Electronic Supplementary Information

AlCl3-mediated heteroarylation-cyclization strategy: one-pot synthesis of fused quinoxalines containing the central core of Lamellarin D

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**Chemistry**

**General methods:** Unless stated otherwise, solvents and chemicals were obtained from commercial sources and were used without further purification. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), visualizing with ultraviolet light or iodine spray. Flash chromatography was performed on silica gel (230-400 mesh) using hexane and ethyl acetate. $^1$H and $^{13}$C NMR spectra were determined in DMSO-$d_6$ solutions by using 400 or 100 MHz spectrometers, respectively. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, δ = 0.00) as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d (doublet), t (triplet) and m (multiplet) as well as b (broad). Coupling constants (J) are given in hertz. Infrared spectra were recorded on a FT-IR spectrometer. Melting points were determined using a melting point apparatus and are uncorrected. MS spectra were obtained on a mass spectrometer. Chromatographic purity by HPLC (Agilent 1200 series Chem Station software) was determined by using area normalization method and the condition specified in each case: column, mobile phase (range used), flow rate, detection wavelength, and retention times.

**Typical procedure for the synthesis of pyrano[3,4-b]indol fused quinoxaline (3a–k):** A mixture of 2,3-dichloroquinoxaline (1, 1.0 equiv), substituted indole-2-carboxylic acids (2a–f) (1.0 equiv) and anhydrous AlCl$_3$ (2.25 equiv) in 1,2-dichloroethane (5 mL) was stirred at 80 °C for 24 h. After completion of the reaction, the mixture was poured into ice-cold water (15 mL), stirred for 10 min and then extracted with ethyl acetate (3×5 mL). The organic layers were collected, combined, washed with water (3×10 mL), dried over anhydrous Na$_2$SO$_4$, filtered and concentrated under vacuum. The residue was purified by column chromatography to afford the expected products.

**Indolo[3’,2’:5,6]pyrano[2,3-b]quinoxalin-7(8H)-one (3a)**

![Chemical structure of Indolo[3’,2’:5,6]pyrano[2,3-b]quinoxalin-7(8H)-one (3a)](image)
Yellow solid; Yield: 85%; mp: 340-350 °C; IR (KBr) \(v_{\text{max}}\): 3281, 1708, 1138, 741 cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 13.24 (s, 1H), 8.66 (d, \(J=8.0\) Hz, 1H), 8.24 (d, \(J=8.4\) Hz, 1H), 8.03 (d, \(J=8.0\) Hz, 1H), 7.89-7.85 (m, 2H), 7.68 (d, \(J=8.0\) Hz, 1H), 7.58 (t, \(J=7.6\) Hz, 1H), 7.46 (t, \(J=7.6\) Hz, 1H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)): \(\delta\) 153.5, 150.4, 144.2, 140.4, 136.2, 136.1, 130.2, 129.2, 128.3 (2C), 128.2, 125.0, 124.9, 123.5, 121.8, 121.5, 113.7; HPLC: 97.8%, column: Agilent eclipse plus C-18 250 × 4.6 mm 5μm, mobile phase A: 0.1% TFA in water, mobile phase B: ACN T/%B: 0/5, 3/5, 10/95, 20/95, 22/5, 30/5; flow rate: 1.0 mL/min, Diluent: ACN : Water (80 : 20); UV 250 nm, retention time 6.1 min; Mass: m/z (CI) 288.35 (M + 1, 100%).

**8-Methylindolo[3',2':4,5]pyrano[2,3-b]quinoxalin-7(8H)-one (3b)**

Pale yellow solid; Yield: 76%; mp: 260-270 °C; IR (KBr) \(v_{\text{max}}\): 1748,1195, 1041, 749 cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 8.72 (d, \(J=7.6\) Hz, 1H), 8.23 (d, \(J= 7.6\) Hz, 1H), 8.02 (d, \(J= 7.8\) Hz, 1H), 7.87 (m, 3H), 7.66 (t, \(J=7.8\) Hz, 1H), 7.51 (t, \(J=7.8\) Hz, 1H), 4.24 (s, 3H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)): \(\delta\) 154.6, 151.3, 141.3, 140.3, 138.5, 136.5, 130.6, 129.9, 128.7, 128.4, 128.3, 125.1, 123.8, 123.7, 121.5, 119.4, 112.6, 32.6; HPLC: 96.12% column: X-Bridge C-18 150 × 4.6 mm 5μm, mobile phase A: 0.1% Formic Acid in water, mobile phase B: ACN T/%B: 0/20, 3/20, 10/95, 23/95, 25/20, 30/20; flow rate: 1.0 mL/min, Diluent : ACN : Water (80 : 20); UV 254 nm, retention time 12.9 min; Mass: m/z (CI) 302.2 (M + 1, 100%).

**8-Ethylindolo[3',2':4,5]pyrano[2,3-b]quinoxalin-7(8H)-one (3c)**
Yellow color solid; Yield: 79%; mp: 201-203 °C; IR : 1736, 1190, 752 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 8.74 (d, $J = 8.0$ Hz, 1H), 8.23 (d, $J = 7.2$ Hz, 1H), 8.04-8.01 (m, 1H), 7.94 (d, $J = 8.2$ Hz, 1H), 7.88-7.83 (m, 2H), 7.67 (t, $J = 7.6$ Hz, 1H), 7.51 (t, $J = 7.6$ Hz, 1H), 4.80 (m, 2H), 1.43 (t, $J = 7.2$ Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$ 154.1, 151.1, 140.7, 140.1, 138.5, 130.5, 129.8, 128.6, 128.4, 128.3, 123.9, 123.6 (2C), 121.6, 121.5, 119.6, 112.3, 40.4, 16.0; HPLC: 92.5%, column: X-Terra RP18 250 × 4.6 mm 5µm, mobile phase A: 0.1% TFA in water, mobile phase B: ACN T/%B: 0/20, 5/20, 15/95, 23/95, 25/20, 30/20; flow rate: 1.0 mL/min, Diluent : ACN : Water (80 : 20), UV 210 nm, retention time 17.36 min; Mass: m/z (CI) 316.2 (M + 1, 100%).

8-Allylindolo[3',2':4,5]pyrano[2,3-b]quinoxalin-7(8H)-one (3d)

Brown colour solid; Yield: 75%; mp: 230-235 °C; IR : 1724, 1535, 1159, 743 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 8.74 (d, $J = 8.0$ Hz, 1H), 8.23 (d, $J = 8.0$ Hz, 1H), 8.03-8.01 (m, 1H), 7.88-7.84 (m, 3H), 7.65 (t, $J = 7.6$ Hz, 1H), 7.51 (t, $J = 7.6$ Hz, 1H), 6.12-6.05 (m, 1H), 5.40 (d, $J = 4.4$ Hz, 2H), 5.16 (d, $J = 10$ Hz, 1H), 5.03 (d, $J = 17.2$ Hz, 1H); $^{13}$C NMR (100 MHz, DMSO-d$_6$): $\delta$ 157.9, 154.9, 144.4, 144.2 (2), 140.1, 137.3, 134.2, 133.5, 132.3, 132.1, 131.9, 128.1, 127.5, 127.4, 125.3, 123.6, 121.0, 116.3, 50.6; HPLC: 98.5%, column: X-Terra RP18 250 × 4.6 mm 5µm, mobile phase A: 0.1% TFA in water, mobile phase B: ACN T/%B: 0/20, 3/20, 12/95, 23/95, 25/20, 30/20; flow rate: 1.0 mL/min, Diluent : ACN : Water (80 : 20), UV 254 nm, retention time 14.7 min; Mass : m/z (CI) 328 (M + 1, 100%).
11-Fluoroindolo[3',2':4,5]pyrano[2,3-b]quinoxalin-7(8H)-one (3e)

Light brown solid; Yield: 68%; mp: 350-360 °C; IR : 1772, 1516, 1111, 761 cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆): δ 13.34 (s, 1H), 8.30-8.26 (m, 2H), 8.01 (d, J= 6.8 Hz, 1H), 7.86-7.85 (m, 2H), 7.72-7.70 (m, 1H), 7.50-7.45 (m, 1H); ¹³C NMR (100 MHz, DMSO-d₆): δ 154.6, 151.3, 140.7 (d, C–F J = 215.0), 136.7, 136.5, 130.5, 129.9, 128.7, 128.3, 127.2, 122.5, 122.4, 119.2, 117.3 (d, C–F J = 26.0), 115.9 (d, C–F J = 9.9), 107.9, 107.6; HPLC: 98.0%, column: X-Bridge C-18 150 × 4.6 mm 5µm, mobile phase A: 0.1% Formic Acid in water, mobile phase B: ACN T/%B: 0/20, 3/20, 10/95, 23/95, 25/20, 30/20; flow rate: 1.0 mL/min, Diluent : ACN : Water (80 : 20), UV 254 nm, retention time 12.1 min; Mass : m/z (Cl) 306.24 (M + 1, 100%).

11-Methylindolo[3',2':4,5]pyrano[2,3-b]quinoxalin-7(8H)-one (3f)

Brown solid; Yield: 83%; mp: 340 °C; IR : 3276, 1710, 1550, 1098,795 cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆) δ 13.15 (s, 1H) 8.42 (s, 1H), 8.24 (d, J=7.2 Hz, 1H), 8.02 (d, J= 7.2 Hz, 1H), 7.87-7.83 (m, 2H), 7.57 (d, J= 8.8 Hz, 1H), 7.40 (d, J= 8.8, 1H), 2.52 (s, 3H); ¹³C NMR (100 MHz, DMSO-d₆) δ 154.7, 151.4, 140.7, 138.5, 138.4, 136.9, 132.5, 130.3, 129.7, 128.6, 128.3, 125.6, 122.7 (2), 118.9, 113.8, 21.7; HPLC: 95.1%, column: X-Bridge C-18 150 × 4.6 mm 5µm, mobile phase A: 0.1% Formic Acid in water, mobile phase B: ACN T/%B: 0/10, 3/20, 10/90, 20/90, 22/10, 25/10; flow rate: 1.0 mL/min, Diluent : ACN : Water (80 : 20), UV 210 nm, retention time 9.6 min; Mass : m/z (Cl) 302.25 (M + 1, 100%).
2-Methylindolo[3',2':4,5]pyrano[2,3-b]quinoxalin-7(8H)-one (3g)

Pale yellow solid; Yield: 82%; mp: 340 °C, IR : 3276, 1718, 1563, 1135,741 cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 13.16 (s , 1H), 8.63 (s, 1H), 8.11-7.90 (m, 2H), 7.78 (d, \(J= 7.6\) Hz, 1H), 7.74-7.67 (m, 2H), 7.57-7.50 (m, 1H), 2.57 (s, 3H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\), 158.8, 152.3, 151.2, 141.3, 138.8, 138.3, 136.8, 134.9, 129.1, 127.5, 126.6, 126.3, 123.1, 122.5, 120.6, 120.1, 116.0, 21.6; HPLC: 96.75% column: X-Bridge C-18 150 × 4.6 mm 5µm, mobile phase A: 0.1% Formic Acid in water, mobile phase B: ACN T/%B: 0/20, 3/20, 12/95, 25/95, 27/20, 30/20; flow rate: 1.0 mL/min, Diluent : ACN : Water (80 : 20), UV 260 nm, retention time 12.67 min; Mass : m/z (CI) 302.15 (M + 1, 100%).

2,8-Dimethylindolo[3',2':4,5]pyrano[2,3-b]quinoxalin-7(8H)-one (3h)

Pale yellow solid; Yield: 77%; mp: 240-245 °C; IR : 1730, 1465, 1201, 1094,738 cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 7.94 (d, \(J= 8\) Hz, 1H), 7.47 (t, \(J= 8\) Hz, 1H), 7.40-7.32 (m, 2H), 7.14-7.07 (m, 2H), 7.04-7.00 (m, 1H), 3.86 (s, 3H), 2.34 (s, 3H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 169.4, 146.3, 143.4, 141.6, 139.8, 131.1, 130.8, 130.2, 125.4, 125.1, 124.6, 123.4, 122.8, 113.9, 113.5, 113.2, 107.8, 35.0, 24.6 ; HPLC: 94.29% column: X-Bridge C-18 150 × 4.6 mm 5µm, mobile phase A: 0.1% Formic Acid in water, mobile phase B: ACN T/%B: 0/20, 3/20, 12/95, 25/95, 27/20,30/20; flow rate: 1.0 mL/min, Diluent : ACN : Water (80 : 20), UV 260 nm, retention time 13.8 min; Mass : m/z (CI) 316 (M + 1, 100%).
2,3-Dimethylindolo[3',2':4,5]pyrano[2,3-b]quinoxalin-7(8H)-one (3i)

Pale yellow solid; Yield: 71%; mp: 350-360 °C; IR: 3272, 1792, 1113, 666 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 13.13 (s, 1H), 8.63 (d, $J$=8.0 Hz, 1H), 7.97 (s, 1H), 7.76 (s, 1H), 7.67 (d, $J$=8.2 Hz, 1H), 7.57 (t, $J$= 8.0 Hz, 1H), 7.44 (t, $J$= 8.0 Hz, 1H), 2.22 (s, 6H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 175.4, 164.9, 153.6, 143.5, 140.7, 134.8, 134.2, 130.8, 129.4, 128.7, 126.6, 123.1, 122.0, 121.7, 118.6, 110.5, 109.0, 20.0, 19.5 ; HPLC: 97.60% column: X-Bridge C-18 150 × 4.6 mm 5µm, mobile phase A: 0.1% Formic Acid in water, mobile phase B: ACN T/%B: 0/20, 3/20, 10/95, 23/95, 25/20, 30/20; flow rate: 1.0 mL/min, Diluent : ACN : Water (80 : 20), UV 254 nm, retention time 13.2 min; Mass: m/z (CI) 316 (M + 1, 100%).

8-Ethyl-2,3-dimethylindolo[3',2':4,5]pyrano[2,3-b]quinoxalin-7(8H)-one (3j)

Yellow solid; Yield: 68%; mp: 245-250 °C; IR : 1727, 1449, 1197, 737 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.73 (d, $J$= 8.0 Hz, 1H), 8.02 (s, 1H), 7.93 (d, $J$= 8.2 Hz, 1H), 7.80 (s, 1H), 7.68-7.64 (m, 1H), 7.52-7.48 (m, 1H), 4.80-4.73 (m, 2H), 2.46 (s, 6H), 1.40 (t, $J$=7.4 Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 169.5, 164.6, 150.8, 141.7, 141.4, 135.4, 134.9, 133.9, 128.2, 128.0, 127.2, 123.5, 121.6, 121.4, 119.6, 110.1, 109.8, 39.8, 20.1, 19.6, 16.0; Mass : m/z (CI) 344 (M + 1, 100%).

1,3-Dimethylindolo[3',2':4,5]pyrano[2,3-b]quinoxalin-7(8H)-one (3k)
Yellow solid; Yield: 79%; mp : 350 °C; IR : 3265, 1709, 1136, 755 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 13.22 (s, 1H), 8.62 (d, $J$= 8.0 Hz, 1H), 7.83 (s, 1H), 7.68 (d, $J$= 8.0 Hz, 1H), 7.59-7.53 (m, 2H), 7.46-7.42 (m, 1H), 2.64 (s, 3H), 2.53 (s, 3H); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 154.8, 150.1, 140.9, 140.0, 139.3, 135.9, 135.7, 132.5, 128.1, 125.6, 125.3, 123.5 (2C), 123.1, 122.3, 119.5, 114.1, 21.7, 17.2; HPLC: 98.3 %, column: X-Terra RP18 250 × 4.6 mm 5µm, mobile phase A: 0.1% TFA in water, mobile phase B: ACN T/%B: 0/20, 3/20, 12/95, 23/95, 25/20, 30/20; flow rate: 1.0 mL/min, Diluent : ACN : Water (80 : 20), UV 260 nm, retention time 15.5 min; Mass : m/z (CI) 316.2 (M + 1, 100%).

**General procedure for the preparation of compound 4a-4d**

A solution of pyrano[3,4-b]indol fused quinoxaline (3) (1.0 mmol) in dry THF (15 mL) was cooled to 0 °C and LiAlH$_4$ (1.2 mmol) was added portion wise with stirring under a nitrogen atmosphere. The duration of addition was 15 min. The mixture was then warmed to room temperature and stirring continued for 2h. After completion of the reaction the mixture was quenched with water (15 mL) and extracted with ethyl acetate (2 × 100 mL). The organic layers were collected, combined, washed with cold water (2 × 50 mL), dried over anhydrous Na$_2$SO$_4$, filtered and concentrated under vacuum. The residue was purified by chromatography on silica gel hexanes/ethyl acetate to to give the desired product 4

3-(2-(Hydroxymethyl)-1H-indol-3-yl)quinoxalin-2-ol (4a)
Brown solid; Yield: 82%; mp: 280 °C; IR: 3256, 3150, 1644, 1181.978, 751 cm⁻¹; H NMR (400 MHz, DMSO-d₆) δ 12.34 (s, 1H), 11.57 (s, 1H), 7.90 (d, J = 7.8 Hz, 1H), 7.74 (d, J = 7.8 Hz, 1H), 7.46-7.41 (m, 2H), 7.31-7.25 (m, 2H), 7.11-7.01 (m, 2H), 5.36 (s, 1H), 4.84 (s, 2H); C NMR (400 MHz, DMSO-d₆) δ 155.1, 154.6, 144.0, 135.6, 132.9, 131.5, 129.2, 128.2, 128.0, 123.6, 121.9, 121.6, 120.2, 115.3, 111.8, 108.2, 57.7; HPLC: 97.6% column: X-Bridge C-18 150 × 4.6 mm 5µm, mobile phase A: 0.1% Formic Acid in water, mobile phase B: ACN T/%B: 0/20, 3/20, 12/95, 25/95, 27/20, 30/20; flow rate: 1.0 mL/min, Diluent : ACN : Water (80 : 20), UV 260 nm, retention time 8.4 min; Mass: m/z (CI) 292.2 (M + 1, 100%).

3-(1-Ethyl-2-(hydroxymethyl)-1H-indol-3-yl)quinoxalin-2-ol (4b)

Yellow solid; Yield: 79%; mp: 245-250 °C; IR: 2842, 1654, 1423, 1022, 747 cm⁻¹; H NMR (400 MHz, DMSO-d₆) δ 12.47 (s, 1H), 7.76 (t, J = 7.6 Hz, 2H) 7.53-7.46 (m, 2H), 7.34-7.28 (m, 2H), 7.21-7.17 (m, 1H), 7.07 (t, J = 7.6 Hz, 1H) 5.03 (t, J = 6.0 Hz, 1H), 4.77 (d, J = 6.0 Hz, 2H), 4.38 (q, J = 7.2 Hz, 2H), 1.37 (s, 3H); C NMR (100 MHz, DMSO-d₆) δ 155.4, 154.4, 141.7, 135.9, 132.9, 131.7, 129.7, 128.5, 127.1, 123.7, 122.3, 121.9, 120.4, 115.5, 110.2, 110.2, 54.2, 38.5, 15.7; HPLC: 97.72% column: X-Bridge C-18 150 × 4.6 mm 5µm, mobile phase A: 0.1% Formic Acid in water, mobile phase B: ACN T/%B: 0/20, 3/20, 10/95, 23/95, 25/20, 30/20; flow rate: 1.0 mL/min, Diluent : ACN : Water (80 : 20), UV 220 nm, retention time 9.8 min; Mass: m/z (CI) 320.2 (M + 1, 100%).

3-(2-(Hydroxymethyl)-5-methyl-1H-indol-3-yl)quinoxalin-2-ol (4c)
Brown solid; Yield: 84%; mp : 250 °C; ; IR : 3246, 2914, 1664, 1023, 791, 744 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 12.31 (s, 1H), 11.45 (s, 1H), 7.74 (d, $J$= 7.6 Hz, 1H), 7.68 (s, 1H), 7.44 (t, $J$= 7.6 Hz, 1H), 7.31-7.25 (m, 3H), 6.91 (d, $J$= 8.0 Hz, 1H), 5.33 (t, $J$= 5.6 Hz, 1H), 4.81 (d, $J$= 5.6 Hz, 2H), 2.35 (s, 3H); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 155.1, 154.7, 144.0, 134.0, 132.9, 131.5, 129.1, 128.5, 128.2 (2C), 123.6, 123.2, 121.5, 115.3, 111.5, 107.8, 57.8, 22.0; HPLC: 97.7% column: X-Bridge C-18 150 × 4.6 mm 5µm, mobile phase A: 0.1% Formic Acid in water, mobile phase B: ACN T/%B: 0/20, 3/20, 10/95, 23/95, 25/20, 30/20; flow rate: 1.0 mL/min, Diluent : ACN : Water (80 : 20), UV 220 nm, retention time 8.9 min; Mass : m/z (CI) 306 (M + 1, 100%).

3-(2-(Hydroxymethyl)-1H-indol-3-yl)-5,7-dimethylquinoxalin-2-ol (4d)

Yellow solid; Yield: 81%; mp: 240 °C ; IR : 3282, 2922, 1656, 1031, 732; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.64 (s, 1H), 11.56 (s, 1H), 7.90 (d, $J$= 7.6 Hz, 1H), 7.43-7.41 (m, 2H), 7.13-7.02 (m, 3H), 5.38 (t, $J$= 7.6 Hz, 1H), 4.85 (d, $J$= 5.6 Hz, 2H), 2.41 (s, 3H), 2.34 (s, 3H); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 155.6, 154.0, 143.8, 135.7, 133.0, 132.4, 131.7, 128.0, 127.7, 126.0, 123.7, 121.9, 121.6, 120.1, 111.8, 108.2, 57.8, 20.7, 17.1; Mass : m/z (CI) 320.2 (M + 1, 100%). ; HPLC: 98.87%, column: X-Bridge C-18 150 × 4.6 mm 5µm, mobile phase A: 0.1% Formic Acid in water, mobile phase B: ACN T/%B: 0/20, 3/20, 12/95, 23/95, 25/20, 30/20; flow rate: 1.0 mL/min, Diluent : ACN : Water (80 : 20), UV 240 nm, retention time 9.9 min; Mass : m/z (CI) 302.25 (M + 1, 100%).

**Cell proliferation Assay**

The anti-proliferative activity and cancer cell selectivity of the synthesized compounds on normal and cancer cells was evaluated using the SRB (Sulforhodamine B) cell proliferation assay. This assay was chosen because of its sensitivity, large dynamic range and the ability to measure cell proliferation over three days with normalization to initial cell number as well as to vehicle-treated cells. Further, this assay is the standardized assay of choice for anticancer
compound screening at the National Cancer Institute (NIH). The SRB assay provides a colorimetric readout which can be spectrophotometrically measured and does not involve antibodies or toxic reagents. The assay is based on detection of total protein content of cells, which increases or decreases in proportion with cell number.

In brief, the assay was performed as follows: TZM-BL (Human cervical carcinoma cells) and A549 (human lung carcinoma cell) were seeded in 96-well plates and incubated overnight. The optimum cell numbers to be seeded were determined by a growth curve analysis for each cell line. In the initial (single dose) screen, compounds (dissolved in 100% DMSO to a stock concentration of 100 mM) were added to the adhered cells at a final concentration of 10 µM. After 72 h of treatment, the cells were washed with phosphate-buffered saline and ice-cold 10% trichloroacetic acid added to the cells to precipitate all proteins for 1h at 4°C. The cells were then washed with water and air-dried. Cellular proteins were then stained using 0.4% SRB solution in 1% acetic acid for 10 min at room temperature. The unbound dye was washed away by destaining with 1% acetic acid and bound dye solubilized with 10 µM Tris solution. Absorbance of solubilized dye was measured at a wavelength of 590 nm. Percentage growth was determined by the formula \([(At-A0/Ac-A0)] \times 100\), where \(At=\)absorbance after 72h of test compound treatment, \(A0=\)Absorbance at time 0, \(Ac=\)Absorbance after 72h without treatment.

References


Docking study

**Method:** We docked all the molecules by using Schrödinger 2015 software. The results are compared with the co-crystal ligand and in vitro activity. The PDB ID 1k4t was used for the docking study. The protein was prepared by giving preliminary treatment like adding hydrogen, adding missing residues, refining the loop with prime and finally minimized by using OPLS 2005 force field. The search grid was generated by picking the co-crystal ligand up to 20 Å search area. The hydroxyl groups of search area were allowed to move.

All the molecules were minimized by using macro module application. We used 1000 iteration for minimization using OPLS 2005 force field and charges were also added from force field only. The PRCG (Polak-Ribier conjugate gradient) method was used for minimization. All the molecules were docked by using glide SP (standard precision) dock application. We performed flexible docking by allowing sample ring conformations and sample nitrogen to move to possible extent in docking. 10 poses were generated for each ligand. The docking results are documented and analyzed.

In order to understand the nature of interactions of these molecules with Human DNA topoisomerase I (1k4t) docking studies were carried out using compounds 3a, 3d, 3f and 4. The SP docking was performed for all the molecules using glide module of Schrödinger 2015. The glide scores and other parameters obtained after docking of these molecules into the 1k4t protein are summarized in Table 1. The data shown in Table suggests that these molecules bind well with 1k4t.

The following Dock scores were obtained after docking with 1k4t proteins:-

**Table S-1:**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Glide score</th>
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<td>3a</td>
<td>-5.81</td>
</tr>
<tr>
<td>3d</td>
<td>-6.62</td>
</tr>
<tr>
<td>3f</td>
<td>-5.51</td>
</tr>
<tr>
<td>Lamellarin D</td>
<td>-6.08</td>
</tr>
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</table>
Fig. S-1. Docking of 3a into the active site of Human DNA topoisomerase I.
Fig. S-2. Docking of 3d into the active site of Human DNA topoisomerase I.
Fig. S-3  Docking of 3f into the active site of Human DNA topoisomerase I.
Copies of NMR spectra

3a $^1$H NMR (400 MHz, DMSO-d$_6$)
$^{13}$C NMR (100 MHz, DMSO-$d_6$)
$3b$ $^1$H NMR (400 MHz DMSO-$d_6$)
$3b^{13}$C NMR (100 MHz, DMSO-d$_6$)
$^{1}$H NMR (400 MHz, DMSO-d$_6$)
$^{13}$C NMR (100 MHz, DMSO-d$_6$)
$^{1}H$ NMR (400 MHz DMSO-$d_{6}$)
$^{13}$C NMR (100 MHz, DMSO-d$_6$)
3e $^1$H NMR (400 MHz, DMSO-d$_6$)
$^{13}$C NMR (100 MHz DMSO-$d_6$)
$^{1}$H NMR (400 MHz, DMSO-d$_6$)
$\text{3f}^{13}\text{C NMR (100 MHz, DMSO-d}_6\text{)}$
3g $^1$H NMR (400 MHz, DMSO-d$_6$)
$^{13}$C NMR (100 MHz DMSO-d$_6$)
$3h \ H NMR (400 MHz, DMSO-d_6)$
$^{13}$C NMR (100 MHz, DMSO-$d_6$)
3i $^1$H NMR (400 MHz DMSO-d$_6$)
$3i$ $^{13}$C NMR (100 MHz, DMSO-$d_6$)
$^{1}$H NMR (400 MHz, DMSO-d$_6$)
$^{13}$C NMR (100 MHz, DMSO-$d_6$)
$3k^1H$ NMR (400 MHz DMSO-d$_6$)
$3k \ ^{13}\text{C} \text{NMR} \ (100 \text{ MHz, DMSO-d}_6)$
4a $^1$H NMR (400 MHz, DMSO-d$_6$)
$^{13}$C NMR (100 MHz, DMSO-$d_6$)
$^1$H NMR (400 MHz, DMSO-$d_6$)
$^{13}$C NMR (100 MHz, DMSO-$d_6$)
$4c \ ^1H \text{NMR (400 MHz, CDCl}_3)$
$^{13}$C NMR (100 MHz, CDCl$_3$)
$^{1}H$ NMR (400 MHz, CDCl$_3$)
$^{13}$C NMR (100 MHz DMSO-$_d_6$)
Heteronuclear Multiple Bond Correlation (HMBC), DEPT and COSY spectras of compound 4d