Supporting Information

Targeting the *c-Kit* Promoter G-quadruplexes with 6-Substituted Indenoisoquinolines

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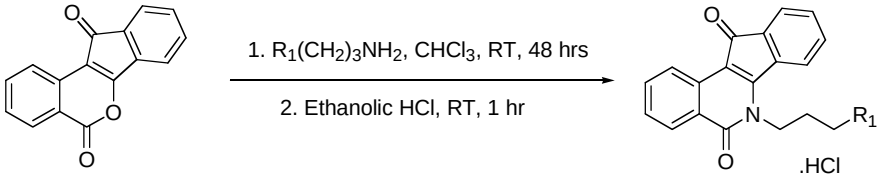
General Methods:

All chemicals and anhydrous solvents were purchased from Aldrich or Fisher Scientific, unless otherwise indicated. Anhydrous reactions were carried out under a nitrogen atmosphere. Thin layer chromatography (TLC) 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 visualized under a UV lamp and stained with iodine. Unless otherwise stated, all the ligand (**1a-e**) solutions were prepared in water for biophysical and biological experiments. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker DRX 500 instrument. Chemical shifts were relative to the deuterated solvent peak

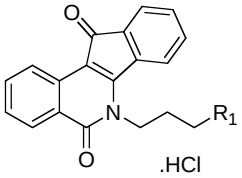
and are reported in parts per million (ppm). The high resolution mass spectra were recorded on Micromass Q-TOF spectrometer using electrospray ionisation technique.

General synthetic route for 6-substituted indenoisoquinolines (1a-e)



2 **1a-e**

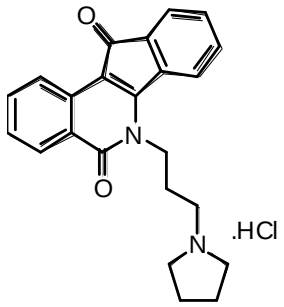
1. General procedure for the synthesis of indenoisoquinolines 1a-e.



1a-e

To a solution of 6-oxa-benzo[a]fluorene-5,11-dione (**2**) (1.0 eq) in $CHCl_3$ [5 mL] was added the appropriate primary amine (1.5 eq). The reaction mixture was stirred at room temperature for 48 hrs. The solvent was removed *in vacuo* and the crude product was purified using 5-15 % methanol in chloroform to afford the free base as an orange solid. Subsequently the free base was treated with ethanolic HCl solution to afford the desired compounds **1a-e** as orange solids in excellent yields (generally >90 %).

2. Synthesis of 6-(3-pyrrolidin-1-yl-propyl)-6*H*-indeno[1,2-*c*]isoquinoline-5,11-dione, hydrochloride salt (1a).

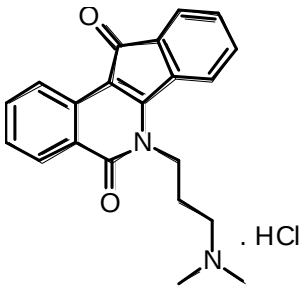


1a

The title compound **1a** was synthesized from **2** and pyrrolidin-1-ylpropylamine using the general procedure. The crude compound was purified by column chromatography using 10 % methanol in chloroform to afford the free base. The free base was treated with ethanolic HCl to afford the title compound **1a** as an orange solid (94 %).

^{1}H NMR (D_2O, 500 MHz) δ_H 7.14 (t, 2 x ArH, *J* = 7.14 Hz), 7.00 (t, ArH, *J* = 7.21 Hz), 6.84 (m, 2 x ArH) (NOTE: generally multiplets in 1H NMR are quoted as a range. Applies to all examples quoted), 6.77 (d, ArH, *J* = 7.4 Hz), 6.68 (t, ArH, *J* = 7.41 Hz), 6.46 (d, ArH, *J* = 6.78 Hz), 3.65 (t, 2H, *J* = 7.85 Hz), 3.21 (bs, 4H), 3.12 (t, 2H, *J* = 7.37 Hz), 1.93 (m, 4H), 1.82 (m, 2H); ^{13}C NMR (D_2O, 125 MHz) δ_C 190.74, 163.28, 154.3, 137.41, 134.0, 133.89, 132.58, 132.12, 131.36, 130.2, 127.16, 127.11, 122.69, 121.25, 121.01, 107.59, 54.02, 51.66, 41.59, 25.03, 22.53; HRMS (ESI): Calculated mass for free base $C_{23}H_{23}N_2O_2$ $[M+H]^+$ 359.1681 and found at 359.1760 $[M+H]^+$.

3. Synthesis of 6-(3-dimethylamino-propyl)-6*H*-indeno[1,2-*c*]isoquinoline-5,11-dione, hydrochloride salt (1b).

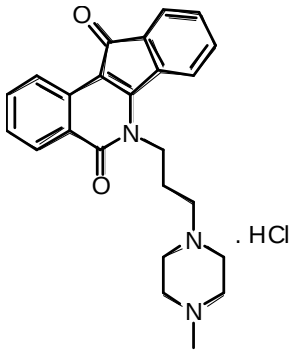


1b

The title compound **1b** was synthesized from **2** and 3-dimethylaminopropylamine using the general procedure. The crude compound was purified by column chromatography using 8 % methanol in chloroform to afford the free base. The free base was treated with ethanolic HCl to afford the title compound **1b** as an orange solid (96 %).

^{1}H NMR (D_2O, 500 MHz) δ_H 7.30 (m, 2 x ArH), 7.05 (t, ArH, *J* = 7.46 Hz), 6.99 (t, ArH, *J* = 7.47 Hz), 6.89 (m, 2 x ArH), 6.78 (t, ArH, *J* = 7.6 Hz), 6.58 (d, ArH, *J* = 6.96 Hz), 3.75 (t, 2H, *J* = 6.7 Hz), 3.06 (t, 2H, *J* = 7.55 Hz), 2.73 (s, 6H), 1.88 (m, 2H); ^{13}C NMR (D_2O, 125 MHz) δ_C 191.0, 163.56, 154.49, 134.56, 134.03, 132.72, 132.01, 131.39, 130.39, 127.27, 127.23, 122.77, 122.55, 121.43, 121.2, 107.84, 54.47, 42.57, 41.38, 23.79; HRMS (ESI): Calculated mass for free base $C_{21}H_{21}N_2O_2$ $[M+H]^+$ 333.1525 and found at 333.1599 $[M+H]^+$.

4. Synthesis of 6-[3-(4-methyl-piperazine-1-yl)-propyl]-6*H*-indeno[1,2-*c*]isoquinoline-5,11-dione, hydrochloride salt (1c).

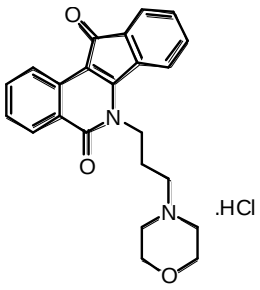


1c

The title compound **1c** was synthesized from **2** and 3-(4-methylpiperazin-1-yl)propylamine using the general procedure. The crude compound was purified by column chromatography using 12 % methanol in chloroform to afford the free base. The free base was treated with ethanolic HCl to afford the title compound **1c** as an orange solid (92 %).

^{1}H NMR (D_2O, 500 MHz) δ_H 7.44 (d, ArH, *J* = 7.83 Hz), 7.30 (d, ArH, *J* = 7.93 Hz), 7.06 (m, 2 x ArH), 6.92 (t, 2 x ArH, *J* = 7.14 Hz), 6.80 (t, ArH, *J* = 7.14 Hz), 6.66 (d, ArH, *J* = 6.96 Hz), 3.85 (t, 2H, *J* = 6.7 Hz), 3.50 (bs, 8H), 3.22 (t, 2H, *J* = 7.22 Hz), 2.83 (s, 3H), 1.96 (m, 2H); ^{13}C NMR (D_2O, 125 MHz) δ_C 193.65, 166.08, 157.05, 137.06, 136.6, 136.53, 135.24, 133.83, 132.93, 129.74, 129.67, 125.28, 125.06, 124.01, 123.72, 110.43, 56.27, 52.56, 51.1, 45.01, 44.0, 27.7; HRMS (ESI): Calculated mass for free base $C_{24}H_{26}N_3O_2$ $[M+H]^+$ 388.1947 and found at 388.2025 $[M+H]^+$.

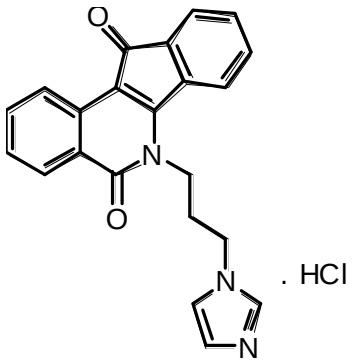
5. Synthesis of 6-[3-(morpholin-4-yl)propyl]-6*H*-indeno[1,2-*c*]isoquinoline-5,11-dione, hydrochloride salt (1d).



1d

The title compound **1d** was synthesized from **2** and 3-(morpholin-4-yl)propylamine using the general procedure. The crude compound was purified by column chromatography using 10 % methanol in chloroform to afford the free base. The free base was treated with ethanolic HCl to afford the title compound **1d** as an orange solid (95 %). ^{1}H NMR (D_2O, 500 MHz) δ_H 7.37 (d, ArH, *J* = 7.89 Hz), 7.28 (d, ArH, *J* = 7.86 Hz), 7.05 (m, 2 x ArH), 7.01 (t, ArH, *J* = 7.53 Hz), 6.89 (t, ArH, *J* = 6.77 Hz), 6.79 (t, ArH, *J* = 7.68 Hz), 6.63 (d, ArH, *J* = 6.98 Hz), 3.93 (bs, 2H), 3.81 (t, 2H, *J* = 6.17 Hz), 3.64 (bs, 2H), 3.34 (bs, 2H), 3.10 (t, 2H, *J* = 7.89 Hz), 3.02 (bs, 2H), 1.94 (m, 2H); ^{13}C NMR (D_2O, 125 MHz) δ_C 191.18, 163.64, 154.6, 134.69, 134.12, 134.07, 132.85, 131.43, 130.52, 127.33, 127.28, 122.85, 122.59, 121.54, 121.31, 107.99, 63.61, 54.05, 51.58, 41.56, 22.94; HRMS (ESI): Calculated mass for free base $C_{23}H_{23}N_2O_3$ $[M+H]^+$ 375.1630 and found at 375.1703 $[M+H]^+$.

6. Synthesis of 6-[3-(imidazol-1-yl)propyl]-6*H*-indeno[1,2-*c*]isoquinoline-5,11-dione, hydrochloride salt (1e).

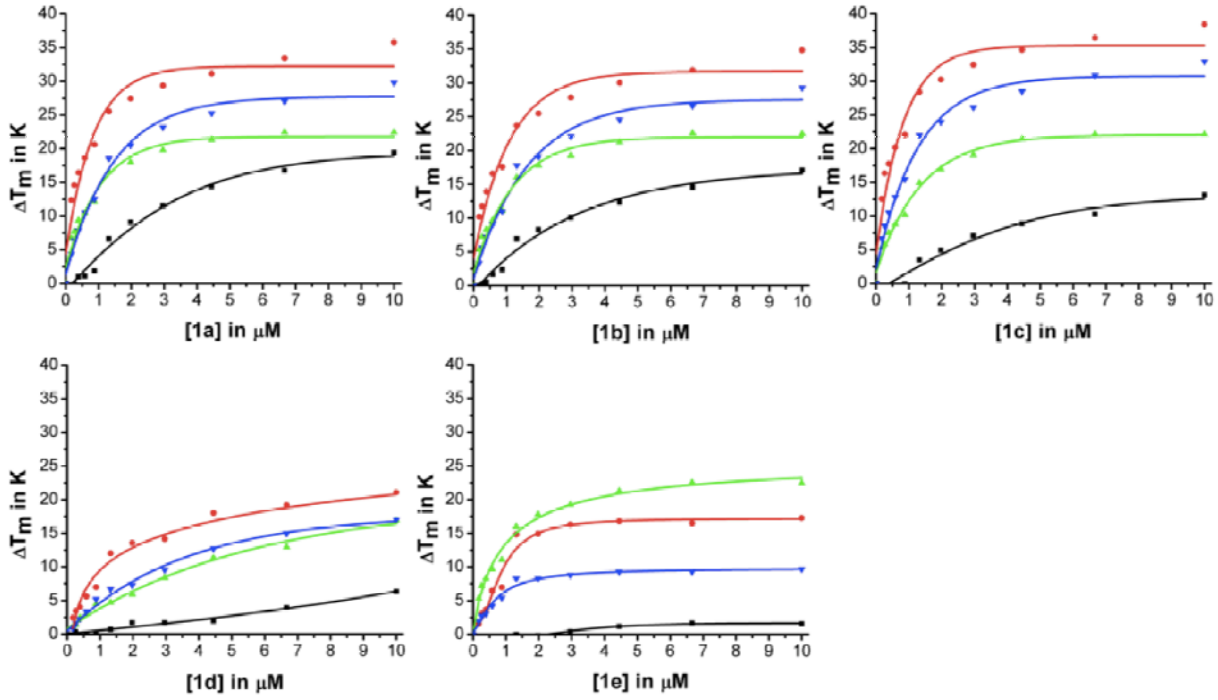


1e

The title compound **1e** was synthesized from **2** and 3-(imidazol-1-yl)propylamine using the general procedure. The crude compound was purified by column chromatography using 15 % methanol in chloroform to afford the free base. The free base was treated with ethanolic HCl to afford the title compound **1e** as an orange solid (94 %).

^{1}H NMR (D_2O, 500 MHz) δ_H 8.51 (s, ArH), 7.28 (d, 2 x ArH, J = 17.48 Hz), 7.08 (d, 2 x ArH, J = 6.66 Hz), 6.87 (m, 2 x ArH), 6.76 (t, ArH, J = 6.78 Hz), 6.68 (t, ArH, J = 6.88 Hz), 6.50 (d, ArH, J = 7.17 Hz), 6.40 (d, ArH, J = 5.88 Hz), 4.08 (t, 2H, J = 5.95 Hz), 3.42 (t, 2H, J = 6.69 Hz), 1.86 (t, 2H, J = 5.5 Hz); ^{13}C NMR (D_2O, 125 MHz) δ_C 190.53, 162.95, 154.23, 134.52, 134.27, 133.9, 133.85, 132.44, 131.3, 130.0, 127.16, 127.06, 122.65, 122.37, 121.37, 121.33, 120.93, 119.89, 107.43, 46.33, 41.25, 28.27; HRMS (ESI): Calculated mass for free base $C_{22}H_{18}N_3O_2$ $[M+H]^+$ 356.1321 and found at 356.1399 $[M+H]^+$.

FRET-melting profiles



FRET-melting assay for *c-Kit* 1 (●), H-telo (▲), *c-Kit* 2 (▲) and ds DNA (■) in the presence of ligands **1a-e**; buffer, 60 mM potassium cacodylate pH 7.4.