J = 5.7 Hz, 1H), 7.66-7.68 (d, J = 5.4 Hz, 1H), 8.26 (s, 1H); LCMS [mobile phase: 30-95% Acetonitrile +0.02% NH_4OAc] purity is >95%, Rt = 3.608 min; MS Calcd.: 296; MS Found: 297 $(M+1)^+$.

4. Appendix

4.1 List of abbreviation

aq	aqueous
^{o}C	degrees Celsius
CDI	*N*,*N*-carbonyl dimidazole
δ_H	chemical shift in parts per million downfield from tetramethylsilane
DIEA	*N*,*N*-diisopropylethylamine
DMF	dimethylformamide
eq	equivalent
ESI	electrospray ionization
Et	ethyl
g	gram(s)
HPLC	high-performance liquid chromatography
Hz	hertz
J	coupling constant (in NMR spectrometry)
LCMS	liquid chromatography mass spectrometry
μ	micro
m	multiplet (spectral); meter(s); milli
M^+	parent molecular ion
Me	methyl
MHz	megahertz
min	minute(s)
mol	mole(s); molecular (as in mol wt)
mL	milliliter
MS	mass spectrometry
N	normal (equivalents per liter)
nm	nanometer(s)
NMR	nuclear magnetic resonance
pH	potential of hydrogen; a measure of the acidity or basicity of an aqueous solution
rt	room temperature
s	singlet (spectral)
t	triplet (spectral)
TFA	trifluoroacetic acid
THF	tetrahydrofuran