Supporting Information

Trisubstituted Isoalloxazines as a New Class of G-Quadruplex Binding Ligands: Small Molecule Regulation of *c-kit* Oncogene Expression

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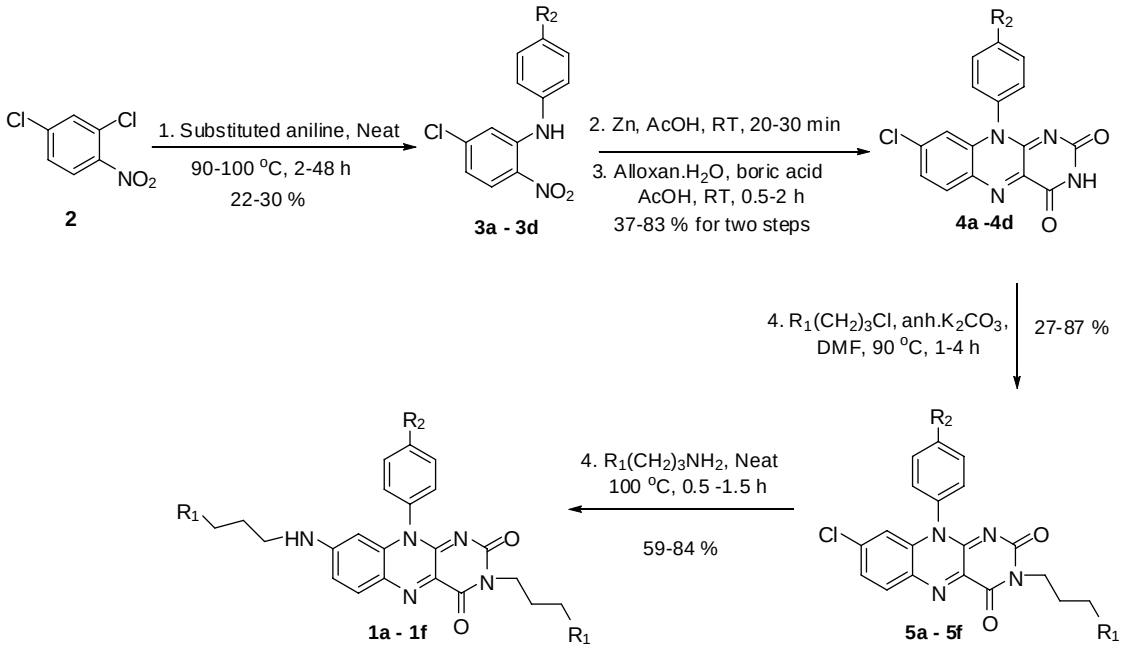
General Methods:

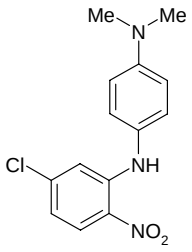
All chemicals and anhydrous solvents were purchased from Aldrich, Alfa Aesar, or Fisher Scientific, unless otherwise indicated. Anhydrous reactions were carried out under nitrogen atmosphere. Thin layer chromatography was performed using silica gel $60F_{254}$ coated on glass plates which were purchased from Merck. The synthesised compounds were purified by flash column chromatography using silica-gel 60 (0.04-0.063 mm). Developed TLC plates were visualised under UV lamp and stained with iodine. Unless otherwise stated, all the ligand (**1a-1f**) solutions were prepared in 10 % DMSO in water, for biophysical and biological experiments. ^{1}H NMR and ^{13}C NMR spectra were recorded on Brucker drx 500 and drx 400 instruments. Chemical shifts were relative to the deuterated solvent peak and are reported in parts per million (ppm). The high resolution mass spectra were recorded on Micromass Q-Tof spectrometer using electrospray ionisation technique. Melting points were determined on Griffin melting point apparatus.

General synthetic route for 3, 8, 10-trisubstituted isoalloxazines (1a-1f)



Experimental Procedures:

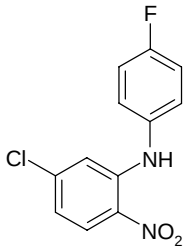
Preparation of *N*-(5-Chloro-2-nitro-phenyl)-*N'*,*N'*-dimethyl-benzene-1,4-diamine (3a)



A 50 mL round bottom flask was charged with 2,4-dichloronitrobenzene (**2**) (2.0 g, 10.4 mmol) and 4-*N,N*-dimethylamino aniline (1.56 g, 11.4 mmol). The reaction mixture was stirred at 100 °C under neat conditions and nitrogen atmosphere for 15 hours. The reaction mixture was cooled to room temperature and diluted with ethyl acetate (20 mL). The crude product was purified by flash chromatography using 5 % ethyl acetate in

petroleum ether to afford the title compound as a brown solid (0.9 g, 30 %). Melting point 228-230 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 9.42 (s, NH), 8.12 (d, ArH, J = 9.13 Hz), 7.12 (dt, 2 × ArH, J = 2.06 Hz, J' = 3.41 Hz), 6.94 (d, ArH, J = 2.17 Hz), 6.76 (dt, 2 × ArH, J = 2.2 Hz, J' = 3.38 Hz), 6.61 (dd, ArH, J = 2.17 Hz, J' = 11.3 Hz), 3.00 (s, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 145.47 (qC), 145.72 (qC), 142.39 (qC), 131.12 (qC), 127.98 (qC), 127.19 (ArC), 126.19 (ArC), 116.80 (ArC), 115.1 (ArC), 113.22 (ArC), 40.58 (CH_3); HRMS: Calculated mass for $C_{14}H_{13}ClN_3O_2$ $[M-H]^-$ 290.0693 found 290.0701 $[M-H]^-$.

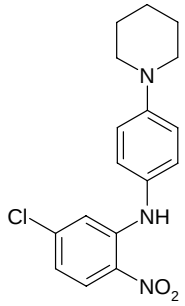
Preparation of (5-Chloro-2-nitro-phenyl)-(4-fluoro-phenyl)-amine (3b)



A 50 mL round bottom flask was charged with 2,4-dichloronitrobenzene (**2**) (3.0 g, 15.6 mmol) and 4-fluoroaniline (4 mL). The reaction mixture was stirred at 100 °C under neat conditions and nitrogen atmosphere for 48 hours. The reaction mixture was cooled to room temperature and diluted with ethyl acetate (15 mL). The crude product was purified by flash chromatography using 1 % ethyl acetate in petroleum ether to afford the title compound as a yellow solid (1.1 g, 27 %). ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 9.42 (s, NH), 8.14 (d, ArH, J = 9.13 Hz), 7.23 (m, 2 × ArH), 7.14 (m, 2 × ArH), 6.95 (d, ArH, J = 2.13 Hz), 6.71 (dd, ArH, J = 2.17 Hz, J' = 11.25 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 161.03 (qC, J_{CF} = 245 Hz), 144.37 (qC), 142.6 (qC), 133.7 (qC), 131.3 (qC), 128.11 (ArC), 127.48 (ArC), 127.38 (ArC), 117.85 (ArC), 117.03 (ArC), 116.84 (ArC), 114.85

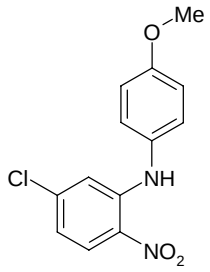
(ArC); HRMS: Calculated mass for $C_{12}H_9ClFN_2O_2$ $[M+H]^+$ 267.0791 found 267.0791 $[M+H]^+$.

Preparation of (5-Chloro-2-nitro-phenyl)-(4-piperidin-1-yl-phenyl)-amine (3c)



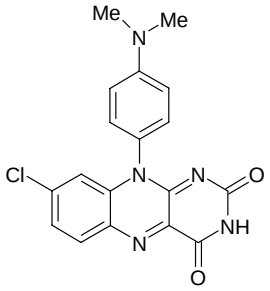
A 50 mL round bottom flask was charged with 2,4-dichloronitrobenzene (**2**) (2.0 g, 10.4 mmol) and 4-piperidino aniline (2.01 g, 11.4 mmol). The reaction mixture was stirred at 100 °C under neat conditions and nitrogen atmosphere for 2 hours. The reaction mixture was cooled to room temperature and diluted with ethyl acetate (20 mL). The crude product was purified by flash chromatography using 5 % ethyl acetate in petroleum ether to afford the title compound as a brown solid (0.9 g, 27 %). Melting point 138-140 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 9.42 (s, NH), 8.10 (d, ArH, J = 9.15 Hz), 7.10 (dt, 2 × ArH, J = 2.06 Hz, J' = 3.41 Hz), 6.95 (m, 3 × ArH), 6.62 (dd, ArH, J = 2.18 Hz, J' = 11.33 Hz), 3.20 (t, 4H, J = 5.37 Hz), 1.73 (quintet, 2H), 1.59 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 150.89 (qC), 145.36 (qC), 142.38 (qC), 130.75 (qC), 128. 37 (qC), 127.92 (ArC), 126.76 (ArC), 117.09 (ArC), 116.99 (ArC), 115.60 (ArC), 50.31 (CH_2), 25.73 (CH_2), 24.20 (CH_2); HRMS: Calculated mass for $C_{17}H_{19}ClN_3O_2$ $[M+H]^+$ 332.1157 found 332.1160 $[M+H]^+$.

Preparation of (5-Chloro-2-nitro-phenyl)-(4-methoxy-phenyl)-amine (3d)



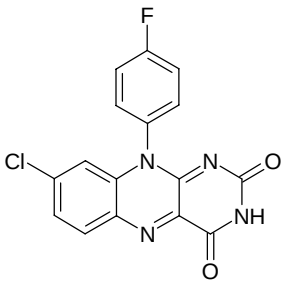
A 50 mL round bottom flask was charged with 2,4-dichloronitrobenzene (**2**) (2.0 g, 10.4 mmol) and *p*-anisidine (1.41 g, 11.4 mmol). The reaction mixture was stirred at 100 °C under neat conditions and nitrogen atmosphere for 8 hours. The reaction mixture was cooled to room temperature and diluted with ethyl acetate (20 mL). The crude product was purified by flash chromatography using 2 % ethyl acetate in petroleum ether to afford the title compound as an orange solid (0.62 g, 22 %). Melting point 228-230 °C ; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 9.41 (s, NH), 8.12 (d, ArH, J = 9.12 Hz), 7.18 (dt, 2 × ArH, J = 2.12 Hz, J' = 3.36 Hz), 6.97 (dt, 2 × ArH, J = 2.24 Hz, J' = 3.36 Hz,), 6.92 (d, ArH, J = 2.22 Hz), 6.64 (dd, ArH, J = 2.16 Hz, J' = 11.33 Hz), 3.84 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 158.32 (qC), 145.16 (qC), 142.48 (qC), 130.92 (qC), 130.31(qC), 128.02 (ArC), 127.34 (ArC), 117.25 (ArC), 115.19 (ArC), 114.95 (ArC), 55.53 (CH_3); HRMS: Calculated mass for $C_{13}H_{12}ClN_2O_3$ $[M+H]^+$ 279.0539 found 279.0531 $[M+H]^+$.

Preparation of 8-Chloro-10-(4-dimethylamino-phenyl)-10H-benzo[g]pteridine-2,4-dione (4a)



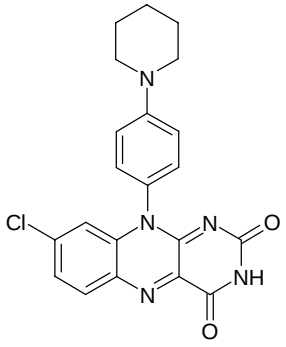
To a stirred solution of compound (**3a**) (0.5 g, 1.71 mmol) in glacial acetic acid (15 mL) was added Zn dust (1.11 g, 17.1 mmol) in portions, whilst maintaining the temperature below 40 °C (using a cold water bath). The reaction mixture was stirred at room temperature under nitrogen atmosphere for 20 minutes. The reaction mixture was quickly filtered through a pad of celite and washed with acetic acid (5 mL). To the same acetic acid solution of diamine was added alloxan.H_2O (0.32 g, 2.0 mmol) and boric acid (0.16 g, 2.6 mmol). The reaction mixture was stirred at room temperature under nitrogen atmosphere for 30 minutes. The solvent was removed under reduced pressure. The crude product was purified by flash chromatography using 5 % MeOH in $CHCl_3$ to afford a green solid (0.23 g, 37 %). Melting point >270 °C; ^{1}H NMR ($CDCl_3$ + CD_3OD-d^4, 400 MHz) δ_H 8.17 (d, ArH, *J* = 8.7 Hz), 7.52 (dd, ArH, *J* = 6.61 Hz, *J'* = 10.93 Hz), 7.04 (dt, 2 × ArH, *J* = 2.2 Hz, *J'* = 3.37 Hz), 7.01 (d, ArH, *J* = 2.06 Hz), 6.84 (dt, 2 × ArH, *J* = 2.15 Hz, *J'* = 3.28 Hz), 3.03 (s, 2 × CH_3); ^{13}C NMR (DMSO-d^6, 125 MHz) δ_C 159.58 (qC), 155.67 (qC), 152.46 (qC), 150.9 (qC),139.83 (qC), 138.87 (qC), 135.97 (qC), 133.59 (qC), 133.2 (ArC), 128.03 (ArC), 126.15 (ArC), 123.53 (qC), 116.25 (ArC), 112.89 (ArC), 40.02 (CH_3); HRMS: Calculated mass for $C_{18}H_{15}ClN_5O_2$ $[M+H]^+$ 368.0923 found 368.0919 $[M+H]^+$.

Preparation of 8-Chloro-10-(4-fluoro-phenyl)-10H-benzo[g]pteridine-2,4-dione (4b)



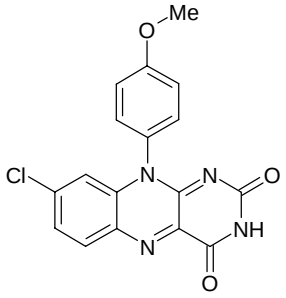
To a stirred solution of compound (**3b**) (0.7 g, 2.62 mmol) in glacial acetic acid (15 mL) was added Zn dust (1.71 g, 26.2 mmol) in portions, whilst maintaining the temperature below 40 °C (using a cold water bath). The reaction mixture was stirred at room temperature under nitrogen atmosphere for 30 minutes. The reaction mixture was quickly filtered through a pad of celite and washed with acetic acid (5 mL). To the same acetic acid solution of diamine was added alloxan.H_2O (0.504 g, 3.14 mmol) and boric acid (0.25 g, 3.93 mmol). The reaction mixture was stirred at room temperature under nitrogen atmosphere for 2 hours. The solvent was removed under reduced pressure. The crude product was purified by flash chromatography using 5 % MeOH in $CHCl_3$ to afford the title compound as a yellow solid (0.35 g, 39 %). Melting point >270 °C; ^{1}H NMR (DMSO-d^6, 400 MHz) δ_H 8.19 (d, ArH, J = 4.87 Hz), 7.65 (dd, ArH, J = 6.61 Hz, J' = 10.89 Hz), 7.52 (m, 4 × ArH), 6.71 (d, ArH, J = 2.11 Hz); ^{13}C NMR (DMSO-d^6, 100 MHz) δ_C 164.8 (qC), 162.32 (qC), 159.91 (qC), 156.33 (qC), 150.95 (qC), 141.08 (qC), 138.28 (qC), 135.21 (qC), 134.18 (qC), 132.95 (ArC), 129.93 (ArC), 129.84 (ArC), 126.97 (ArC), 117.46 (ArC), 117.22 (ArC), 116.32 (ArC); HRMS: Calculated mass for $C_{16}H_9ClFN_4O_2$ $[M+H]^+$ 343.0384 found 343.0392 $[M+H]^+$.

Preparation of 8-Chloro-10-(4-piperidin-1-yl-phenyl)-10H-benzo[g]pteridine-2,4-dione (4c)



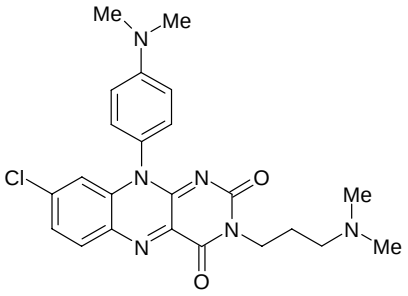
To a stirred solution of compound (**3c**) (0.5 g, 1.5 mmol) in glacial acetic acid (15 mL) was added Zn dust (0.98 g, 15.1 mmol) in portions, whilst maintaining the temperature below 40 °C (using a cold water bath). The reaction mixture was stirred at room temperature under nitrogen atmosphere for 30 minutes. The reaction mixture was filtered through a pad of celite and washed with acetic acid (5 mL). To the same acetic acid solution of diamine was added alloxan.H_2O (0.29 g, 1.8 mmol) and boric acid (0.14 g, 2.25 mmol). The reaction mixture was stirred at room temperature under nitrogen atmosphere for 1 hour. The solvent was removed under reduced pressure. The crude product was purified by flash chromatography using 3 % MeOH in $CHCl_3$ to afford the title compound as a brown solid (0.51 g, 83 %). Melting point >270 °C; ^{1}H NMR (CD_3OD-d^4, 400 MHz) δ_H 8.18 (d, ArH, J = 8.76 Hz), 7.62 (dd, ArH, J = 2.16 Hz, J' = 10.92 Hz), 7.22 (m, 4 × ArH), 6.79 (d, ArH, J = 2.19 Hz), 3.42 (quintet, 4H), 1.75 (m, 4H), 1.66 (m, 2H); ^{13}C NMR (DMSO-d^6, 125 MHz) δ_C 172.1 (qC), 159.55 (qC), 155.66 (qC), 152.35 (qC), 139.83 (qC), 138.91 (qC), 135.8 (qC), 133.58 (qC), 133.21 (ArC), 128.13 (ArC), 126.19 (ArC), 125.19 (qC), 116.19 (ArC), 116.06 (ArC), 48.71 (CH_2), 25.23 (CH_2), 23.94 (CH_2); HRMS: Calculated mass for $C_{21}H_{19}ClN_5O_2$ $[M+H]^+$ 408.1219 found 408.1221 $[M+H]^+$.

Preparation of 8-Chloro-10-(4-methoxy-phenyl)-10H-benzo[g]pteridine-2,4-dione (4d)



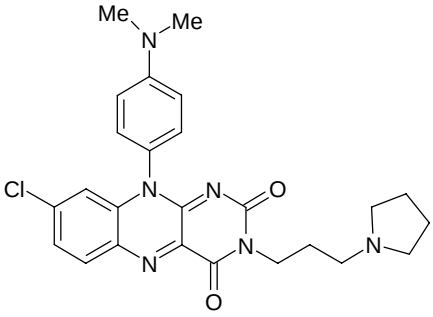
To a stirred solution of compound (**3d**) (0.2 g, 0.71 mmol) in glacial acetic acid (10 mL) was added Zn dust (0.47 g, 7.1 mmol) in portions, whilst maintaining the temperature below 40 °C (using a cold water bath). The reaction mixture was stirred at room temperature under nitrogen atmosphere for 20 minutes. The reaction mixture was quickly filtered through a pad of celite and washed with acetic acid (5 mL). To the same acetic acid solution of diamine was added alloxan.H_2O (0.14 g, 0.85 mmol) and boric acid (0.066 g, 1.07 mmol). The reaction mixture was stirred at 100 °C under nitrogen atmosphere for 0.5 hours. The solvent was removed under reduced pressure. The crude product was purified by flash chromatography using 4 % MeOH in $CHCl_3$ to afford the title compound as a greenish solid (0.16 g, 64 %). Melting point > 270 °C; ^{1}H NMR (DMSO-d^6, 500 MHz) δ_H 11.49 (s, NH), 8.22 (d, ArH, J = 7.7 Hz), 7.68 (dd, ArH, J = 2.08 Hz, J' = 10.79 Hz), 7.36 (d, 2 × ArH, J = 8.86 Hz), 7.26 (d, 2 × ArH, J = 8.88 Hz), 6.74 (d, ArH, J = 2.04 Hz), 3.90 (s, OCH_3); ^{13}C NMR (DMSO-d^6, 125 MHz) δ_C 160.12 (qC), 159.47 (qC), 155.57 (qC), 152.25 (qC), 139.83 (qC), 139.01 (qC), 135.54 (qC), 133.54 (qC), 133.23 (ArC), 128.98 (ArC), 128.04 (qC), 126.27 (ArC), 116.07 (ArC), 115.69 (ArC), 55.64 (CH_3); HRMS: Calculated mass for $C_{17}H_{12}ClN_4O_3$ $[M+H]^+$ 355.0604 found 355.0592 $[M+H]^+$.

Preparation of 8-Chloro-10-(4-dimethylamino-phenyl)-3-(3-dimethylamino-propyl)-10H-benzo [g] pteridine-2,4-dione (5a)



To a stirred suspension of compound (**4a**) (0.1 g, 0.27 mmol) and anhydrous potassium carbonate (0.11 g, 0.81 mmol) in anhydrous DMF (5 mL) was added *N,N*-dimethylaminopropyl chloride.HCl (0.13 g, 0.81 mmol). The reaction mixture was stirred at 90 °C under nitrogen atmosphere for 2 hours. The reaction mixture was filtered and washed with DMF (5 mL). The solvent was removed under reduced pressure. The crude product was purified by flash chromatography using 15 % MeOH in $CHCl_3$ to afford the title compound as a brown solid (55 mg, 45 %). Melting point 250 °C, decomposed; ^{1}H NMR (CD_3OD-d^4, 500 MHz) δ_H 8.19 (d, ArH, J = 8.77 Hz), 7.63 (dd, ArH, J = 2.15 Hz, J' = 10.9 Hz), 7.18 (d, 2 × ArH, J = 9.03 Hz), 6.99 (d, ArH, J = 2.11 Hz), 6.82 (d, 2 × ArH, J = 9.03 Hz), 4.06 (t, 2H, J = 7.27 Hz), 3.13 (s, 2 × CH_3), 2.49 (t, 2H, J = 7.4 Hz), 2.30 (s, 2 × CH_3), 1.91 (quintet, 2H); ^{13}C NMR (CD_3OD-d^4, 125 MHz) δ_C 161.61 (qC), 158.13 (qC), 153.13 (qC), 152.74 (qC), 142.29 (qC), 139.65 (qC), 137.6 (qC), 135.7 (qC), 134.17 (qC), 129.12 (ArC), 128.2 (ArC), 124.52 (ArC), 118.33 (ArC), 114.16 (ArC), 57.88 (CH_2), 45.71 (CH_3), 41.71 (CH_2), 41.11 (CH_3), 26.4 (CH_2); HRMS: Calculated mass for $C_{23}H_{26}ClN_6O_2$ $[M+H]^+$ 453.1823 found 453.1811 $[M+H]^+$.

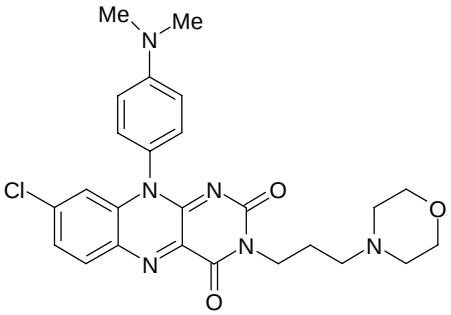
Preparation of 8-Chloro-10-(4-dimethylamino-phenyl)-3-(3-pyrrolidin-1-yl-propyl)-10H-benzo [g] pteridine-2,4-dione (5b)



To a stirred suspension of compound (**4a**) (0.4 g, 1.08 mmol) and anhydrous potassium carbonate (0.45 g, 3.26 mmol) in anhydrous DMF (5 mL) was added

3-chloropropylpyrrolidine (0.48 g, 3.26 mmol) in anhydrous DMF (2 mL). The reaction mixture was stirred at 90 °C under nitrogen atmosphere for 2 hours. The reaction mixture was filtered and washed with DMF (10 mL). The solvent was removed under reduced pressure. The crude product was purified by flash chromatography using 20 % MeOH in $CHCl_3$ to afford the title compound as a brown solid (0.25 mg, 48 %). Melting point 260 °C, decomposed; ^{1}H NMR (DMSO-d^6, 500 MHz) δ_H 8.21 (d, ArH, J = 8.72 Hz), 7.67 (dd, ArH, J = 2.2 Hz, J' = 10.92 Hz), 7.16 (dt, 2 × ArH, J = 2.02 Hz, J' = 12.23 Hz), 6.94 (dt, 2 × ArH, J = 2.02 Hz, J' = 12.26 Hz), 6.82 (d, ArH, J = 2.19 Hz), 3.90 (t, 2H, J = 7.11 Hz), 3.02 (s, 2 × CH_3), 2.62 (m, 6H), 1.80 (quintet, 2H), 1.70 (m, 4H); ^{13}C NMR (DMSO-d^6, 125 MHz) δ_C 159.19 (qC), 154.88 (qC), 151.06 (qC), 139.08 (qC), 135.82 (qC), 133.8 (qC), 133.25 (ArC), 128.03 (ArC), 126.32 (ArC), 123.15 (qC), 116.29 (ArC), 112.86 (ArC), 53.53 (CH_2), 53.37 (CH_2), 30.76 (CH_3), 23.1 (CH_2), 23.04 (CH_2); HRMS: Calculated mass for $C_{25}H_{28}ClN_6O_2$ $[M+H]^+$ 479.1859 $[M+H]^+$ found 479.1876 $[M+H]^+$.

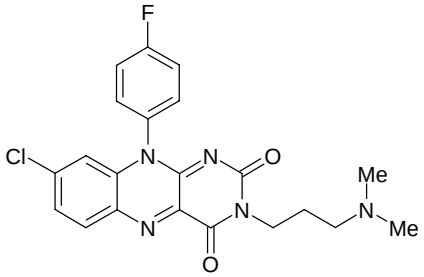
Preparation of 8-Chloro-10-(4-dimethylamino-phenyl)-3-(3-morpholin-4-yl-propyl)-10H-benzo [g] pteridine-2,4-dione (5c)



To a stirred suspension of compound (**4a**) (0.25 g, 0.68 mmol) and anhydrous potassium carbonate (0.28 g, 2.03 mmol) in anhydrous DMF (6 mL) was added *N*-(3-chloropropyl)-morpholine (0.33 g, 2.03 mmol). The reaction mixture was stirred at 80 °C under

nitrogen atmosphere for 2 hours. The reaction mixture was filtered and washed with DMF (5 mL). The solvent was removed under reduced pressure. The crude product was purified by flash chromatography using 8 % MeOH in $CHCl_3$ to afford the title compound as a brown solid (100 mg, 30 %). Melting point 240 °C, decomposed; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 8.22 (d, ArH, *J* = 4.96 Hz), 7.47 (dd, ArH, *J* = 4.84 Hz, *J'* = 13.58 Hz), 7.08 (dt, 2 × ArH, *J* = 2.2 Hz, *J'* = 3.35 Hz,), 7.04 (d, ArH, *J* = 2.12 Hz), 6.87 (dt, 2 × ArH, *J* = 2.2 Hz, *J'* = 3.31 Hz,), 4.15 (t, 4H, *J* = 7.17 Hz), 3.62 (t, 2H, *J* = 4.62 Hz), 3.07 (s, 2 × CH_3), 2.46 (m, 4H), 2.41 (m, 2H), 1.87 (quintet, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 159.45 (qC), 155.21 (qC), 151.33 (qC), 150.65 (qC), 141.74 (qC), 137.65 (qC), 135.86 (qC), 134.1 (qC), 133.46 (qC), 127.7 (ArC), 127.1 (ArC), 122.3 (ArC), 117.17 (ArC), 113.16 (ArC), 67.05 (CH_2), 56.30 (CH_2), 53.49 (CH_2), 40.52 (CH_2), 40.31 (CH_3), 24.18 (CH_2); HRMS: Calculated mass for $C_{25}H_{28}ClN_6O_3$ $[M+H]^+$ 495.1911 found 495.1901 $[M+H]^+$.

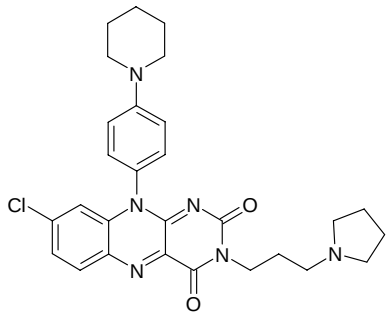
Preparation of 8-Chloro-3-(3-dimethylamino-propyl)-10-(4-fluoro-phenyl)-10H-benzo [g] pteridine-2,4-dione (5d)



To a stirred suspension of compound (**4b**) (0.15 g, 0.48 mmol) and anhydrous potassium carbonate (0.18 g, 1.31 mmol) in anhydrous DMF (6 mL) was added *N,N*-dimethylaminopropyl chloride.HCl (0.21 g, 1.31 mmol). The reaction mixture was stirred at 90 °C under nitrogen atmosphere for 4 hours. The reaction mixture was filtered

and washed with DMF (5 mL). The solvent was removed under reduced pressure. The crude product was purified by flash chromatography using 17 % MeOH in $CHCl_3$ to afford the title compound as a yellow solid (50 mg, 27 %). ^1H NMR (CD_3OD-d^4, 400 MHz) δ_H 8.20 (d, ArH, J = 8.76 Hz), 7.67 (dd, ArH, J = 2.14 Hz, J' = 10.89 Hz), 7.47 (m, 4 × ArH), 6.95 (d, ArH, J = 2.07 Hz), 4.04 (t, 2H, J = 7.27 Hz), 2.53 (t, 2H, J = 7.34 Hz), 2.32 (s, 2 × CH_3), 1.91 (quintet, 2H); ^{13}C NMR (CD_3OD-d^4, 100 MHz) δ_C 164.8 (qC), 162.33 (qC), 159.91(qC), 156.34 (qC), 150.95 (qC), 141.09 (qC), 138.28 (qC), 135.21 (qC), 134.18 (qC), 132.92 (ArC), 131.12 (qC), 129.93 (ArC), 129.84 (ArC), 126.96 (ArC), 117.46 (ArC), 117.25 (ArC), 116.34 (ArC), 56.39 (CH_2), 43.71 (CH_3), 39.65 (CH_2), 24.64 (CH_2); HRMS: Calculated mass for $C_{21}H_{20}ClFN_5O_2$ $[M+H]^+$ 428.1290 found 428.1302 $[M+H]^+$.

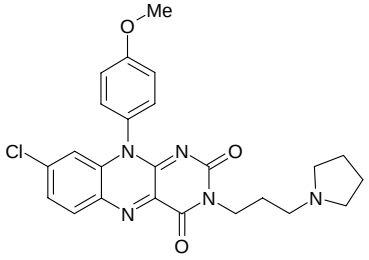
Preparation of 8-Chloro-10-(4-piperidin-1-yl-phenyl)-3-(3-pyrrolidin-1-yl-propyl)-10H-benzo [g] pteridine-2,4-dione (5e)



To a stirred suspension of compound (**4c**) (0.15 g, 0.37 mmol) and anhydrous potassium carbonate (0.15 g, 1.1 mmol) in anhydrous DMF (6 ml) was added *N*-(3-chloropropyl) pyrrolidine (0.16 g, 1.1 mmol). The reaction mixture was stirred at 90 °C under nitrogen atmosphere for 1 hour. The reaction mixture was filtered and washed with DMF (5 mL). The solvent was removed under reduced pressure. The crude product was purified by flash chromatography using 15 % MeOH in $CHCl_3$ to afford the title compound as a

brown solid (165 mg, 87 %). Melting point 256 °C, decomposed; ^{1}H NMR (CD_3OD-d^4, 500 MHz) δ_H 8.18 (d, ArH, *J* = 8.76 Hz), 7.64 (dd, ArH, *J* = 2.16 Hz, *J'* = 10.92 Hz), 7.19 (s, 4 × ArH), 6.98 (d, ArH, *J* = 2.12 Hz), 4.09 (dt, 2H, *J* = 6.38 Hz, *J'* = 2.15 Hz), 3.35 (t, 4H, *J* = 5.23 Hz), 2.83 (m, 4H), 2.71 (m, 2H), 2.0 (m, 6H), 1.84 (m, 6H); ^{13}C NMR (CD_3OD-d^4, 125 MHz) δ_C 172.81 (qC) 161.61 (qC), 158.19 (qC), 154.59 (qC), 152.61 (qC), 142.42 (qC), 139.61 (qC), 137.33 (qC), 135.74 (qC), 134.23 (ArC), 129.25 (ArC), 128.34 (ArC), 126.43 (ArC), 118.25 (ArC), 63.59 (CH_2), 55.18 (CH_2), 54.6 (CH_2), 53.93 (CH_2), 50.9 (CH_2), 40.85 (CH_2), 28.42 (CH_2), 27.2 (CH_2), 26.7 (CH_2), 25.41 (CH_2), 24.12 (CH_2), 20.73 (CH_2); HRMS: Calculated mass for $C_{28}H_{32}ClN_6O_2$ $[M+H]^+$ 519.2252 $[M+H]^+$ found 519.2269 $[M+H]^+$.

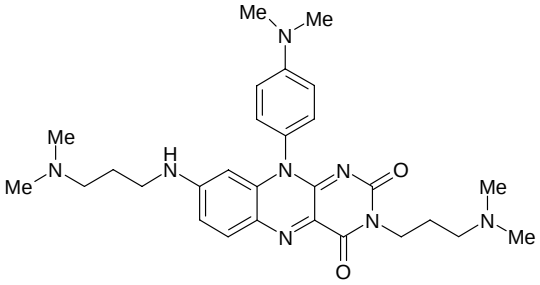
Preparation of 8-Chloro-10-(4-methoxy-phenyl)-3-(3-pyrrolidin-1-yl-propyl)-10H-benzo [g] pteridine-2,4-dione (5f)



To a stirred suspension of compound (**4d**) (0.12 g, 0.34 mmol) and anhydrous potassium carbonate (0.14 g, 1.01 mmol) in anhydrous DMF (6 mL) was added *N*-(3-chloropropyl) pyrrolidine (0.15 g, 1.01 mmol). The reaction mixture was stirred at 90 °C under nitrogen atmosphere for 1 hour. The reaction mixture was filtered and washed with DMF (5 mL). The solvent was removed under reduced pressure. The crude product was purified by flash chromatography using 16 % MeOH in $CHCl_3$ to afford the title compound as a yellow solid (60 mg, 39 %). Melting point >270 °C; ^{1}H NMR (CD_3OD-

d^4, 500 MHz) δ_H 8.19 (d, ArH, J = 8.78 Hz), 7.64 (dd, ArH, J = 2.13 Hz, J' = 10.9 Hz), 7.32 (d, 2 × ArH, J = 6.75 Hz), 7.23 (d, 2 × ArH, J = 6.84 Hz), 6.92 (d, ArH, J = 2.1 Hz), 4.08 (t, 2H, J = 7.01 Hz), 3.11 (s, 3H), 2.87 (m, 6H), 2.0 (quintet, 2H), 1.89 (quintet, 4H); ^{13}C NMR (CD_3OD-d^4, 125 MHz) δ: 162.63 (qC) 161.56 (qC), 158.03 (qC), 152.52 (qC), 142.49 (qC), 139.65 (qC), 137.65 (qC), 135.68 (qC), 134.26 (ArC), 130.01 (qC), 128.85 (ArC), 128.4 (ArC), 118.06 (ArC), 116.87 (ArC), 56.26 (CH_2), 55.11 (CH_3), 54.48 (CH_2), 40.62 (CH_2), 26.91 (CH_2), 24.07 (CH_2); HRMS: Calculated mass for $C_{24}H_{25}ClN_5O_3$ $[M+H]^+$ 466.1655 $[M+H]^+$ found 466.1640 $[M+H]^+$.

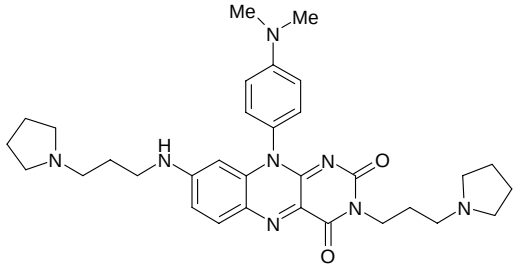
Preparation of 10-(4-Dimethylamino-phenyl)-3-(3-dimethylamino-propyl)-8-(3-dimethylamino-propylamino)-10H-benzo[g]pteridine-2,4-dione (1a)



A 10 mL round bottom flask was charged with compound (**5a**) (60 mg, 0.13 mmol) and *N,N*-dimethylaminopropyl amine (2 mL). The reaction mixture was stirred at 100 °C, under nitrogen atmosphere for 30 minutes. The reaction mixture was cooled to room temperature and cold ether (5 mL) was added to afford an orange solid. The solid was separated by centrifugation and was washed with diethyl ether (3 × 10 mL) to afford the title compound as an orange solid (40 mg, 59 %). Melting point 248-250 °C; ^{1}H NMR (CD_3OD-d^4, 400 MHz) δ_H 7.79 (d, ArH, J = 9.11 Hz), 7.16 (d, 2 × ArH, J = 8.95 Hz), 7.01 (d, ArH, J = 8.79 Hz), 6.93 (d, 2 × ArH, J = 8.95 Hz), 5.85 (s, ArH), 3.98 (t, 2H, J = 7.82 Hz), 3.07 (m, 2H), 3.03 (s, 2 × CH_3), 2.41 (t, 2H, J = 7.54 Hz), 2.29 (t, 2H, J = 7.33

Hz), 2.23 (s, 2 × CH_3), 2.14 (s, 2 × CH_3), 1.85 (quintet, 2H), 1.69 (quintet, 2H); ^{13}C NMR (CD_3OD-d^4, 100 MHz) δ_C 163.09 (qC), 158.63 (qC), 152. 7 (qC), 152.6 (qC), 133.73 (qC), 129.0 (ArC), 127.98 (qC), 125.68 (qC), 114.25 (ArC), 58.06 (CH_2), 57.92 (CH_2), 45.44 (CH_3), 45.32 (CH_3), 42.22 (CH_2), 40.82 (CH_2), 40.62 (CH_3), 27.12 (CH_2), 26.72 (CH_2); HRMS: Calculated mass for $C_{28}H_{39}N_8O_2$ $[M+H]^+$ 519.3196 found 519.3206 $[M+H]^+$.

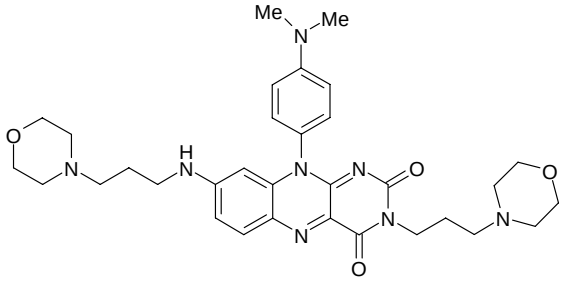
Preparation of 10-(4-Dimethylamino-phenyl)-3-(3-pyrrolidin-1-yl-propyl)-8-(3-pyrrolidin-1-yl-propylamino)-10H-benzo[g]pteridine-2,4-dione (1b)



A 10 mL round bottom flask was charged with compound (**5b**) (90 mg, 0.19 mmol) and 3-aminopropyl pyrrolidine (2 mL). The reaction mixture was stirred at 100 °C, under nitrogen atmosphere for 20 minutes. The reaction mixture was cooled to room temperature and cold ether (5 mL) was added to afford a red solid. The solid was separated by centrifugation and was washed with diethyl ether (3 × 10 mL) to afford the title compound as a red solid (95 mg, 88 %). Melting point 244-246 °C, decomposed; ^{1}H NMR (CD_3OD-d^4, 500 MHz) δ_H 7.70 (d, ArH, J = 8.82 Hz), 7.19 (d, 2 × ArH, J = 8.77 Hz), 7.01 (d, ArH, J = 8.79 Hz), 6.96 (d, 2 × ArH, J = 8.96 Hz), 5.77 (s, ArH), 4.02 (t, 2H, J = 6.78 Hz), 3.05 (s, 6H), 2.86 (t, 2H, J = 7.06 Hz), 2.70 (m, 6H), 2.61 (t, 4H, J = 5.49 Hz), 2.50 (m, 4H), 1.95 (quintet, 2H), 1.82 (m, 8H); ^{13}C NMR (CD_3OD-d^4, 125 MHz) δ_C 163.06 (qC), 158.95 (qC), 152.79 (qC), 152.51 (qC), 133.93 (qC), 129.27

(ArC), 127.40 (qC), 125.59 (qC), 114.28 (ArC), 54.99 (CH_2), 54.89 (CH_2), 54.48 (CH_2), 42.46 (CH_2), 40.64 (CH_3), 40.49 (CH_2), 40.45 (CH_2), 29.29 (CH_2), 27.59 (CH_2), 24.20 (CH_2), 24.03 (CH_2); HRMS: Calculated mass for $C_{32}H_{43}N_8O_2$ $[M+H]^+$ 571.3509 found 571.3535 $[M+H]^+$.

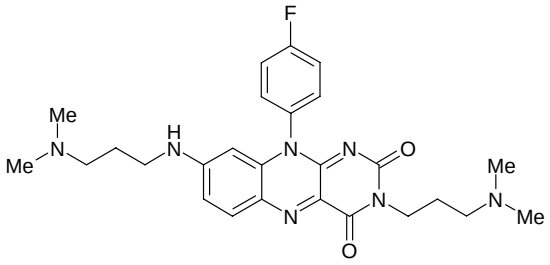
Preparation of 10-(4-Dimethylamino-phenyl)-3-(3-morpholin-4-yl-propyl)-8-(3-morpholin-4-yl-propylamino)-10H-benzo[g]pteridine-2,4-dione (1c)



A 10 mL round bottom flask was charged with compound (**5c**) (60 mg, 0.12 mmol) and 3-aminopropyl morpholine (2 mL). The reaction mixture was stirred at 100 °C, under nitrogen atmosphere for 90 minutes. The reaction mixture was cooled to room temperature and cold ether (5 mL) was added to afford a red solid. The solid was separated by centrifugation and was washed with diethyl ether (3 × 10 mL). The crude product was purified by flash chromatography using MeOH: $CHCl_3$: Et_3N (1:4:0.1) to afford the title compound as a red solid (25 mg, 35 %). Melting point 284-286 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 7.94 (d, ArH, J = 9.07 Hz), 7.53 (s, NH), 7.05 (d, 2 × ArH, J = 8.97 Hz), 6.83 (d, 2 × ArH, J = 8.99 Hz), 6.75 (dd, ArH, J = 2.21 Hz, J' = 11.31 Hz), 5.72 (d, ArH, J = 1.93 Hz), 4.13 (t, 2H, J = 7.27 Hz), 3.64 (t, 8H, J = 4.39 Hz), 3.03 (s, 2 × CH_3), 2.51 (t, 2H, J = 5.69 Hz), 2.45 (m, 12H), 1.88 (quintet, 2H), 1.73 (quintet, 2H); ^{13}C NMR (CD_3OD-d^4, 125 MHz) δ_C 161.16 (qC), 156.22 (qC), 154.44 (qC), 151.18 (qC), 150.98 (qC), 139.38 (qC), 131.31 (qC), 127.81 (ArC), 123.84 (qC), 113.53 (ArC),

66.98 (CH_2), 66.72 (CH_2), 58.62(CH_2), 57.78 (CH_2), 56.36 (CH_2), 53.54 (CH_2), 43.77 (CH_2), 40.41 (CH_3), 39.94 (CH_2), 24.45 (CH_2), 23.15 (CH_2); HRMS: Calculated mass for $C_{32}H_{43}N_8O_4$ $[M+H]^+$ 603.3407 found 603.3412 $[M+H]^+$.

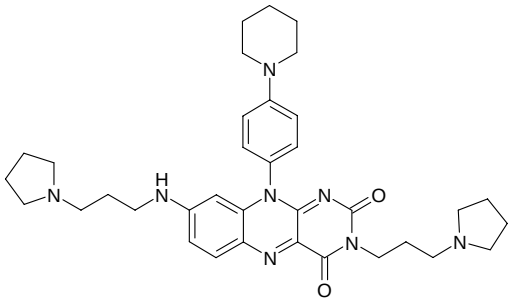
Preparation of 3-(3-Dimethylamino-propyl)-8-(3-dimethylamino-propylamino)-10-(4-fluoro-phenyl)-10H-benzo[g]pteridine-2,4-dione (1d)



A 10 mL round bottom flask was charged with compound (**5d**) (25 mg, 0.06 mmol) and *N,N*-dimethylaminopropyl amine (1 mL). The reaction mixture was stirred at 100 °C, under nitrogen atmosphere for 30 minutes. The reaction mixture was cooled to room temperature and cold ether (5 mL) was added to afford an orange solid. The solid was separated by centrifugation and was washed with diethyl ether (3 × 10 mL). The crude product was purified by flash chromatography using MeOH: $CHCl_3$: Et_3N (1:4:0.1) to afford the title compound as an orange solid (20 mg, 70 %). ^{1}H NMR (CD_3OD-d^4, 500 MHz) δ_H 7.84 (d, ArH, J = 8.67 Hz), 7.42 (m, 4H), 7.05 (d, ArH, J = 7.67 Hz), 5.70 (s, ArH), 4.03 (t, 2H, J = 7.25 Hz), 2.46 (t, 2H, J = 7.5 Hz), 2.31 (m, 2H), 2.27 (s, 2 × CH_3), 2.23 (s, 2 × CH_3), 2.19 (m, 2H), 1.83 (quintet, 2H), 1.69 (quintet, 2H); ^{13}C NMR (CD_3OD-d^4, 100 MHz) δ_C 163.01 (qC), 158.48 (qC), 152.32 (qC), 133.65 (qC), 133.56 (qC), 131.41 (qC), 131.33 (ArC), 128.01 (qC), 118.7 (ArC), 118.52 (ArC), 58.06 (CH_2), 57.92 (CH_2), 45.44 (CH_3), 45.03 (CH_3), 42.22 (CH_2), 40.82 (CH_2), 40.62 (CH_2), 26.72

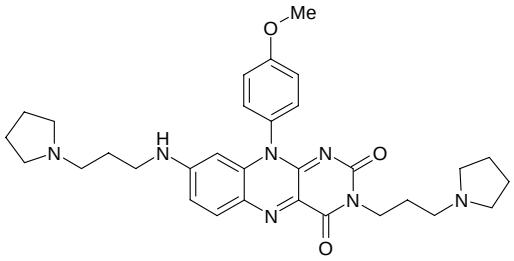
(CH_2); HRMS: Calculated mass for $C_{26}H_{33}FN_7O_2$ $[M+H]^+$494.2680 found 494.2696 $[M+H]^+$.

Preparation of 10-(4-Piperidin-1-yl-phenyl)-3-(3-pyrrolidin-1-yl-propyl)-8-(3-pyrrolidin-1-yl-propylamino)-10H-benzo[g]pteridine-2,4-dione (1e)



A 10 ml round bottom flask was charged with compound (**5e**) (75 mg, 0.144 mmol) and 3-aminopropyl pyrrolidine (2 mL). The reaction mixture was stirred at 100 °C, under nitrogen atmosphere for 20 minutes. The reaction mixture was cooled to room temperature and cold ether (5 mL) was added to afford a red solid. The solid was separated by centrifugation and was washed with diethyl ether (3 × 10 mL) to afford the title compound as a red solid (80 mg, 91 %). Melting point >270 °C; ^{1}H NMR (CD_3OD-d^4, 500 MHz) δ_H 7.72 (d, ArH, J = 6.25 Hz), 7.20 (q, 4 × ArH, J = 16.14 Hz, J' = 25.46 Hz), 6.97 (d, ArH, J = 6.95 Hz), 5.82 (s, ArH), 4.03 (t, 2H, J = 6.82 Hz), 3.21 (m, 2H), 2.75 (m, 8H), 2.49 (m, 8H), 1.95 (quintet, 2H), 1.72 (m, 16H); ^{13}C NMR (CD_3OD-d^4, 125 MHz) δ_C 161.64 (qC), 157.49 (qC), 152.8 (qC), 150.99 (qC), 132.45 (qC), 127.92 (ArC), 126.16 (qC), 126.09 (qC), 116.61 (ArC), 53.6 (CH_2), 53.38 (CH_2), 53.3 (CH_2), 53.08 (CH_2), 49.57 (CH_2), 40.98 (CH_2), 39.04 (CH_2), 26.12 (CH_2), 25.32 (CH_2), 23.96 (CH_2), 22.77 (CH_2), 22.61 (CH_2); HRMS: Calculated mass for $C_{35}H_{47}N_8O_2$ $[M+H]^+$ 611.3841 found 611.3816 $[M+H]^+$.

Preparation of 10-(4-Methoxy-phenyl)-3-(3-pyrrolidin-1-yl-propyl)-8-(3-pyrrolidin-1-yl-propylamino)-10H-benzo[g]pteridine-2,4-dione (1f)



A 10 mL round bottom flask was charged with compound (**5f**) (45 mg, 0.96 mmol) and 3-aminopropyl pyrrolidine (2 mL). The reaction mixture was stirred at 100 °C, under nitrogen atmosphere for 20 minutes. The reaction mixture was cooled to room temperature and cold ether (5 mL) was added to afford a red solid. The solid was separated by centrifugation and was washed with diethyl ether (3 × 10 mL) to afford the title compound as a red solid (32 mg, 84 %). Melting point >270 °C; ^{1}H NMR (CD_3OD-d^4, 500 MHz) δ_H 7.63 (d, ArH, J = 6.73 Hz), 7.37 (d, 2 × ArH, J = 8.6 Hz), 7.22 (d, 2 × ArH, J = 8.8 Hz), 6.98 (d, ArH, J = 7.4 Hz), 5.57 (d, ArH, J = 7.4 Hz), 3.99 (t, 2H, J = 6.37 Hz), 3.90 (s, 3H), 3.73 (t, 2H, J = 7.82 Hz), 2.72 (m, 6H), 2.61 (m, 6H), 1.98 (quintet, 4H), 1.81 (m, 8H); ^{13}C NMR (CD_3OD-d^4, 125 MHz) δ_C 162.93 (qC), 162.29 (qC), 158.93 (qC), 152.23 (qC), 131.95 (qC), 130.25 (ArC), 129.9 (qC), 127.07 (qC), 116.93 (ArC), 56.3 (CH_3), 54.73 (CH_2), 54.52 (CH_2), 54.52 (CH_2), 54.16 (CH_2), 53.51 (CH_2), 42.39 (CH_2), 40.18 (CH_2), 24.19 (CH_2), 23.96 (CH_2), 22.95 (CH_2); HRMS: Calculated mass for $C_{31}H_{40}N_7O_3$ $[M+H]^+$ 558.3190 found 558.3187 $[M+H]^+$.

Surface plasmon resonance

Surface plasmon resonance measurements were performed on a four-channel BIAcore 3000 optical biosensor system (Biacore Inc.) using a streptavidin-coated sensor chip (Biacore SA-chip). In a typical experiment, biotinylated *ds-DNA* comprising the oligonucleotide d(biotin-[$G_2CATAGTGCGTG_3CGT_2AGC$]) hybridized with its complementary sequence, biotinylated *htelo* d(biotin-[$GT_2A(G_3T_2A)_4G_2$]), biotinylated *c-kit2* d(biotin-[$C_3G_3CG_3CGCGAG_3AG_4AG_2$]) and biotinylated *c-kit1* d(biotin-[$AG_3AG_3CGCTG_3AG_2AG_3$]) were folded in filtered and degassed running buffer (Tris-HCl 50 mM pH 7.4, 100 mM KCl; 95 °C for 5 min then cooled to room temperature overnight) and immobilized (600 RU) in flow cells 2, 3 and 4, leaving the first flow cell empty as a blank. DNA binding experiments were carried out with running buffer at a flow rate of 20 μl min^{-1}. Ligand solutions (**1a-1f**) (at 0.39, 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, 100 and 200 μM) were prepared with running buffer by serial dilutions from stock solutions. These solutions were injected using the KINJECT command (Biacore 3000 Control Software version 3.0.1) for 2 min followed by a 30 s 1 M KCl injection and a 30 s running buffer injection for chip regeneration. Each sample injection was repeated in duplicate. The response at equilibrium (R_{eq}) was plotted against concentration of analyte to generate a hyperbolic binding curve. The final graphs were obtained by subtracting blank sensorgrams from the duplex or quadruplex sensorgrams. For all compounds dissociation constants were determined by fitting the binding curve (from at least 5 concentrations) using the steady state affinity algorithm (Biaevaluation 3.0.2). The ligand stoichiometry for quadruplex binding was always either 1:1 or 2:1 by SPR, (Table 1) which is consistent with a general binding model involving ligand binding at one or both external G-quartets.

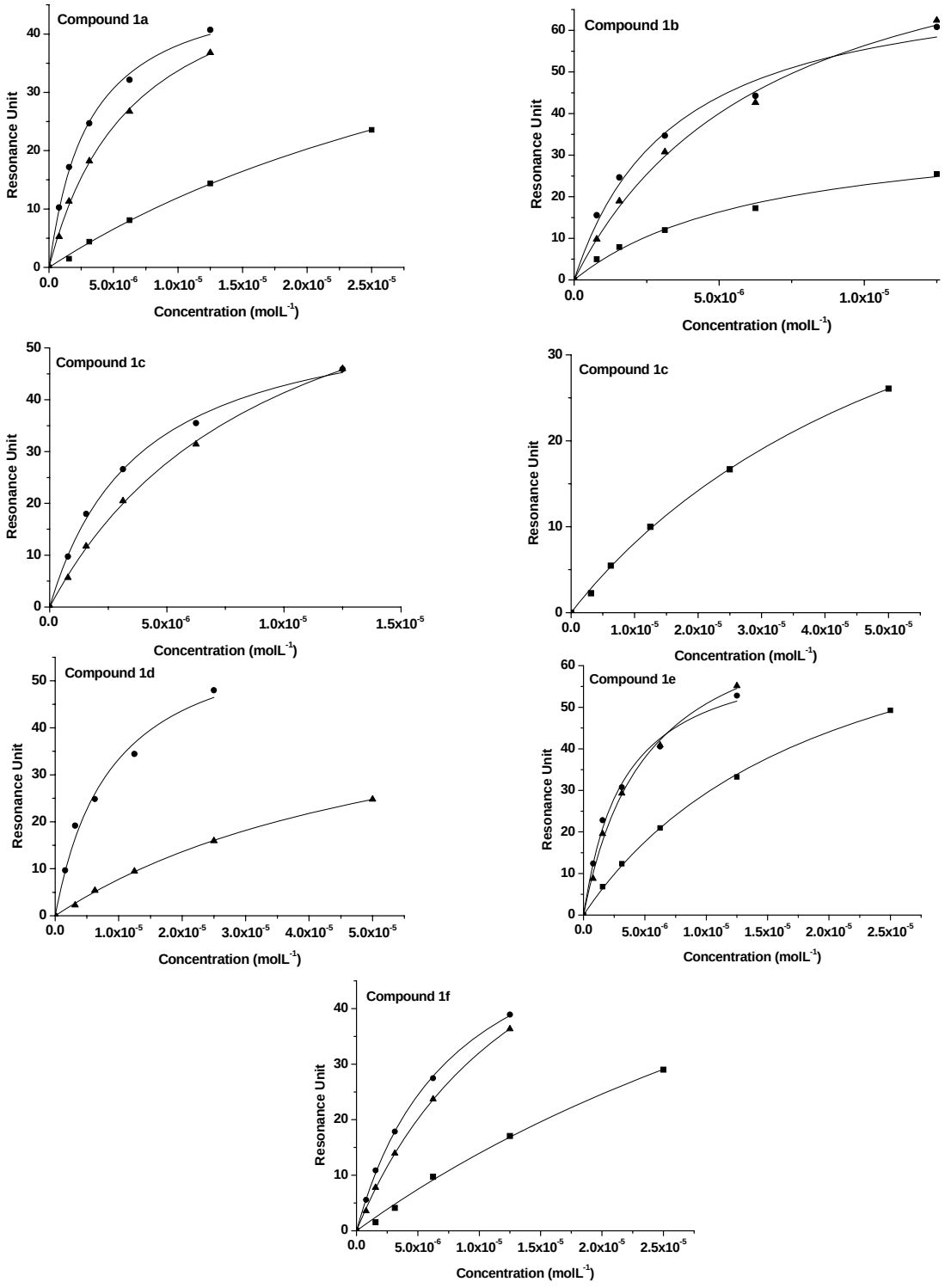


Figure 1: SPR binding curves for compounds **1a**, **1b**, **1c**, **1d**, **1e** and **1f**. (●) c-kit2, (▲) c-kit1, (■) htelo

Table 1. G-quadruplex binding stoichiometry of ligands **1a-1f**

Ligand	Target quadruplex DNA		
	c-kit2	c-kit1	htelo
1a	1	1	2
1b	2	2	1
1c	1	1	2
1d	1	1	-[a]
1e	2	2	2
1f	2	2	2

[a] stoichiometry could not be measured because no detectable binding was observed.

Fluorescence Resonance Energy Transfer (FRET) Assay

All the oligonucleotides and their fluorescent conjugates (Eurogentec, Southampton, UK) were initially dissolved as a 100 μM stock solution in purified water; further dilutions were carried out in the relevant buffer. The ability of the compounds to stabilize G-quadruplex DNA was investigated using a fluorescence resonance energy transfer (FRET) assay modified to be used as a high-throughput screen in a 96-well format. The labelled oligonucleotides htelo: 5'-FAM-d(GGG[TTAGGG]3)-TAMRA-3', c-kit2: 5'-FAM-d(GGG CGG GCG CGA GGG AGG GG)-TAMRA-3', c-kit1: 5'-FAM-d(GGG AGG GCG CTG GGA GGA GGG)-TAMRA-3', DNA duplex: 5'-FAM-d(TAT AGC TAT A-HEG-TAT AGC TAT A)-TAMRA-3'; donor fluorophore FAM is 6-carboxyfluorescein; acceptor fluorophore TAMRA is 6-carboxytetramethyl-rhodamine] were prepared as a 400 nM solution in a 60 mM potassium cacodylate buffer (pH 7.4) and then annealed by heating to 90 °C for 2 min, followed by cooling to room temperature. Compounds were stored at -80 °C and dilutions were done with 60 mM potassium cacodylate buffer (pH 7.4) (1 mM stock solution of ligands **1a, 1b, 1c, 1e** and **1f** were made up in 10 % DMSO in water). The 96-well plates (MJ Research, Waltham, MA) were prepared by aliquoting 50 μl of the annealed DNA into each well, followed by 50 μl of the compound solutions using Beckman Coulter liquid-handling robot. For each

compound, a minimum of 10 different concentrations were tested. Fluorescence melting curves were determined in a Roche light cycler 480, using a total reaction volume of 100 µl. Measurements were made in duplicate with excitation at 483 nm and detection at 533 nm. Final analysis of the data was carried out using Origin 7.5 (OriginLab Corp., Northampton, MA).

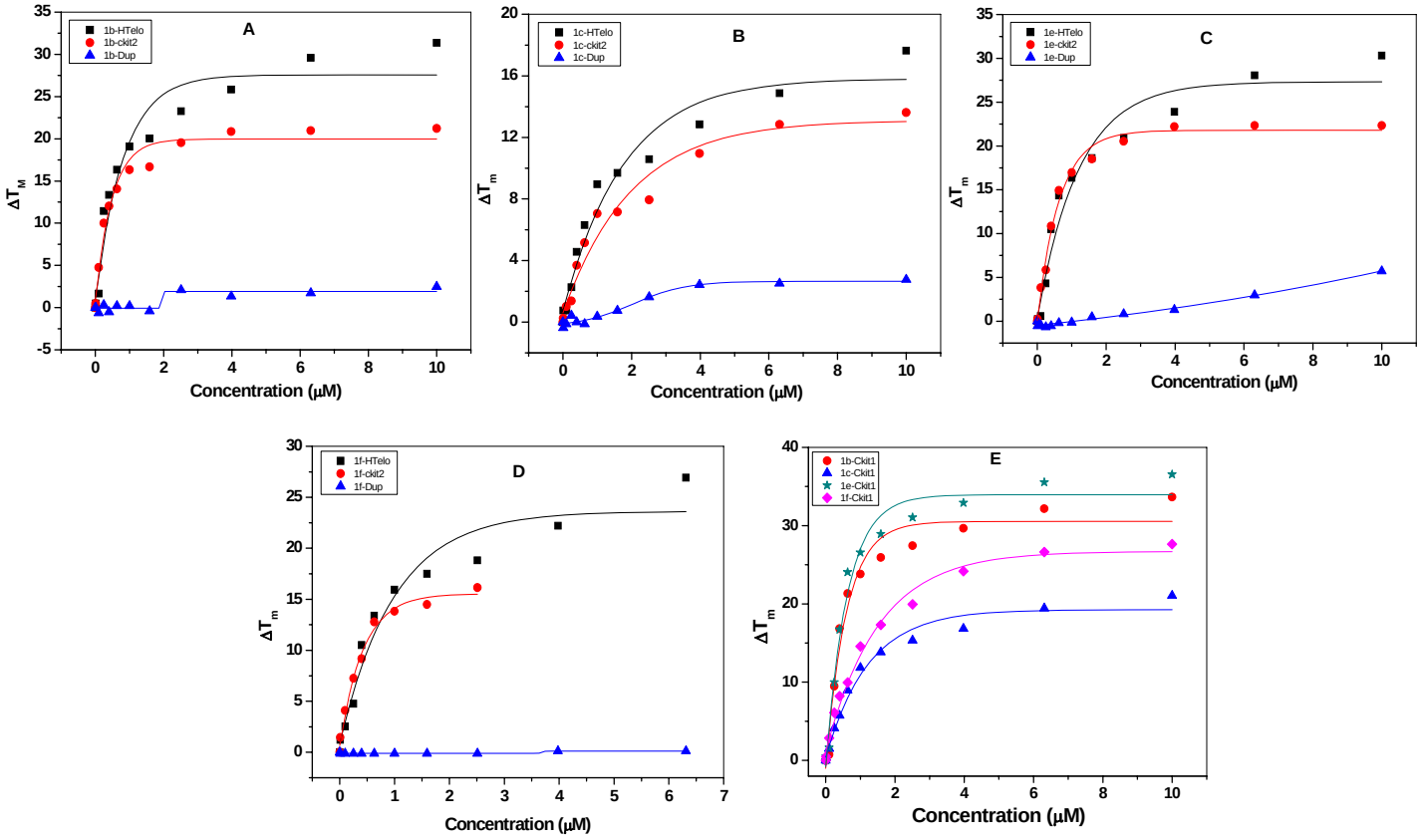


Figure 2. FRET-melt graphs for ligands **1b-1f**

Cell Based Experiment Procedure:

Gene expression analysis was carried out using MCF-7 or HGC-27 cell lines. MCF-7 cells were cultured in DMEM supplemented with 10 % fetal bovine serum (FBS), HGC-27 in EMEM, supplemented with 10 % FBS and 2 mM glutamine. Cells were cultured in T-75 flasks and were split at 70-80 % confluency. MCF-7 was a gift from Prof. Ashok

Venkitaraman, Hutchison/MRC Research Centre, MRC Cancer Cell Unit and University of Cambridge/Cancer Research UK, Cambridge, U.K. HGC-27 cell line was obtained from the European Collection of Cell Cultures (Cat. No. 94042256).

The ligands (**1a** and **1d**) were added after sterile filtration directly to the media from a 1 mM stock solution, which contained 10 % DMSO, to make final concentration of 5 μM in cell media. The control experiments consisted of the addition of water with 10 % DMSO. After incubation for the indicated time periods, cells were harvested for RNA isolation. RNA was extracted using the Qiagen RNeasy Kit according to the manufacturer's instructions. The quantity of the RNA was measured by UV spectrometry. Synthesis of cDNA was performed using 1 μg of total RNA and SuperScript III reverse transcriptase (Invitrogen), with one hour elongation time at 55 °C and oligo dT primers. The cDNA was quantified on a Roche LightCycler 480 real time PCR machine, using the SYBR Green I Master kit (Roche). The sequences of the primers used were CGTGGAAAAGAGAAAACAGTCA and CACCGTGATGCCAGCTA TTA for c-kit and CTCCTCCTGAGCGCAAGTACTC and TCCTGCTTGCTGATCCACA TC for *β*-actin. The annealing temperature was 64 °C for all primers. The levels of relative gene expression were calculated according to Pfaffl, *et al.*[1] The presence of single amplicon in the RT-PCR reaction was confirmed by the presence of just one peak in a thermal melting analysis carried out on the sample at the end of the RT-PCR. We carried out three independent experiments on each of two different days and the variation in c-kit expression was within 10 %.

Reference:

(1) Pfaffl, M.W.; Horgan, G. W.; Dempfle, L. *Nucleic Acids Res.* **2002**, *30*, e36.