Electronic Supplementary Information

A greener approach towards double heteroarylation of N, O and S nucleophiles: synthesis of bioactive polynuclear fused N-heteroarenes

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Chemistry

General Methods

All reagents were used as received from commercial sources without further purification or prepared as described in the literature. Reactions were stirred using Teflon-coated magnetic stirring bars. TLC plates were visualized by ultraviolet light or by treatment with a spray of Pancaldi reagent \((\text{NH}_4)_6\text{MoO}_4\), Ce(SO\(_4\))\(_2\), H\(_2\)SO\(_4\), H\(_2\)O\}. Chromatographic purification of products was carried out by flash column chromatography on silica gel (60-120 mesh). Melting points were determined using an electro thermal melting point apparatus and are uncorrected. NMR spectra were recorded in CDCl\(_3\) or DMSO-\(d_6\) (all with TMS as internal standard) on a Varian Gemini 400 MHz FT NMR spectrometer. Chemical shifts (\(\delta\)) are reported in ppm, and coupling constants (\(J\)) are in Hz. The following abbreviations were used to explain the multiplicities: \(s\) = singlet, \(d\) = doublet, \(t\) = triplet, \(q\) = quartet, \(m\) = multiplet. Mass spectra were recorded on an HP-5989A quadrupole mass spectrometer.

Preparation of compound 1

Preparation of methyl 2-(2-chloro-1\textit{H}-indol-3-yl)-2-oxoacetate (4):\(^1\)

1. \((\text{COCl})_2\), CH\(_2\)Cl\(_2\), rt, 20 h
2. MeOH, Et\(_2\)O, rt, 15 h, 85%

3. \(\text{H}_2\text{N}, 100-110^\circ\text{C}, 3\ h, 85\%\)

4. \(\text{POCl}_3, \text{toluene/DMF}, 100-110^\circ\text{C}, 3\ h, 90\%\)

\(R^1 = \text{H}\), Mel, NaH/DMF, 0 \(^\circ\text{C}\), 15 min, 92%  

\(R^1 = \text{Me}\)
Methyl 2-chloro-indole-3-glyoxylate (5):

To a vigorously stirred solution of oxalyl chloride (122 mL, 1.4 mol, 2 eq.) in 500 mL of dichloromethane was added oxindole (93 g, 0.7 mol, 1 equiv.) in portions at room temperature and stirring continued for 20 h at room temperature. The formed beige slurry was filtered, washed with dichloromethane (4x100 mL) and dried in vacuum. The solid was resuspended in diethyl ether (400 mL) and methanol (46 mL, 1.1 mol, 2 equiv.) was added in one portion at room temperature. After a few seconds a beige precipitate appeared and the reaction mixture was stirred for additional 15 min. The precipitate was filtered, carefully washed with diethyl ether (3x250 mL) and dried under vacuum to afford the desired methyl 2-chloroindole-3-glyoxylate as a beige solid (140 g, 85% yield).

$^1$H NMR (400 MHz, DMSO-d$_6$): δ 13.44 (s, br, 1H, NH), 8.04 (dd, $J_1$ = 6.8 Hz, $J_1$ = 2.0 Hz, 1H, ArH), 7.46 (dd, $J_1$ = 7.2 Hz, $J_1$ = 1.6 Hz, 1H, ArH), 7.33-7.26 (m, 2H, ArH), 3.91 (s, 3H); HRMS: m/z[M+1] calcd for C$_{11}$H$_9$NO$_3$Cl (M+H): 238.0271; found: 238.0260.

Preparation of 3-(2-chloro-1H-indol-3-yl)quinoxalin-2(1H)-one (6)

To a suspension of methyl 2-(2-chloro-1H-indol-3-yl)-2-oxoacetate (1 mmol) in glacial acetic acid (10 mL) was added o-phenylenediamine (1 mmol) and the reaction mixture was heated at 110 °C for 3 h. The reaction was monitored by TLC. Upon completion of the reaction the mixture was cooled to 0 °C and diluted with water (10 mL). Solid was filtered under vacuum and
dried under vacuum at 55 °C to give the desired product as a yellow solid; yield: 85%; mp: 256-258 °C; ¹H NMR (400 MHz, DMSO-d6): δ 12.49 (s, br, 1H, NH), 12.37 (s, br, 1H, NH), 7.81 (d, J = 8.4 Hz, 1H, ArH), 7.68 (d, J = 8.4 Hz, 1H, ArH), 7.56 (t, J = 7.6 Hz, 1H, ArH), 7.39-7.31 (m, 3H, ArH), 7.21 (t, J = 7.6 Hz, 1H, ArH), 7.12 (d, J = 7.2 Hz, 1H, ArH); ¹³C NMR (100 MHz, DMSO-d6): δ 153.92, 152.50, 134.30, 132.21, 131.68, 129.80, 128.37, 126.84, 125.04, 123.25, 122.16, 120.54, 120.29, 115.14, 110.87, 108.7; HRMS: m/z[M+1] calcd for C₁₆H₁₁N₃OCl (M+H): 296.0591; found: 296.0560.

**Preparation of 2-chloro-3-(2-chloro-1H-indol-3-yl)quinoxaline (1a)**

![Chemical structure of 2-chloro-3-(2-chloro-1H-indol-3-yl)quinoxaline](image)

To a suspension of 3-(2-chloro-1H-indol-3-yl)quinoxalin-2(1H)-one (1mmol) in toluene-DMF (30:1) was added POCl₃ slowly and the reaction mixture was stirred at 110 °C for 3 h. The reaction was monitored by TLC. Upon completion of the reaction, the mixture was cooled to 0 °C, diluted with water (10 mL) and extracted with EtOAc (2x10 mL). The EtOAc layers were collected, and washed with DM water (10 mL) followed by brine solution (15 mL), dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The residue obtained was purified by column chromatography (petroleum ether-EtOAc) to give the desired product.

Light yellow solid; Yield: 90%; mp: 171-173 °C; ¹H NMR (400 MHz, DMSO-d6): δ 12.54 (s, br, 1H, NH), 8.20-8.13 (m, 2H, ArH), 7.98-7.95 (m, 2H, ArH), 7.45 (t, J = 8.2 Hz, 2H, ArH), 7.23 (t, J = 7.2 Hz, 1H, ArH), 7.11 (t, J = 7.6 Hz, 1H, ArH); ¹³C NMR (100 MHz, DMSO-d6): δ 147.16, 140.53, 140.26, 134.33, 131.37, 130.97 (2C), 128.83, 127.86, 126.59, 124.0, 122.54, 120.67, 119.17, 111.33, 108.69; HRMS: m/z[M+1] calcd for C₁₆H₁₁N₃Cl₂ (M+H): 314.0252; found: 314.0194.

**Preparation of 2-chloro-3-(2-chloro-1-methyl-1H-indol-3-yl)quinoxaline (1b)**
To a suspension of 2-chloro-3-(2-chloro-1H-indol-3-yl)quinoxaline (1mmol) in DMF (10 mL) was added sodium hydride (1mmol) followed by methyl iodide (1mmol) at 0±5°C. The mixture was stirred at 0±5°C for 1h. The reaction was monitored by TLC. Upon completion of the reaction, the mixture was diluted with water (10 mL) and solid was filtered under vacuum, dried to give the desired product.

Yellow solid; yield: 92%; mp: 220-222 °C; \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta 8.18-8.16\) (m, 1H, ArH), \(8.11-8.09\) (m, 1H, ArH), \(7.84-7.79\) (m, 2H, ArH), \(7.55\) (d, \(J = 7.6\) Hz, 1H, ArH), \(7.40\) (d, \(J = 8.0\) Hz, 1H, ArH), \(7.33-7.29\) (m, 1H, ArH), \(7.26-7.19\) (m, 1H, ArH), \(3.88\) (s, 3H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta 147.93, 141.06, 135.66, 130.84, 130.72, 130.29, 129.11, 128.16, 126.19, 123.15, 122.76, 121.38, 121.28, 119.55, 110.73, 109.55, 30.16\); HRMS: m/z[M+1] calcd for C\(_{17}\)H\(_{12}\)N\(_3\)Cl\(_2\)(M+H): 328.0408; found: 328.0409.

**General procedure for the preparation of compound (3):**

A mixture of dichloro compound 1 (1.0 mmol) and amine or Na\(_2\)S or NaOH (2, 1.1 mmol) was heated to 135±5°C till reaching to its molten state and then stirred at the same temperature under open air for the time mentioned in Table S-1. The reaction was monitored by TLC (by preparing the sample solution for TLC via dissolving a very small portion of the reaction mixture in EtOAc). Upon completion of the reaction, the mixture was cooled to room temp and diluted with cold water (10 mL). The mixture was stirred vigorously at room temp for 10 min. The solid separated was filtered and washed thoroughly with cold water (3 x 5 mL). The solid obtained was then titurated in cold methyl \(t\)-butyl ether (MTBE, 10 mL) and filtered. After repeating the tituration / filtration process twice the solid obtained was dried under vacuum to give the desired product 3.

**Table S-1. Synthesis of indole fused pyrrolo-, furo- and thieno[2,3-b]quinoxalines (3)**
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<th>Product (3)</th>
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<th>Yield (%)</th>
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6-Benzyl-6,7-dihydroindolo[3',2':4,5]pyrrolo[2,3-b]quinoxaline (3a)

![Chemical Structure of 3a]

Yellow solid; yield: 87%; mp: 320-322 °C; \(^1\)H NMR (400 MHz, CDCl\(_3\)+DMSO-\(d_6\)): \(\delta\) 11.6 (s, 1H, NH), 8.23 (dd, \(J_1 = 8.0, J_2 = 1.2\) Hz, 1H, ArH), 8.16 (d, \(J = 7.6\) Hz, 1H, ArH), 8.07 (dd, \(J_1 = 8.4\) Hz, \(J_2 = 1.6\) Hz, 1H, ArH), 7.66-7.59 (m, 2H, ArH), 7.46 (d, \(J = 8.0\) Hz, 2H, ArH), 7.37-7.21 (m, 6H, ArH), 5.71 (s, 2H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)+DMSO-\(d_6\)): \(\delta\) 152.45, 145.86, 139.43, 138.61, 136.77, 136.20, 128.78 (3C), 127.79 (2C), 127.66 (2C), 127.08 (2C), 126.36, 125.73, 121.58 (2C), 121.28, 118.71, 112.59, 45.24; HRMS: m/z[M+1] calcd for C\(_{23}\)H\(_{17}\)N\(_4\)(M+H): 349.1453; found: 349.1443.

6-Phenethyl-6,7-dihydroindolo[3',2':4,5]pyrrolo[2,3-b]quinoxaline (3b)

![Chemical Structure of 3b]
Light yellow solid; yield: 89%; mp: 288-289 °C; $^1$H NMR (400 MHz, DMSO-$_d_6$): $\delta$ 12.5 (s, br, 1H, NH), 8.12 (d, $J = 6.0$, 1H, ArH), 8.04 (d, $J = 6.0$, 1H, ArH), 7.91 (d, $J = 6.0$ Hz, 1H, ArH), 7.68-7.56 (m, 3H, ArH), 7.29-7.14 (m, 7H, ArH), 4.7 (t, $J = 6.0$ Hz, 2H), 3.35 (t, $J = 6.2$ Hz, 2H); $^{13}$CNMR (100 MHz, DMSO-$_d_6$): $\delta$ 152.58, 145.81, 139.23, 138.65, 138.03, 137.17, 136.14, 128.77 (3C), 128.35 (2C), 127.70, 126.49, 126.14, 125.53, 121.55, 121.46, 121.13, 118.64, 112.45, 94.88, 43.38, 34.13; HRMS: m/z [M+1] calcd for C$_{24}$H$_{19}$N$_4$ (M+H): 363.1610; found: 363.1614

6-(Pyridin-2-ylmethyl)-6,7-dihydroindolo[3',2':4,5]pyrrolo[2,3-b]quinoxaline (3c)

![Chemical Structure](image)

Yellow solid; Yield: 85%; mp: 300-302 °C; $^1$H NMR (400 MHz, DMSO-$_d_6$): $\delta$ 12.5 (s, br, 1H, NH), 8.46 (d, $J = 4.8$ Hz, 1H, ArH), 8.15 (d, $J = 8.0$, 1H, ArH), 7.96 (dd, $J_1 = 13.2$, $J_2 = 5.6$, 2H, ArH), 7.78-7.52 (m, 4H, ArH), 7.33-7.20 (m, 4H, ArH), 5.8 (s, 2H); $^{13}$CNMR (100 MHz, DMSO-$_d_6$): $\delta$ 155.73, 153.26, 149.43, 146.13, 139.47, 138.66, 137.46, 137.28, 136.16, 127.75, 127.60, 126.34, 125.68, 122.87, 121.64, 121.57, 121.25, 121.18, 118.75, 112.62, 95.18, 59.83; HRMS: m/z [M+1] calcd for C$_{22}$H$_{16}$N$_5$ (M+H): 350.1406; found: 350.1391.

6-(3-Ethoxypropyl)-6,7-dihydroindolo[3',2':4,5]pyrrolo[2,3-b]quinoxaline (3d)

![Chemical Structure](image)
Yellow solid; Yield: 86%; mp: 221-222 °C; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 12.53 (s, 1H, NH), 8.14 (dd, $J_1 = 7.6$ Hz, $J_2 = 1.2$ Hz, 1H, ArH), 8.05 (dd, $J_1 = 8.0$, $J_2 = 1.6$ Hz, 1H, ArH), 7.91 (d, $J = 7.2$ Hz, 1H, ArH), 7.68-7.57 (m, 3H, ArH), 7.29-7.20 (m, 2H, ArH), 4.54 (t, $J = 6.8$ Hz, 2H), 3.44 (t, $J = 5.8$ Hz, 2H), 3.33-3.25 (m, 2H), 2.21-2.15 (m, 2H), 0.95 (t, $J = 7.0$ Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 152.76, 145.94, 139.22, 138.64, 137.23, 136.14, 127.71, 127.54, 126.01, 125.44, 121.57, 121.39, 121.0, 118.52, 112.39, 94.86, 67.16, 65.39, 57.96, 28.48, 14.85; HRMS: m/z [M+1] calcd for C$_{21}$H$_{21}$N$_4$O (M+H): 345.1715; found: 345.1701.

6-(4-Methoxybenzyl)-6,7-dihydroindolo[3',2':4,5]pyrrolo[2,3-b]quinoxaline (3e)

![Image of 6-(4-Methoxybenzyl)-6,7-dihydroindolo[3',2':4,5]pyrrolo[2,3-b]quinoxaline (3e)](image)

Yellow solid; yield: 87%; mp: 340-342 °C; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 12.6 (s, 1H, NH), 8.15 (d, $J = 7.6$ Hz, 1H, ArH), 8.07 (dd, $J_1 = 8.0$ Hz, $J_2 = 1.2$ Hz, 1H, ArH), 7.91 (d, $J = 7.6$ Hz, 1H, ArH), 7.68-7.56 (m, 3H, ArH), 7.4-7.2 (m, 4H, ArH), 6.88 (d, $J = 8.0$, 2H, ArH), 5.61 (s, 2H), 3.67 (s, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 158.77, 152.31, 145.77, 139.39, 138.57, 137.27, 131.18, 128.75 (2C), 128.65, 127.76, 127.66, 126.26, 125.64, 121.51, 121.49, 121.19, 118.62, 114.09 (2C), 112.52, 95.12, 55.02, 44.77; HRMS: m/z [M+1] calcd for C$_{24}$H$_{19}$N$_4$O (M+H): 379.1559; found: 379.1543.

6-(3-Methoxypropyl)-6,7-dihydroindolo[3',2':4,5]pyrrolo[2,3-b]quinoxaline (3f)

![Image of 6-(3-Methoxypropyl)-6,7-dihydroindolo[3',2':4,5]pyrrolo[2,3-b]quinoxaline (3f)](image)
Pale yellow solid; yield: 87%; mp: 277-279 °C; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 12.5 (s, br, 1H, NH), 8.14 (d, $J = 8.0$ Hz, 1H, ArH), 8.06 (d, $J = 7.6$ Hz, 1H, ArH), 7.92 (d, $J = 7.6$ Hz, 1H, ArH), 7.69-7.56 (m, 3H, ArH), 7.29-7.20 (m, 2H, ArH), 4.53 (t, $J = 7.2$ Hz, 2H), 3.42-3.29 (m, 2H), 3.18 (s, 3H), 2.22-2.15 (m, 2H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 152.73, 145.91, 139.23, 138.69, 137.23, 136.15, 127.71, 127.59 (2C), 126.06, 125.48, 121.57, 121.42, 121.04, 118.55, 112.46, 94.86, 69.21, 57.96, 28.42; HRMS: m/z [M+1] calcd for C$_{20}$H$_{19}$N$_4$O (M+H): 331.1559; found: 331.1567.

6-Hexyl-6,7-dihydroindolo[3',2':4,5]pyrrolo[2,3-b]quinoxaline (3g)

Yellow solid; yield: 85%; mp: 242-243°C; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 12.54 (s, 1H, NH), 8.14 (dd, $J_1 = 8.4$ Hz, $J_2 = 1.2$ Hz, 1H, ArH), 8.04 (dd, $J_1 = 7.6$ Hz, $J_2 = 1.6$ Hz, 1H, ArH), 7.91 (d, $J = 6.8$ Hz, 1H, ArH), 7.68-7.56 (m, 3H, ArH), 7.28-7.20 (m, 2H, ArH), 4.43 (t, $J = 7.0$ Hz, 2H), 1.92 (t, $J = 6.6$ Hz, 2H), 1.10-1.33 (m, 6H), 0.78 (t, $J = 7.0$ Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 152.69, 145.84, 139.21, 138.63, 137.12, 136.14, 127.72, 127.57, 126.02, 125.46, 121.56, 121.42, 121.04, 118.54, 112.43, 94.80, 42.05, 30.75, 28.29, 25.80, 21.94, 13.80; HRMS: m/z [M+1] calcd for C$_{22}$H$_{23}$N$_4$ (M+H): 343.1923; found: 343.1911.

6-(3,4-Dimethylbenzyl)-6,7-dihydroindolo[3',2':4,5]pyrrolo[2,3-b]quinoxaline (3h)
Yellow solid; yield: 79.3 %; mp: 205-206 °C; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 12.54 (s, 1H, NH), 8.16 (dd, $J_1 = 8.4$ Hz, $J_2 = 1.2$ Hz, 1H, ArH), 8.06 (dd, $J_1 = 8.4$ Hz, $J_2 = 1.2$ Hz, 1H, ArH), 7.93 (d, $J = 7.6$ Hz, 1H, ArH), 7.70-7.55 (m, 3H, ArH), 7.30-7.21 (m, 3H, ArH), 7.06 (s, 2H, ArH), 5.61 (s, 2H), 2.14 (s, 6H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 152.45, 145.86, 139.40, 138.62, 136.51, 136.21, 135.64, 134.05, 129.77 (2C), 128.36 (2C), 127.78, 127.67, 126.30, 125.69, 124.55, 121.55, 121.23, 118.67, 112.59, 95.11, 45.03, 19.43, 18.99; HRMS: m/z[M+1]
calcd for C$_{25}$H$_{21}$N$_4$(M+H): 377.1766; found: 377.1748.

6-Butyl-6,7-dihydroindolo[3',2':4,5]pyrrolo[2,3-b]quinoxaline (3i)

Light orange solid; yield: 80%; mp: 342-344°C; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 12.55 (s, 1H, NH), 8.14 (d, $J = 6.8$ Hz, 1H, ArH), 8.05 (d, $J = 8.0$ Hz, 1H, ArH), 7.91 (d, $J = 7.6$ Hz, 1H, ArH), 7.69-7.58 (m, 3H, ArH), 7.29-7.21 (m, 2H, ArH), 4.44 (t, $J = 6.8$ Hz, 2H), 1.99-1.90 (m, 2H), 1.40-1.23 (m, 2H), 0.95 (t, $J = 7.4$ Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 152.69, 145.84, 139.20, 138.63, 137.11, 136.13, 127.71, 127.59, 126.04, 125.48, 121.55, 121.42, 121.05, 118.54, 112.43, 95.79, 41.85, 30.45, 19.51, 13.55; HRMS: m/z [M+1] calcd for C$_{20}$H$_{19}$N$_4$(M+H): 315.1610; found: 315.1609.

6-(3,4-Dimethoxyphenethyl)-6,7-dihydroindolo[3',2':4,5]pyrrolo[2,3-b]quinoxaline (3j)
Pale yellow solid; yield: 82%; mp: 262-264 °C; $^1$H NMR (400 MHz, DMSO-$d_6$): δ 12.51 (s, 1H, NH), 8.13 (dd, $J = 8.4$ Hz, $J = 1.6$ Hz, 1H, ArH), 8.05 (dd, $J = 8.0$ Hz, $J = 1.6$ Hz, 1H, ArH), 7.90 (d, $J = 7.6$ Hz, 1H, ArH), 7.68-7.56 (m, 3H, ArH), 7.29-7.2 (m, 2H, ArH), 6.83-6.71 (m, 3H, ArH), 4.66 (t, $J = 6.8$ Hz, 2H), 3.63 (s, 3H), 3.60 (s, 3H), 3.20 (t, $J = 7.4$ Hz, 2H); $^{13}$CNMR (100 MHz, DMSO-$d_6$): δ 152.60, 148.52, 147.41, 145.82, 139.20, 138.61, 137.16, 136.10, 130.31, 127.70, 127.61, 126.07, 125.47, 121.50, 121.42, 121.06, 120.78, 118.54, 112.49, 112.39, 111.83, 97.85, 55.40, 55.27, 43.34, 33.54; HRMS: m/z [M+1] calcd for C$_{26}$H$_{23}$N$_4$O$_2$ (M+H): 423.1821; found: 423.1826.

2-(Indolo[3',2':4,5]pyrrolo[2,3-b]quinoxalin-6(7H)-yl)ethanol (3k)

![Image of 2-(Indolo[3',2':4,5]pyrrolo[2,3-b]quinoxalin-6(7H)-yl)ethanol (3k)](image)

Yellow solid; yield: 78%; mp: 227-229 °C; $^1$H NMR (400 MHz, DMSO-$d_6$): δ 12.43 (s, 1H, NH), 8.14 (t, $J = 4.2$ Hz, 1H, ArH), 8.04 (t, $J = 4.8$ Hz, 1H, ArH), 7.91 (d, $J = 8.0$ Hz, 1H, ArH), 7.68-7.57 (m, 3H, ArH), 7.28-7.19 (m, 2H, ArH), 5.04 (t, $J = 5.2$ Hz, 1H), 4.50 (t, $J = 5.6$ Hz, 2H), 3.96 (q, $J = 4.6$ Hz, 2H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): δ 153.36, 146.06, 139.24, 138.56, 137.35, 136.09, 127.70, 127.52, 126.01, 125.41, 121.53, 121.34, 120.97, 118.47, 112.43, 94.81, 58.78, 44.93; HRMS: m/z [M+1] calcd for C$_{18}$H$_{15}$N$_4$O (M+H): 303.1246; found: 303.1258.

(R)-6-(1-Phenylethyl)-6,7-dihydroindolo[3',2':4,5]pyrrolo[2,3-b]quinoxaline (3l)

![Image of (R)-6-(1-Phenylethyl)-6,7-dihydroindolo[3',2':4,5]pyrrolo[2,3-b]quinoxaline (3l)](image)

Yellow solid; yield: 77.8%; mp: 291-293 °C; $^1$H NMR (400 MHz, DMSO-$d_6$): δ 12.29 (s, 1H, NH), 8.16 (t, $J = 4.4$ Hz, 1H, ArH), 8.04 (dd, $J = 8.0$ Hz, $J = 1.2$ Hz, 1H, ArH), 7.94 (d, $J = 8.4$ Hz,
$^1$H, ArH), 7.70-7.61 (m, 2H, ArH), 7.55 (d, $J = 7.6$ Hz, 1H, ArH), 7.42-7.21 (m, 7H, ArH), 6.43 (q, $J = 7.2$ Hz, 1H), 2.19 (d, $J = 7.6$ Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 150.88, 145.73, 140.85, 139.40, 138.98, 136.94, 136.11, 128.71 (2C), 127.73, 127.66, 127.52, 126.30, 126.04 (2C), 125.67, 121.52, 121.26, 121.12, 118.54, 112.56, 95.99, 51.81, 18.66; HRMS: m/z [M+1] calcd for C$_{24}$H$_{19}$N$_4$(M+H): 363.1610; found: 363.1621.

7H-Indolo[3',2':4,5]furo[2,3-b]quinoxaline (3m)

Yellow solid; yield: 67%; mp: 279-281 °C; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 13.21 (s, br, 1H, NH), 8.16 (d, $J = 8.4$ Hz, 1H, ArH ), 8.05 (d, $J = 8.0$ Hz, 1H, ArH ), 7.94 (dd, $J = 6.8$ Hz, $J = 3.6$ Hz, 1H, ArH), 7.82 (t, $J = 7.6$ Hz, 1H, ArH), 7.73 (t, $J = 7.6$ Hz, 1H, ArH), 7.62-7.60 (m, 1H, ArH), 7.35-7.33 (m, 2H, ArH); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 161.49, 157.03, 140.94, 139.48, 136.78, 135.51, 128.33, 128.08, 127.56, 126.73, 122.65, 122.08, 119.76, 119.54, 113.36, 95.09; HRMS: m/z [M+1] calcd for C$_{16}$H$_{10}$N$_3$O (M+H): 260.0824; found: 260.0832.

7H-Indolo[3',2':4,5]thieno[2,3-b]quinoxaline (3n)

Pale yellow solid; yield: 91%; mp: 354-356 °C; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 12.57 (s, br, 1H, NH), 8.21-8.11 (m, 3H, ArH), 7.88-7.68 (m, 3H, ArH), 7.37-7.33 (m, 2H, ArH); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 158.29, 145.35, 144.72, 140.66, 140.21, 137.66, 129.36, 128.22, 128.07, 127.40, 122.96, 122.05, 121.46, 119.05, 112.65, 111.76; HRMS: m/z [M+1] calcd for C$_{16}$H$_{10}$N$_3$S (M+H): 276.0595; found: 276.0603.

Indolo[3',2':4,5]pyrrolo[2,3-b]quinoxalin-6(7H)-amine (3o)
Yellow solid; yield: 85%; mp: 298-300 °C; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 12.57 (s, br, 1H, NH), 8.15 (t, $J$ = 3.6 Hz, 1H, ArH), 8.08 (t, $J$ = 4.8 Hz, 1H, ArH), 7.9 (d, $J$ = 7.2 Hz, 1H, ArH), 7.68-7.64 (m, 2H, ArH), 7.52 (d, $J$ = 8.0 Hz, 1H, ArH), 7.25-7.20 (m, 2H, ArH), 6.17 (s, 2H, NH$_2$); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 153.749, 146.39, 139.42, 138.54, 136.10, 135.9, 127.76, 127.40, 126.04, 125.49, 121.71, 121.32, 120.84, 118.47, 112.44, 92.87; HRMS: m/z [M+1] calcd for C$_{16}$H$_{12}$N$_5$ (M+H): 274.1093; found: 274.1098.

7-Methyl-7H-indolo[3',2':4,5]furo[2,3-b]quinoxaline (3p)

Yellow solid; yield: 70%; mp: 175-177 °C; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.19 (dd, $J$ = 8.4 Hz, $J$ = 1.2 Hz, 1H, ArH), 8.13 (dd, $J$ = 6.4 Hz, $J$ = 1.2 Hz, 1H, ArH), 8.05 (dd, $J$ = 8.0 Hz, $J$ = 1.2 Hz, 1H, ArH), 7.75-7.71 (m, 1H, ArH), 7.66-7.62 (m, 1H, ArH), 7.41-7.26 (m, 3H, ArH), 3.94 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 161.03, 157.04, 141.52, 139.80, 137.77, 135.93, 128.42, 128.32, 127.86, 126.74, 122.59, 122.49, 120.83, 119.97, 110.16, 95.29, 29.28; HRMS: m/z [M+1] calcd for C$_{17}$H$_{12}$N$_3$O (M+H): 274.0980; found: 274.0991.

7-Methyl-7H-indolo[3',2':4,5]thieno[2,3-b]quinoxaline (3q)

Yellow solid; yield: 91.5%; mp: 190-192 °C; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.37-8.34 (m, 1H, ArH), 8.24 (dd, $J_1$ = 8.4 Hz, $J_2$ = 1.2 Hz, 1H, ArH), 8.09 (dd, $J_1$ = 8.4 Hz, $J_2$ = 1.2 Hz, 1H, ArH),
7.79-7.75 (m, 1H, ArH), 7.70-7.66 (m, 1H, ArH), 7.46-7.26 (m, 3H, ArH), 3.95 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 158.09, 146.98, 146.03, 141.29, 140.95, 138.09, 128.99, 128.61, 128.19, 127.09, 122.87, 122.79, 121.69, 120.39, 111.76, 109.36, 32.31; HRMS: m/z [M+1] calcd for C$_{17}$H$_{12}$N$_3$S (M+H): 290.0752; found: 290.0751.

2-Chloro-3-(2-chloro-1H-indol-3-yl)quinoxaline (Intermediate)

Pale yellow solid; yield: 52%; mp: 220-221°C; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 12.36 (s, br, 1H, NH), 7.8 (d, $J$ = 7.2 Hz, 1H, ArH), 7.61-7.54 (m, 2H, ArH), 7.44-7.30 (m, 5H, 4ArH, 1NH), 7.28-7.17 (m, 5H, ArH), 7.09 (t, $J$ =7.2 Hz, 1H, ArH), 4.63 (d, $J$ = 5.6 Hz, 2H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 150.9, 141.01, 140.87, 139.89, 136.39, 134.81, 129.49, 128.33, 128.02 (2C), 127.52 (2C), 126.77, 126.47, 125.48, 123.67, 123.41, 122.28, 120.28, 118.77, 111.18, 107.64, 43.94; HRMS: m/z [M+1] calcd for C$_{23}$H$_{18}$N$_4$Cl (M+H): 385.1220; found: 385.1207.

References:

Biology

Sulphorhodamine B (SRB) Assay:

The principle: The anti-proliferative activity and cancer cell selectivity of the synthesized compounds on 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 screening of anticancer compounds 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.

**The methodology:** Cancer cells (around 5000 in number) were seeded in 96-well plates and incubated overnight. The optimum cell number to be seeded was determined by a growth curve analysis for the cell line. Compounds (dissolved in 100% DMSO to a stock concentration of 200mM) were added to the adhered cells at a final concentration of 10µM. After 72h of treatment, the cells were washed with phosphate-buffered saline and ice-cold 10% trichloroacetic acid was added to the cells to precipitate the proteins. It was incubated 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 30 min at room temperature. The unbound dye was washed away by destaining with 1% acetic acid and bound dye was solubilized with 10mM Tris solution (pH 10.5). Absorbance of solubilized dye was measured at a wavelength of 590 nm. Percentage growth was determined by the formula

\[
\frac{(At-A0/Ac-A0)}{\times100}
\]

where At=absorbance after 72h of test compound treatment, A0=Absorbance at time 0, Ac=Absorbance after 72h without treatment.

The known cytotoxic agent, gemcitabine was used as a positive control in the assay.