Electronic Supplementary Information:

Kinome chemoproteomics characterization of pyrrolo[3,4-*c*]pyrazoles as potent and selective inhibitors of glycogen synthase kinase 3

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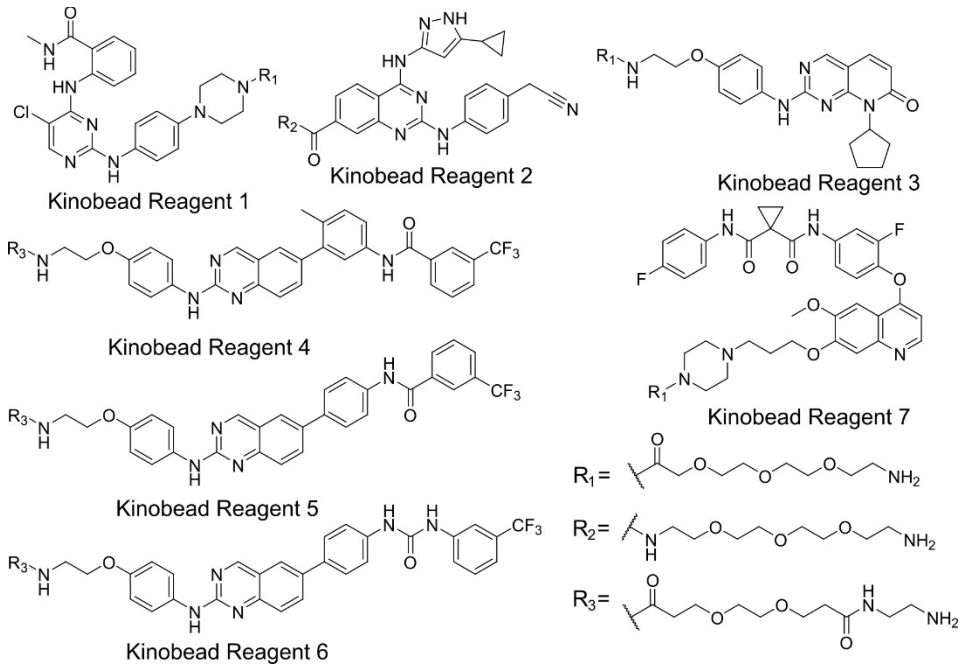
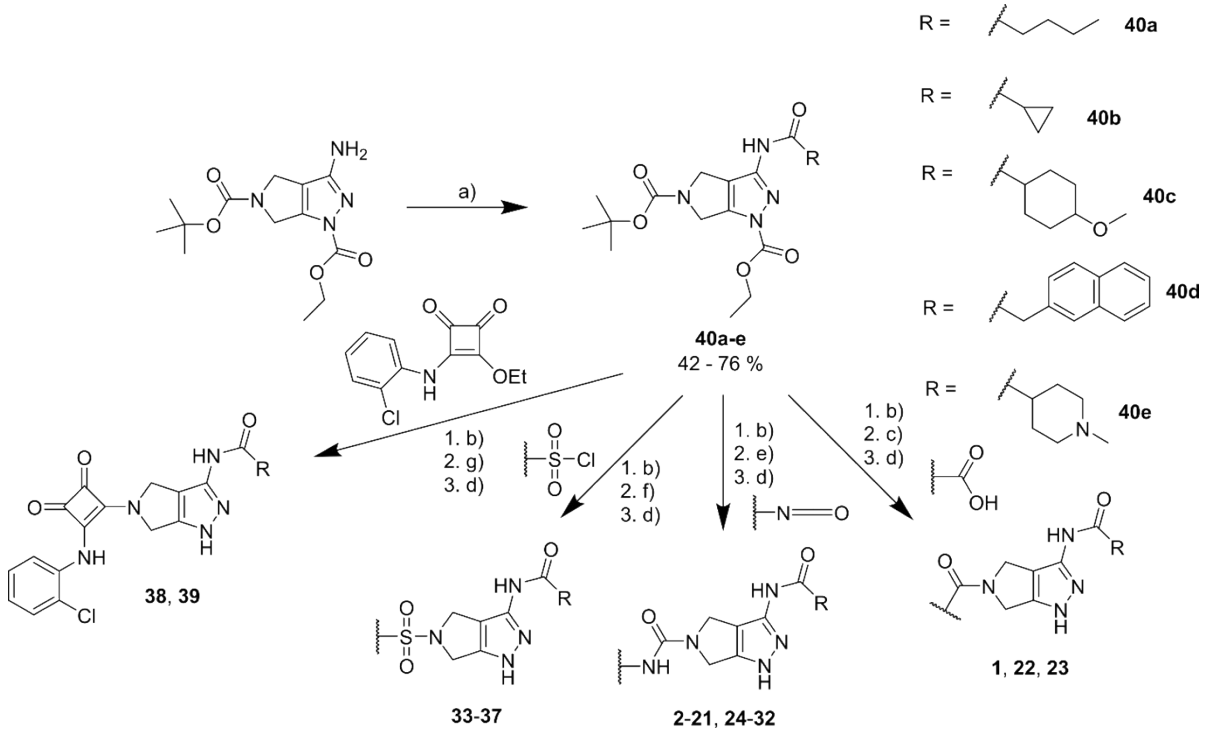


SI-Figure 1: Structures of the kinobead affinity capture reagents used in this study.



SI-Figure 2: Scheme for the synthesis of the 39-member pyrrolo[3,4-*c*]pyrazol inhibitor library. **a)** Carboxylic acid chloride (1.2 eq), DIPEA (2.2 eq), DCM, 0°C – RT, 16 h; **b)** 20% TFA in DCM, RT, 1h; **c)** Carboxylic acid (1.2 eq), EDC (1.2 eq), HOBt (1.2 eq), DIPEA (2.4 eq), DMF, 0°C – RT, 16 h; **d)** TEA (3 eq), MeOH, 40°C, 30 min; **e)** Isocyanate (1.5 eq), DIPEA (1.5 eq), THF, RT, 16h; **f)** Sulfonyl chloride (1.2 eq), DIPEA (1.2 eq), DCM, RT, 16 h; **g)** Squaric acid ethyl ester (1.2 eq), DIPEA (1.2 eq), EtOH, microwave, 80°C (25W), 2h.

Materials and Methods

General Methods

Unless otherwise noted, all chemicals were purchased from commercial suppliers and used without further purification. Microwave synthesis was performed using a Biotage Initiator Microwave Synthesizer EXP US 300W 355302. Reactions were monitored with thin-layer chromatography using silica gel 60 F254 coated glass plates (EM Sciences). Compound purification was performed with an IntelliFlash 280 automated flash chromatography system/Combi flash Rf+ using prepacked Varian Super Flash/Redi sep Rf silica gel columns (hexanes/EtOAc or DCM/MeOH gradient solvent systems). Chemical reactions were conducted on a 1 g scale for *General synthetic procedure A* and on a 20 mg scale for *General synthetic procedure B-G*.

^{1}H-NMR were recorded on a Bruker AV300 NMR at an operation frequency of 300 MHz. Chemical shifts (δ) are given in ppm. Mass spectra were determined with a Bruker Esquire 3000 Ion Trap instrument. All HPLC analyses were performed utilizing a Varian Microsorb-MV C18 reverse phase analytical column (4.6 mm x 150 mm) using either MeOH or ACN as the solvent. UV trace was recorded at 220 nm absorption.

General synthetic procedure A

1g of 3-Amino-5-((tert-butoxy)carbonyl)-4,6-dihydropyrrolo[3,4-c]pyrazole-1-carboxylic acid ethyl ester (3.37 mmol) was dissolved in 30 ml of dry DCM and DIPEA (1.29 ml, 2.2 eq., 7.41 mmol) was added under a nitrogen atmosphere. The mixture was cooled to 4°C and the corresponding acyl chloride (1.2 eq., 4.04 mmol) dissolved in 5 ml dry DCM was added dropwise over 10 min. The reaction was stirred for another 2 h at 4°C and then stirred at RT overnight. Then the solvent was removed under reduced pressure, the residue taken up in EtOAc and transferred to a separating funnel. The organic phase was washed twice with 1 N HCl, twice with sat. $NaHCO_3$ and twice with brine, dried over $MgSO_4$ and the solvent removed under reduced pressure. The residue was purified by flash chromatography and the identity of compounds **40a**-**e** confirmed by ESI-MS.

General synthetic procedure B

20 mg of either of the compounds **40a**-**e** was dissolved in 1 ml of 20% TFA in dry DCM and stirred for 1 h at RT. Then the solvent was removed under reduced pressure, the residue was dried under high vacuum overnight and used for the next step without further purification.

General synthetic procedure C

The crude product from *General synthetic procedure B* was dissolved in 1 ml of dry DMF, DIPEA (2.4 eq), the corresponding carboxylic acid (1.2 eq), HOBt (1.2 eq) were added and the mixture cooled to 4°C. Then EDC (1.2 eq) was added and the mixture stirred for 2 h at 4°C and at RT overnight. The mixture was

taken up in 20 ml EtOAc and transferred to a separating funnel. The organic phase was washed twice with 1 N HCl, twice with sat. $NaHCO_3$ and twice with brine, dried over $MgSO_4$ and the solvent removed under reduced pressure. The residue was subjected to *General synthetic procedure D* without further purification.

General synthetic procedure D

The crude product from *General synthetic procedure C, E, F and G* were dissolved in 1 ml of dry MeOH and TEA (3 eq) was added. The mixture was heated to 40°C for 30 min and then the solvent was removed under reduced pressure. The residue was purified by flash chromatography and the pure product characterized by ESI-MS, ^{1}H-NMR and analytical HPLC analyses (see below).

General synthetic procedure E

The crude product from *General synthetic procedure B* was dissolved in 1 ml of dry THF, the corresponding isocyanate (1.5 eq) and DIPEA (1.5 eq) were added and the mixture was stirred overnight at RT under a nitrogen atmosphere. Then the solvent was removed under reduced pressure and the crude product used for the *General synthetic procedure D* without further purification.

General synthetic procedure F

The crude product from *General synthetic procedure B* was dissolved in 1 ml of dry DCM, the corresponding sulfonyl chloride (1.2 eq) and DIPEA (1.2 eq) were added and the mixture was stirred overnight at RT under a nitrogen atmosphere. Then the solvent was removed under reduced pressure and the crude product used for the *General synthetic procedure D* without further purification.

General synthetic procedure G

The crude product from *General synthetic procedure B* was dissolved in 1 ml of dry EtOH, 3-[(2-chlorophenyl)amino]-4-ethoxy- 3-Cyclobutene-1,2-dione (1.2 eq) and DIPEA (1.2 eq) were added and the mixture was heated in the microwave reactor at 80°C (25W) for 2 h. Then the solvent was removed under reduced pressure and the crude product used for the *General synthetic procedure D* without further purification.

Compound yield, ^{1}H-NMR, ESI-MS, analytical HPLC data

Compound **1**

1 was prepared according to the *General synthetic procedures B, C* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless

solid. Yield: 9.4 mg (65%, 35.4 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 4.71 (d, *J* = 17.2 Hz, 2H), 4.55 (d, *J* = 10.8 Hz, 2H), 2.46 – 2.30 (m, 4H), 1.76-1.64 (m, 4H), 1.05-0.94 (m, 6H). MS (ESI) Calcd for $C_{13}H_{21}N_4O_2$ $(M+H)^+$ 265.2, found 265.4. Analytical reverse-phase HPLC (t_R = 11.4 min, ACN), purity >95%.

Compound **2**

2 was prepared according to the *General synthetic procedures B, E* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 12.5 mg (78%, 42.5 µmol).^{1}H NMR (300 MHz, Chloroform-*d*) δ 4.65 (s, 2H), 4.54 (s, 2H), 3.40 – 3.18 (m, 2H), 2.49 – 2.18 (m, 2H), 1.72 (q, *J* = 7.3 Hz, 2H), 1.61 – 1.42 (m, 2H), 1.43 – 1.20 (m, 2H), 1.0-0.91 (m, 6H). MS (ESI) Calcd for $C_{14}H_{24}N_5O_2$ $(M+H)^+$ 294.2, found 294.3. Analytical reverse-phase HPLC (t_R = 10.5 min, ACN), purity >95%.

Compound **3**

3 was prepared according to the *General synthetic procedures B, E* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 6.2 mg (37%, 15.8 µmol). ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.95 (d, *J* = 8.1 Hz, 1H), 7.90 – 7.71 (m, 2H), 7.66 – 7.36 (m, 4H), 4.53 (s, 4H), 4.14 (s, 2H), 3.22 (s, 2H), 1.63 – 1.19 (m, 4H), 1.05 – 0.79 (m, 3H). MS (ESI) Calcd for $C_{22}H_{26}N_5O_2$ $(M+H)^+$ 392.5, found 392.7. Analytical reverse-phase HPLC (t_R = 15.4 min, ACN), purity >95%.

Compound **4**

4 was prepared according to the *General synthetic procedures B, E* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 13.9 mg (81%, 44.2 µmol). MS (ESI) Calcd for $C_{16}H_{20}N_5$ $(M+H)^+$ 314.2, found 314.6. Analytical reverse-phase HPLC (t_R = 12.5 min, ACN), purity >95%.

Compound **5**

5 was prepared according to the *General synthetic procedures B, E* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 8.7 mg (44%, 23.9 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 8.06 (d, *J* = 7.5 Hz, 1H), 7.89 (d, *J* = 7.5 Hz, 1H), 7.78 (d, *J* = 6.7 Hz, 1H), 7.55-746 (m, 4H), 4.78 (s, 2H), 4.70 (s, 2H), 2.38 (t, *J* = 7.4 Hz, 2H), 1.85 – 1.63 (m, 2H), 1.07 – 0.95 (m, 3H). MS (ESI) Calcd for $C_{20}H_{22}N_5O_2$ $(M+H)^+$ 364.2, found 364.4. Analytical reverse-phase HPLC (t_R = 20.5 min, MeOH), purity >95%.

Compound **6**

6 was prepared according to the *General synthetic procedures B, E* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 4.6 mg (23%, 10.6 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 8.06 (d, *J* = 7.5 Hz, 1H), 7.88 (s, 1H), 7.78 (d, *J* = 6.7 Hz, 1H), 7.63 – 7.37 (m, 4H), 4.77 (s, 2H), 4.70 (s, 2H), 3.37 (s, 3H), 3.20 (s, 1H), 2.38 (s, 1H), 2.17 (d, *J* = 12.1 Hz, 2H), 1.97 (d, *J* = 13.2 Hz, 2H), 1.68-1.50 (m, 2H), 1.33 – 1.19 (m, 2H). MS (ESI) Calcd for $C_{24}H_{28}N_5O_3$ $(M+H)^+$ 434.2, found 434.4. Analytical reverse-phase HPLC (t_R = 14.6 min, ACN), purity >95%.

Compound **7**

7 was prepared according to the *General synthetic procedures B, E* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 14.1 mg (74%, 40.3 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.30 (t, *J* = 7.3 Hz, 1H), 7.21 – 6.88 (m, 2H), 4.75 (s, 2H), 4.58 (s, 2H), 2.37 (t, *J* = 7.7 Hz, 2H), 1.72 (q, *J* = 7.4 Hz, 2H), 1.15 – 0.89 (m, 3H). MS (ESI) Calcd for $C_{16}H_{18}F_2N_5O_2$ $(M+H)^+$ 350.1, found 350.3. Analytical reverse-phase HPLC (t_R = 13.5 min, ACN), purity >95%.

Compound **8**

8 was prepared according to the *General synthetic procedures B, E* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 15.9 mg (83%, 45.4 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.54 (s, 1H), 7.14 (s, 1H), 6.85 (s, 1H), 4.69 (s, 2H), 4.63 (s, 2H), 2.36 (t, *J* = 7.1 Hz, 2H), 1.77-1.66 (m, 2H), 1.05-0.95 (m, 3H). MS (ESI) Calcd for $C_{16}H_{18}F_2N_5O_2$ $(M+H)^+$ 350.1, found 350.4. Analytical reverse-phase HPLC (t_R = 14.1 min, ACN), purity >95%.

Compound **9**

9 was prepared according to the *General synthetic procedures B, E* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 13.6 mg (71%, 38.8 µmol). MS (ESI) Calcd for $C_{16}H_{18}F_2N_5O_2$ $(M+H)^+$ 350.1, found 350.3. Analytical reverse-phase HPLC (t_R = 12.4 min, ACN), purity >95%.

Compound **10**

10 was prepared according to the *General synthetic procedures B, E* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 14.3 mg (75%, 41.1 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.76 (d, *J* = 8.5 Hz, 1H), 7.44 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.35 – 7.28 (m, 1H), 7.19 – 7.07 (m, 1H), 4.70 (s, 2H), 4.63 (s, 2H), 2.36 (t, *J* = 7.5 Hz, 2H), 1.79 – 1.65 (m, 2H), 1.00 (td, *J* = 7.5, 2.6 Hz, 3H). MS (ESI) Calcd for $C_{16}H_{19}ClN_5O_2$ $(M+H)^+$ 348.1, found 348.4. Analytical reverse-phase HPLC (t_R = 18.2 min, MeOH), purity >95%.

Compound **11**

11 was prepared according to the *General synthetic procedures B, E* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 7.8 mg (41%, 22.5 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.75 (d, *J* = 7.8 Hz, 1H), 7.44 (d, *J* = 8.0 Hz, 1H), 7.28 (d, *J* = 8.7 Hz, 1H), 7.19 – 7.08 (m, 1H), 4.66 (d, *J* = 14.5 Hz, 4H), 1.79 (s, 1H), 1.00 – 0.85 (m, 4H). MS (ESI) Calcd for $C_{16}H_{17}ClN_5O_2$ $(M+H)^+$ 346.1, found 346.3. Analytical reverse-phase HPLC (t_R = 12.6 min, ACN), purity >95%.

Compound **12**

12 was prepared according to the *General synthetic procedures B, E* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 13.2 mg (69%, 31.6 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.75 (d, *J* = 8.4 Hz, 1H), 7.45 (d, *J* = 8.5 Hz, 1H), 7.30 (t, *J* = 7.9 Hz, 1H), 7.16 (d, *J* = 8.6 Hz, 1H), 4.70 (s, 2H), 4.64 (s, 1H), 2.35 (d, *J* = 11.9 Hz, 1H), 2.17 (d, *J* = 12.6 Hz, 2H), 1.96 (d, *J* = 13.9 Hz, 2H), 1.59 (q, *J* = 13.1 Hz, 2H), 1.26 (t, *J* = 12.6 Hz, 2H). MS (ESI) Calcd for $C_{20}H_{25}ClN_5O_3$ $(M+H)^+$ 418.2, found 418.7. ACN, 14.0 min, >95%. Analytical reverse-phase HPLC (t_R = 14.0 min min, ACN), purity >95%.

Compound **13**

13 was prepared according to the *General synthetic procedures B, E* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 16.0 mg (84%, 46.0 µmol).^{1}H NMR (300 MHz, Methanol-d_4) δ 7.67 – 7.60 (m, 1H), 7.37 (s, 1H), 7.25 (t, *J* = 7.1 Hz, 1H), 7.04 (s, 1H), 4.66 (s, 2H), 4.62 (s, 2H), 2.37 (t, *J* = 7.3 Hz, 2H), 1.73 (q, *J* = 7.1 Hz, 2H), 1.07 – 0.93 (m, 3H). MS (ESI) Calcd for $C_{16}H_{19}ClN_5O_2$ $(M+H)^+$ 348.1, found 348.6. Analytical reverse-phase HPLC (t_R = 15.3 min, ACN), purity >95%.

Compound **14**

14 was prepared according to the *General synthetic procedures B, E* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 14.7 mg (77%, 35.2 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.75 (d, *J* = 8.4 Hz, 1H), 7.45 (d, *J* = 8.5 Hz, 1H), 7.30 (t, *J* = 7.9 Hz, 1H), 7.16 (d, *J* = 8.6 Hz, 1H), 4.70 (s, 3H), 4.64 (s, 2H), 3.21 (s, 1H), 2.17 (d, *J* = 12.6 Hz, 2H), 1.96 (d, *J* = 13.9 Hz, 2H), 1.68-1.48 (m, 2H), 1.26 (t, *J* = 12.6 Hz, 2H). MS (ESI) Calcd for $C_{20}H_{25}ClN_5O_3$ $(M+H)^+$ 418.2, found 418.3. Analytical reverse-phase HPLC (t_R = 14.8 min, ACN), purity >95%.

Compound **15**

15 was prepared according to the *General synthetic procedures B, E* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 11.2 mg (64%, 29.3 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.74 (d, *J* = 8.7 Hz, 1H), 7.54 – 7.44 (m, 1H), 7.31 (dd, *J* = 8.7, 2.4 Hz, 1H), 4.70 (s, 2H), 4.63 (s, 2H), 2.37 (t, *J* = 7.4 Hz, 2H), 1.72 (h, *J* = 7.3 Hz, 2H), 1.00 (t, *J* = 7.4 Hz, 3H). MS (ESI) Calcd for $C_{16}H_{18}Cl_2N_5O_2$ $(M+H)^+$ 382.1, found 382.1. Analytical reverse-phase HPLC (t_R = 20.0 min, MeOH), purity >95%.

Compound **16**

16 was prepared according to the *General synthetic procedures B, E* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 15.2 mg (73%, 39.8 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.73 (d, *J* = 7.9 Hz, 1H), 7.39 – 7.25 (m, 2H), 4.95 (s, 2H), 4.64 (s, 2H), 2.37 (t, *J* = 7.5 Hz, 2H), 1.72 (q, *J* = 7.5 Hz, 2H), 1.00 (t, *J* = 7.4 Hz, 3H). MS (ESI) Calcd for $C_{16}H_{18}Cl_2N_5O_2$ $(M+H)^+$ 382.1, found 382.2. Analytical reverse-phase HPLC (t_R = 16.3 min, ACN), purity >95%.

Compound **17**

17 was prepared according to the *General synthetic procedures B, E* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 16.1 mg (77%, 42.1 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.51 – 7.40 (m, 2H), 7.32-7.23 (m, 1H), 4.69 (s, 2H), 4.62 (s, 2H), 3.74 (s, 4H), 3.41 – 3.32 (m, 9H), 2.37 (t, *J* = 7.4 Hz, 2H), 1.73 (p, *J* = 7.3 Hz, 2H), 1.00 (t, *J* = 7.3 Hz, 3H). MS (ESI) Calcd for $C_{16}H_{18}Cl_2N_5O_2$ $(M+H)^+$ 382.1, found 382.5. Analytical reverse-phase HPLC (t_R = 12.6 min, ACN), purity >95%.

Compound **18**

18 was prepared according to the *General synthetic procedures B, E* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 7.9 mg (38%, 20.8 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.50 – 7.41 (m, 2H), 7.26 (ddd, *J* = 8.8, 7.5, 4.7 Hz, 1H), 4.63 (s, 4H), 1.78 (s, 1H), 1.01 – 0.84 (m, 4H). MS (ESI) Calcd for $C_{16}H_{16}Cl_2N_5O_2$ $(M+H)^+$ 380.1, found 380.3. Analytical reverse-phase HPLC (t_R = 12.9 min, ACN), purity >95%.

Compound **19**

19 was prepared according to the *General synthetic procedures B, E* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 17.8 mg (86%, 39.4 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.51 – 7.41 (m, 2H), 7.31 – 7.22 (m, 1H), 4.80 – 4.55 (m, 4H), 2.45 (t, *J* = 12.6 Hz, 1H), 2.08 – 1.76 (m, 4H), 1.72 – 1.43 (m, 4H). MS (ESI) Calcd for $C_{20}H_{24}Cl_2N_5O_3$ $(M+H)^+$ 452.1, found 452.2. Analytical reverse-phase HPLC (t_R = 14.2 min, ACN), purity >95%.

Compound **20**

20 was prepared according to the *General synthetic procedures B, E* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 6.6 mg (32%, 13.7 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.63 – 7.42 (m, 3H), 7.42 – 7.08 (m, 4H), 4.18 (d, *J* = 4.2 Hz, 2H), 7.51 – 7.40 (m, 2H), 7.32-7.23 (m, 1H) 4.69 (s, 2H), 4.62 (s, 2H). MS (ESI) Calcd for $C_{24}H_{20}Cl_2N_5O_2$ $(M+H)^+$ 480.1, found 480.2. Analytical reverse-phase HPLC (t_R = 13.4 min, ACN), purity >95%.

Compound **21**

21 was prepared according to the *General synthetic procedures B, E* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 7.0 mg (34%, 16.0 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.46 (t, *J* = 6.8 Hz, 2H), 7.28 (t, *J* = 8.3 Hz, 1H), 4.76 – 4.50 (m, 4H), 3.27 – 2.99 (m, 4H), 2.90 (s, 3H), 2.68 (t, *J* = 13.6 Hz, 1H), 2.17 (d, *J* = 14.6 Hz, 2H), 2.10 – 1.85 (m, 2H). MS (ESI) Calcd for $C_{19}H_{23}Cl_2N_6O_2$ $(M+H)^+$ 437.1, found 437.4. Analytical reverse-phase HPLC (t_R = 10.0 min, ACN), purity >95%.

Compound **22**

22 was prepared according to the *General synthetic procedures B, C* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 15.0 mg (79%, 43.2 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.52 – 7.09 (m, 4H), 4.77 (d, *J* = 21.1 Hz, 2H), 4.58 (d, *J* = 9.6 Hz, 2H), 3.80 (s, 2H), 2.35 (dd, *J* = 8.5, 6.4 Hz, 2H), 1.71 (h, *J* = 7.4 Hz, 2H), 0.99 (td, *J* = 7.4, 2.6 Hz, 3H). MS (ESI) Calcd for $C_{17}H_{20}ClN_4O_2$ $(M+H)^+$ 347.1, found 347.4. Analytical reverse-phase HPLC (t_R = 14.0 min, ACN), purity >95%.

Compound **23**

23 was prepared according to the *General synthetic procedures B, C* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 5.2 mg (27%, 11.6 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 8.06 (d, *J* = 8.1 Hz, 1H), 7.98 – 7.75 (m, 2H), 7.63 – 7.42 (m, 3H), 7.42 – 7.08 (m, 4H), 4.72 (d, *J* = 5.2 Hz, 2H), 4.55 (d, *J* = 6.2 Hz, 2H), 4.18 (d, *J* = 4.2 Hz, 2H), 3.74 (d, *J* = 18.7 Hz, 2H). MS (ESI) Calcd for $C_{25}H_{22}ClN_4O_2$ $(M+H)^+$ 445.1, found 445.1. Analytical reverse-phase HPLC (t_R = 16.8 min, ACN), purity >95%.

Compound **24**

24 was prepared according to the *General synthetic procedures B, E* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 10.9 mg (58%, 31.7 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.84 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.12 – 6.96 (m, 2H), 6.91 (ddd, *J* = 7.9, 7.0, 1.9 Hz, 1H), 4.67 (s, 2H), 4.61 (s, 2H), 3.92 (s, 3H), 2.37 (t, *J* = 7.4 Hz, 2H), 1.73 (h, *J* = 7.3 Hz, 2H), 1.01 (t, *J* = 7.4 Hz, 3H). MS (ESI) Calcd for $C_{17}H_{22}N_5O_3$ $(M+H)^+$ 344.2, found 344.5. Analytical reverse-phase HPLC (t_R = 13.0 min, ACN), purity >95%.

Compound **25**

25 was prepared according to the *General synthetic procedures B, E* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 6.6 mg (32%, 13.7 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 8.07 (d, *J* = 9.1 Hz, 1H), 7.96 – 7.73 (m, 3H), 7.66 (d, *J* = 9.1 Hz, 1H), 7.60 – 7.33 (m, 4H), 7.29 (s, 1H), 4.61 (s, 4H), 4.20 (s, 2H). MS (ESI) Calcd for $C_{25}H_{21}F_3N_5O_2$ $(M+H)^+$ 480.2, found 480.3. Analytical reverse-phase HPLC (t_R = 17.8 min, ACN), purity >95%.

Compound **26**

26 was prepared according to the *General synthetic procedures B, E* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 15.0 mg (66%, 36.0 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 8.22 (s, 1H), 7.65 (d, *J* = 8.7 Hz, 1H), 7.40 (d, *J* = 8.7 Hz, 1H), 4.73 (s, 2H), 4.66 (s, 2H), 2.37 (t, *J* = 7.3 Hz, 2H), 1.72 (q, *J* = 7.5 Hz, 2H), 1.06 – 0.93 (m, 3H). MS (ESI) Calcd for $C_{17}H_{18}ClF_3N_5O_2$ $(M+H)^+$ 416.1, found 416.4. Analytical reverse-phase HPLC (t_R = 23.8 min, MeOH), purity >95%.

Compound **27**

27 was prepared according to the *General synthetic procedures B, E* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 13.6 mg (61%, 28.0 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 8.22 (s, 1H), 7.65 (d, *J* = 8.4 Hz, 1H), 7.41 (d, *J* = 8.4 Hz, 1H), 4.73 (s, 2H), 4.65 (s, 2H), 2.48 – 2.33 (m, 1H), 2.17 (d, *J* = 12.7 Hz, 1H), 1.98-1.88 (m, 4H), 1.71 – 1.39 (m, 4H). MS (ESI) Calcd for $C_{21}H_{24}ClF_3N_5O_3$ $(M+H)^+$ 486.1, found 486.7. Analytical reverse-phase HPLC (t_R = 20.5 min, MeOH), purity >95%.

Compound **28**

28 was prepared according to the *General synthetic procedures B, E* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 16.6 mg (76%, 41.5 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 8.07 (d, *J* = 7.6 Hz, 1H), 7.45 (s, 1H), 7.34 (t, *J* = 9.5 Hz, 1H), 4.7 (s, 2H), 4.64 (s, 2H), 2.43 – 2.30 (m, 2H), 1.72 (q, *J* = 7.4 Hz, 2H), 0.98 (t, *J* = 7.1 Hz, 3H). MS (ESI) Calcd for $C_{17}H_{18}F_4N_5O_2$ $(M+H)^+$ 400.1, found 400.4. Analytical reverse-phase HPLC (t_R = 15.4 min, ACN), purity >95%.

Compound **29**

29 was prepared according to the *General synthetic procedures B, E* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 10.7 mg (54%, 29.5 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.50 (s, 1H), 7.31 (d, *J* = 8.9 Hz, 1H), 6.76 – 6.63 (m, 1H), 4.71 (s, 2H), 4.64 (s, 2H), 3.79 (s, 3H), 2.37 (t, *J* = 7.4 Hz, 2H), 1.72 (q, *J* = 7.4 Hz, 2H), 1.00 (t, *J* = 7.3 Hz, 3H). MS (ESI) Calcd for $C_{17}H_{21}FN_5O_3$ $(M+H)^+$ 362.2, found 362.3. Analytical reverse-phase HPLC (t_R = 14.5 min, ACN), purity >95%.

Compound **30**

30 was prepared according to the *General synthetic procedures B, E* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 10.5 mg (51%, 23.4 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.50 (s, 1H), 7.32 (dd, *J* = 8.8, 1.2 Hz, 1H), 6.76 – 6.65 (m, 1H), 4.70 (s, 2H), 4.64 (s, 2H), 3.80 (s, 3H), 3.37 (s, 3H), 2.43-2.30 (m, 1H), 2.18 (d, *J* = 12.3 Hz, 2H), 1.97 (d, *J* = 13.5 Hz, 2H), 1.68-1.50 (m, 2H), 1.26 (t, *J* = 11.9 Hz, 2H). MS (ESI) Calcd for $C_{21}H_{27}ClN_5O_4$ $(M+H)^+$ 448.2, found 448.3. Analytical reverse-phase HPLC (t_R = 23.5 min, ACN), purity >95%.

Compound **31**

31 was prepared according to the *General synthetic procedures B, E* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 8.8 mg (43%, 23.5 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.50 (d, *J* = 8.7 Hz, 1H), 6.63 – 6.53 (m, 1H), 6.49 (dd, *J* = 8.7, 2.7 Hz, 1H), 4.93 (s, 2H), 4.60 (s, 2H), 3.87 (s, 3H), 3.79 (s, 3H), 2.37 (t, *J* = 7.4 Hz, 2H), 1.71 (p, *J* = 7.3 Hz, 2H), 1.00 (t, *J* = 7.4 Hz, 3H). MS (ESI) Calcd for $C_{18}H_{24}N_5O_4$ $(M+H)^+$ 374.2, found 374.4. Analytical reverse-phase HPLC (t_R = 12.6 min, ACN), purity >95%.

Compound **32**

32 was prepared according to the *General synthetic procedures B, E* and *D* and purified by flash chromatography using EtOAc/hexanes as the solvent system. The product was obtained as colorless solid. Yield: 4.0 mg (19%, 10.4 µmol). MS (ESI) Calcd for $C_{19}H_{24}N_5O_4$ $(M+H)^+$ 386.2, found 386.4. Analytical reverse-phase HPLC (t_R = 14.6 min, ACN), purity >95%.

Compound **33**

33 was prepared according to the *General synthetic procedures B, F* and *D* and purified by flash chromatography using DCM/MeOH as the solvent system. The product was obtained as colorless solid. Yield: 16.1 mg (73%, 40.0 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.64 – 7.54 (m, 2H), 7.53 – 7.41 (m, 1H), 4.67 (s, 2H), 4.66 (s, 2H), 2.33 (t, *J* = 7.4 Hz, 2H), 1.69 (h, *J* = 7.4 Hz, 2H), 0.98 (t, *J* = 7.4 Hz, 3H). MS (ESI) Calcd for $C_{15}H_{17}Cl_2N_4O_3S$ $(M+H)^+$ 403.00, found 403.1. Analytical reverse-phase HPLC (t_R = 16.0 min, ACN), purity >95%.

Compound **34**

34 was prepared according to the *General synthetic procedures B, F* and *D* and purified by flash chromatography using DCM/MeOH as the solvent system. The product was obtained as colorless solid. Yield: 16.5 mg (76%, 34.9 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.64 – 7.53 (m, 2H), 7.53 – 7.41 (m, 1H), 4.66 (d, *J* = 7.1 Hz, 4H), 2.44 – 2.30 (m, 1H), 2.15 (d, *J* = 12.3 Hz, 1H), 1.91 (dt, *J* = 26.9, 14.3 Hz, 3H), 1.60 (d, *J* = 17.0 Hz, 2H), 1.51 (s, 1H), 1.21 (d, *J* = 12.0 Hz, 1H). MS (ESI) Calcd for $C_{19}H_{23}Cl_2N_4O_4S$ $(M+H)^+$ 473.1, found 473.5. Analytical reverse-phase HPLC (t_R = 15.7 min, ACN), purity >95%.

Compound **35**

35 was prepared according to the *General synthetic procedures B, F* and *D* and purified by flash chromatography using DCM/MeOH as the solvent system. The product was obtained as colorless solid. Yield: 14.8 mg (67%, 36.6 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 8.04 (ddd, *J* = 1.8, 1.1, 0.6 Hz, 1H), 7.66 – 7.59 (m, 2H), 4.63 – 4.56 (m, 4H), 2.38 – 2.26 (m, 2H), 1.68 (p, *J* = 7.5 Hz, 2H), 0.96 (t, *J* = 7.4, 3H). MS (ESI) Calcd for $C_{15}H_{17}Cl_2N_4O_3S$ $(M+H)^+$ 404.3, found 404.6. Analytical reverse-phase HPLC (t_R = 19.2 min, MeOH), purity >95%.

Compound **36**

36 was prepared according to the *General synthetic procedures B, F* and *D* and purified by flash chromatography using DCM/MeOH as the solvent system. The product was obtained as colorless solid. Yield: 16.9 mg (71%, 38.7 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 8.30 (s, 1H), 7.99 – 7.81 (m, 2H), 4.61 (s, 4H), 2.32 (t, *J* = 7.4 Hz, 2H), 1.67 (q, *J* = 7.4 Hz, 2H), 0.96 (t, *J* = 7.4 Hz, 3H). MS (ESI) Calcd for $C_{16}H_{17}ClF_3N_4O_3S$ $(M+H)^+$ 437.1, found 437.2. Analytical reverse-phase HPLC (t_R = 17.0 min, ACN), purity >95%.

Compound **37**

37 was prepared according to the *General synthetic procedures B, F* and *D* and purified by flash chromatography using DCM/MeOH as the solvent system. The product was obtained as colorless solid. Yield: 13.7 mg (59%, 27.0 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 8.31 (s, 1H), 7.99 – 7.82 (m, 2H), 4.62 (s, 4H), 3.25 – 2.99 (m, 1H), 2.35-2.25 (m, 1H), 2.15 (d, *J* = 12.4 Hz, 2H), 1.92 (d, *J* = 14.0 Hz, 2H), 1.64-1.44 (m, 2H), 1.29-1.11 (m, 2H). MS (ESI) Calcd for $C_{20}H_{23}ClF_3N_4O_4S$ $(M+H)^+$ 507.1, found 507.2. Analytical reverse-phase HPLC (t_R = 16.6 min, ACN), purity >95%.

Compound **38**

38 was prepared according to the *General synthetic procedures B, G* and *D* and purified by flash chromatography using DCM/MeOH as the solvent system. The product was obtained as colorless solid. Yield: 6.1 mg (28%, 15.2 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.60 (d, *J* = 8.0 Hz, 1H), 7.53 – 7.40 (m, 1H), 7.34 (td, *J* = 7.7, 1.6 Hz, 1H), 7.19 (t, *J* = 7.7 Hz, 1H), 5.07 (s, 4H), 2.35 (s, 2H), 1.70 (d, *J* = 7.3 Hz, 2H), 1.08 – 0.87 (m, 3H). MS (ESI) Calcd for $C_{19}H_{19}ClN_5O_3$ $(M+H)^+$ 400.1, found 400.3. Analytical reverse-phase HPLC (t_R = 13.6 min, ACN), purity >95%.

Compound **39**

38 was prepared according to the *General synthetic procedures B, G* and *D* and purified by flash chromatography using DCM/MeOH as the solvent system. The product was obtained as colorless solid. Yield: 5.0 mg (23%, 10.6 µmol). ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.58 (d, *J* = 8.0 Hz, 1H), 7.46 (t, *J* = 8.7 Hz, 1H), 7.40 – 7.28 (m, 1H), 7.26 – 7.09 (m, 1H), 5.05 (s, 4H), 2.46 – 2.28 (m, 1H), 2.17 (d, *J* = 7.4 Hz, 1H), 2.07 – 1.91 (m, 1H), 1.91 – 1.77 (m, 1H), 1.68 – 1.45 (m, 3H), 1.42 – 1.18 (m, 3H). MS (ESI) Calcd for $C_{23}H_{25}ClN_5O_4$ $(M+H)^+$ 470.2, found 470.6. Analytical reverse-phase HPLC (t_R = 12.1 min, ACN), purity >95%.