"Synthesis of 4-aryl-6-indolylpyridine-3-carbonitriles and Evaluation of Their Antiproliferative Activity" data files

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Supplementary Data

Synthesis of 4-aryl-6-indolylpyridine-3-carbonitriles and evaluation of their antiproliferative activity

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Experimental

Chemical protocols

All reagents and solvents used were purchased from common commercial of analytical grad, melting points are uncorrected and were measured using an electro-thermal IA 9100 apparatus (Shimadzu, Japan). IR spectra were carried out on JASCO FT/IR 6100 Japan spectrometer (National Research Center Cairo, Egypt) using KBr disc. $^1$H-NMR spectra were determined using JEOL ECA-500 run for $^1$H-NMR at 500 MHz and run for $^{13}$C-NMR at 125 MHz spectrometer (National Research Center, Cairo, Egypt). Chemical shifts were expressed in part per million $\delta$ (ppm) against tetramethylsilane (TMS) as an internal standard. The coupling constant $J$ is expressed in Hz. The Mass spectra were recorded on GCMS Finnigan mat SSQ 7000 spectrometer (National Research Centre, Cairo, Egypt). Reactions under microwave irradiation were performed using CEM Discover model I908005 using closed vessel with magnetic stirring. All reactions were followed up by thin layer chromatography (TLC) using aluminum sheets recoated with UV fluorescent silica gel (Merck Kieselgel 60 F$_{245}$), UV lamp, iodine vapor, and ninhydrin reagent.

Synthesis of 6-(1H-indol-3-yl)-2-oxo-4-substituted-1,2-dihydropyridine-3-carbonitrile (13a-e).

Method (A). A mixture of ethyl cyanoacetate (1.13 g, 10 mmol), 3-indolymethyl ketone 1 (1.59 g, 10 mmol), aromatic aldehydes (2-thiophenaldehyde (1.12 g, 10 mmol), 4-methoxybenzaldehyde (1.36 g, 10 mmol), 4-fluorobenzaldehyde (1.24 g, 10 mmol), 4-chlorobenzaldehyde (1.40 g, 10 mmol), or 4-bromobenzaldehyde (1.85 g, 10 mmol)), ammonium acetate (4.62 g, 6 mmol) in anhydrous ethylene glycol (3 mL) and pipredine (1 mL) as a catalyst
were placed in a (100 mL) round flask and heated under reflux for given time 10-17 h. The reaction progress was monitored by TLC. Then it was cooled to room temperature after completion. The crude solid product was filtrated off, dried, and recrystallized from methanol/DMF (12 mL, 3:1 v/v) giving the corresponding derivatives **13a-e** as yellow crystals.

*Method (B).* The procedure was similar to that described in method A except that the solvent used is ethylene glycol (1 mL) and the mixture was placed in closed (10 mL) vessels and irradiated in a microwave reactor at 250 W and 150 °C for a given time. The reaction progress was monitored by TLC. The reaction mixture was cooled to room temperature after completion, then the crude material boiled in ethanol (20 mL) where a yellow solid product was separated, filtrated off, dried, and recrystallized from methanol/DMF (12 mL 3:1 v/v) to give derivatives **13a-e** as yellow crystals.

**6-(1H-Indol-3-yl)-2-oxo-4-(thiophen-2-yl)-1,2-dihydropyridine-3-carbonitrile** (13a). Yield (77%); mp: 259-261 °C; IR (KBr) cm⁻¹: 3275 (NH indole), 3116 (NH pyridone), 2209 (CN), and 1661 (CO amide). ¹H-NMR (DMSO-d₆) σ 6.67 (s, 1H, H-5 pyridone), 7.10 (m, 2H, indole); 7.30 (d, J = 7.5 Hz, 1H, indolyl); 7.43 (t, J = 7.3 Hz, 1H, thiophenyl ring), 7.67 (d, J = 6.4 Hz, 1H, thiophenyl ring), 7.95 (d, J = 6.2 Hz ,1H, thiophenyl ring), 8.02 (d, J = 7.5 Hz, 1H, indolyl), 8.33 (d, 1H, indolyl), 11.62 (s,1H, NH pyridone, D₂O exchangeable); 11.81 (broad, 1H, NH indole, D₂O exchangeable). ¹³C NMR (DMSO-d₆), σ 102.91 (CN), 108.20, 113.35, 118.06, 119.88, 122.10, 123.37, 124.56, 129.29, 130.08, 130.98, 131.65, 137.58, 137.86, 147.01, 148.23, 151.05
(aromatic carbons), 162.84 (CO amide). MS (m/z, %): calcd. for C_{12}H_{11}N_{3}O_{5}: 317.06; found 317.00 [M^+, 100%].

6-(1H-Indol-3-yl)-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile  (13b). Yield (79%); mp: 283-286 °C; IR (KBr) cm\(^{-1}\): 3448 (NH indolyl ring), 3262 (NH pyridone), 2210 (CN), and 1644 (CO amide). \(^1\)H-NMR (DMSO-d\(^6\)) \(\sigma\) 3.8 (s, 3H, OCH\(_3\)), 6.57 (s, 1H, H-5 pyridone), 7.08 (d, \(J = 8.4\) Hz, 2H, aromatic), 7.18 (m, 2H, indolyl), 7.49 (d, \(J = 8.5\) Hz, 1H, indolyl), 7.69 (d, \(J = 8.4\) Hz, 2H, aromatic), 7.86 (d, \(J = 8.5\) Hz, 1H, indolyl), 8.27 (d, 1H, indolyl), 12.06 (s, 1H, NH pyridone, D\(_2\)O exchangeable), 12.35 (1H, d, NH indole, D\(_2\)O exchangeable). \(^13\)C-NMR (DMSO-d\(^6\)), \(\sigma\) 108.82 (CN), 113.28, 114.81, 117.96, 120.09, 121.99, 123.33, 124.56, 129.13, 129.71, 129.88, 130.32, 137.35, 137.51, 147.59, 159.9, 161.47 (aromatic carbons), 162.43 (CO amide). MS (m/z, %) calcd. for C\(_{21}\)H\(_{15}\)N\(_3\)O\(_3\): 341.12; found 341.00 [M^+, 100%].

6-(1H-Indol-3-yl)-(4-florophenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile  (13c). Yield (87%); mp > 310 °C; IR (KBr) cm\(^{-1}\): 3356 (NH indolyl), 3109 (NH pyridone), 2210 (CN), and 1635 (CO amide). \(^1\)H-NMR (DMSO-d\(^6\)) \(\sigma\) 6.69 (s, 1H, H5-pyridone), 7.24 (m, 2H, indolyl), 7.44 (d, \(J = 8.7\) Hz, 2H, aromatic); 7.51 (d, \(J = 7.71\)Hz, 1H, indolyl), 7.82 (d, \(J = 8.5\) Hz, 2H, aromatic), 7.92 (d, \(J = 8.2\) Hz, 1H, indolyl), 8.31 (d, 1H, indolyl), 12.11 (s, 1H, NH pyridone, D\(_2\)O exchangeable), 12.42 (d, 1H, NH indolyl, D\(_2\)O exchangeable). \(^13\)C-NMR, (DMSO-d\(^6\)) \(\sigma\) 104.48 (CN), 112.25, 113.21, 115.19, 115.34, 116.31, 116.45, 120.76, 121.98, 122.44, 123.33,
6-(1H-Indol-3-yl)-4-(4-chlorophenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (13d). Yield (82%); mp > 310 °C; IR (KBr) cm$^{-1}$: 3418 (NH indolyl), 3128 (NH pyridone), 2219 (CN), and 1636 (CO amide). $^1$H-NMR (DMSO-d$_6$) $\sigma$ 6.76 (s, 1H, H-5 pyridone), 7.17-22 (m, 2H, indolyl), 7.52 (d, $J = 7.7$ Hz, 1H, indolyl), 7.65 (d, $J = 8.1$ Hz, 2H, aromatic), 7.77 (d, $J = 8.2$ Hz, 2H, aromatic), 7.93 (1H, $J = 8.76$ Hz, d, indolyl), 8.32 (d, 1H, indolyl), 12.12 (s, 1H, NH pyridone, D$_2$O exchangeable); 12.41 (d, 1H, NH indole, D$_2$O exchangeable). $^{13}$C-NMR, (DMSO-d$_6$) $\sigma$ 108.40 (CN), 108.46, 109.90, 113.23, 117.51, 120.23, 122.01, 123.35, 124.56, 129.40, 130.14, 130.53, 135.58, 135.90, 137.53, 148.53, 159.05 (aromatic carbons), 162.60 (CO amide). MS (m/z, %) calcd. for C$_{20}$H$_{12}$FN$_3$O: 345.07; found 345.00 [M$^+$, 15].

6-(1H-Indol-3-yl)-4-(4-bromophenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (13e). Yield (83%); mp > 310 °C; IR (KBr) cm$^{-1}$: 3259 (NH indolyl), 3114 (NH pyridone), 2210 (CN), and 1638 (CO amide). $^1$H-NMR (DMSO-d$_6$) $\sigma$ 6.69 (s, 1H, H-5 pyridone); 7.17-7.23 (m, 2H, indolyl), 7.47 (d, $J = 6.7$ Hz, 1H, indolyl), 7.65 (d, $J = 8.4$ Hz, 1H, aromatic), 7.76 (2H, $J = 8.4$ Hz, d, aromatic), 7.87 (d, $J = 6.7$ Hz, 1H, indolyl), 8.28 (d, 1H, indolyl), 12.11 (s, 1H, NH pyridone, D$_2$O exchangeable); 12.54 (d, 1H, NH indolyl, D$_2$O exchangeable). $^{13}$C-NMR (DMSO-d$_6$) $\sigma$ 104.32 (CN), 113.21, 117.50, 120.24, 122.02, 123.37, 124.35, 124.56, 130.16, 130.76, 132.35, 136.29, 137.52, 159.17 (aromatic carbons), 162.60 (CO amide), MS m/z calcd. for C$_{20}$H$_{12}$FN$_3$O: 389.02; found 389.00 [M$^+$, 100].
2. 2-Chloro-6-(1H-indol-3-yl)-4 substituted nicotinonitrile (14a-e). A mixture of compound 2 (0.01 mmol) and phosphoryl chloride (10 mL) was subjected to gentle heating under reflux for 18-24 h. Then, it was poured onto crushed ice and neutralized by a saturated solution of NaHCO₃. Whereby, a yellow solid product was separated, filtered off, washed with water for several times, dried, and crystallized from ethanol (25 mL) affording pale yellow crystals of compounds 14a-e.

2-Chloro-6-(1H-indol-3-yl)-4-(thiophen-2-yl)nicotinonitrile (14a). Yield (95%); mp: 274-277 °C; IR (KBr) cm⁻¹: 3305 (NH indolyl), and 2224 (CN). ¹H-NMR (DMSO-d₆) σ 7.23 (t, J = 6.2 Hz, 2H, indolyl ring), 7.30 (t, J = 9.2 Hz, 1H, thiophene), 7.52 (d, J = 9.2 Hz, 1H, indolyl), 7.85 (d, J = 9.9 Hz, 1H, thiophenyl), 7.93 (d, J = 9.9 Hz, 1H, thiophenyl), 8.03 (s, 1H, pyridine), 8.46 (d, J = 9.2 Hz, 1H, indolyl), 8.50 (d, 1H, indolyl), 12.10 (broad, 1H, NH indolyl, D₂O exchangeable). ¹³C-NMR (DMSO-d₆) σ 100.02 (CN), 112.82, 113.49, 116.45, 116.45, 121.79, 122.13, 123.19, 125.52, 129.04, 130.75, 131.24, 131.33, 136.83, 137.74, 146.54, 153.71, 158.87 (aromatic carbons). MS (m/z, %) calcd. for C₁₈H₁₀ClN₃S: 335.03; found 335.00 [M⁺, 100].

2-Chloro-6-(1H-indol-3-yl)-4-(4-methoxyphenyl)nicotinonitrile (14b). Yield (98%); mp: 255-257 °C; IR (KBr) cm⁻¹: 3319 (NH indolyl), and 2219(CN). ¹H-NMR (DMSO-d₆): σ 3.83 (s, 3H, OCH₃), 7.12 (d, J = 6.9 Hz, 2H, aromatic), 7.20 (t, J = 6.2 Hz, 2H, indolyl), 7.45 (d, J = 6.1 Hz, 1H, indolyl), 7.70 (d, J = 6.9 Hz, 2H, aromatic), 7.99 (s, 1H, H-5 pyridine), 8.41 (d, J = 6.1 Hz, 1H, indolyl), 8.53 (d, 1H, indolyl), 12.03 (broad, 1H, NH indolyl, D₂O exchangeable). ¹³C-NMR
(DMSO-d6): \( \sigma \) 55.95 (OCH\(_3\)); 102.09 (CN), 112.83, 113.64, 114.80, 116.74, 117.88, 121.79, 122.15, 123.21, 125.55, 128.21, 130.77, 131.13, 137.71, 153.00, 154.59, 158.83, 161.35 (aromatic carbons). MS (m/z, %) calcd. for C\(_{21}\)H\(_{14}\)ClN\(_3\)O: 358.08; found 358.00 [M\(^+\), 100].

2-Chloro-4-(4-fluorophenyl)-6-(1H-indol-3-yl)nicotinonitrile (14c). Yield (95%); mp: 290-293 °C; IR (KBr) cm\(^{-1}\): 3227 (NH indolyl) and 2217 (CN). \(^1\)H-NMR (DMSO-d\(_6\)): \( \sigma \) 7.19 (d, \( J = 8.4 \) Hz, 2H, aromatic), 7.34-7.41 (m, 2H, indolyl), 7.47 (d, \( J = 7.6 \) Hz, 1H, indolyl), 7.75 (2H, \( J = 8.4 \) Hz, d, aromatic), 7.96 (s, 1H, H-5 pyridine), 8.38 (d, \( J = 7.6 \) Hz,1H, indolyl), 8.45 (d,1H, indolyl), 11.27 (broad, 1H, NH indolyl, D\(_2\)O exchangeable). \(^13\)C-NMR (DMSO-d\(_6\)): \( \sigma \) 102.44 (CN), 112.84, 113.61, 116.29, 116.65, 118.29, 121.86, 122.19, 123.26, 125.55, 131.43, 131.64, 131.71, 137.74, 152.85, 153.84, 159.05, 162.72, 164.69 (aromatic carbons). MS (m/z) calcd. for C\(_{20}\)H\(_{11}\)ClN\(_3\): 347.06; found 347 [M\(^+\)].

2-Chloro-4-(4-chlorophenyl)-6-(1H-indol-3-yl)nicotinonitrile (14d). Yield (98%); mp: 270-273 °C; IR (KBr) cm\(^{-1}\): 3290 (NH indolyl) and 2224 (CN). \(^1\)H-NMR (DMSO-d6): \( \sigma \) 7.21-7.25 (m, 2H, indolyl), 7.52 (d, \( J = 7.7 \) Hz, 1H, indolyl), 7.60 (d, \( J = 8.4 \) Hz, 2H, aromatic), 7.78 (d, \( J = 8.4 \) Hz, 2H, aromatic), 7.99 (s, 1H, H-5 pyridine), 8.44 (d, \( J = 7.7 \) Hz, 1H, indolyl), 8.53 (d, 1H, indolyl), 11.27 (broad,1H, NH indolyl, D\(_2\)O exchangeable). \(^13\)C (DMSO-d\(_6\)) \( \sigma \) 102 (CN), 112.87, 113.59, 116.36, 118.27, 121.91, 122.19, 123.31, 125.54, 129.43, 131.13, 131.53, 135.00, 135.63, 137.75, 152.86, 153.66, 159.12 (aromatic carbons). MS (m/z, %) calcd. for C\(_{20}\)H\(_{11}\)Cl\(_2\)N\(_3\): 363.03; found 363.00 [M\(^+\), 100].
2-Chloro-4-(4-bromophenyl)-6-(1H-indol-3-yl)nicotinonitrile (14e). Yield (97%); mp: 272-275 °C; IR (KBr) cm⁻¹: 3288 (NH indolyl), and 2220 (CN). \(^1^H\) NMR (DMSO-d₆): \(\delta\) 7.19-7.20 (t, \(J = 7.7\) Hz, 2H, indolyl), 7.45 (d, \(J = 6.9\) Hz, 1H, indolyl), 7.67 (d, \(J = 8.4\) Hz, 2H, aromatic), 7.79 (d, \(J = 8.4\) Hz, 2H, aromatic), 8.05 (s, 1H, H-5 pyridine), 8.43 (1H, \(J = 6.9\) Hz, d, indolyl), 8.54 (d, 1H, indolyl), 12.07 (broad, 1H, NH indolyl, D₂O exchangeable). \(^1^C\) (DMSO-d₆): \(\delta\) 102.30 (CN); 112.86, 113.61, 116.31, 118.21, 121.91, 122.21, 123.29, 124.38, 125.55, 131.34, 131.55, 132.35, 135.37, 137.75, 152.87, 152.87, 153.7, 159.13 (aromatic carbons). MS (m/z) calcd. for C₂₀H₁₁BrClN₃: 428.98; found 428.00 [M⁺ + Na].

3. 2-((2-Aminoethyl)amino)-6-(1H-indol-3-yl)-4-substitued nicotinonitrile (15a-e). A mixture of compound 14a-e (10 mmol) and ethylenediamine (15 mmol) in ethanol (10 mL) was refluxed. The progress of the reaction was monitored by TLC using eluent system (DCM: Methanol 2:1 v/v) for 48 h. Then the reaction mixture was poured onto crushed ice and neutralized by HCl (1 M), filtered off, washed with water, dried and crude products crystallized from methanol (25 mL) pale yellow crystals of compounds 15a-e.

2-((2-Aminoethyl)amino)-6-(1H-indol-3-yl)-4-((thiophen-2yl)nicotinonitrile (15a). Yield (91%); mp: 214-217 °C; IR (KBr) cm⁻¹: 3361 (NH), 3307 (NH₂), and 2191(CN). \(^1^H\)-NMR (DMSO-d₆): \(\delta\) 2.94 (t, \(J = 6.1\)Hz, H, CH₂), 3.47 (broad, 2H, NH₂, D₂O exchangeable), 3.69 (m, 2H, CH₂), 4.09 (broad, 1H, NH, D₂O exchangeable), 7.18 (t, \(J = 6.9\) Hz, 2H, indolyl), 7.30 (t, \(J = 8.8\) Hz, 1H, thiophenyl), 7.46 (d, \(J = 7.2\) Hz, 1H, thiophenyl), 7.47 (d, \(J = 8.6\) Hz, 1H, thiophenyl), 7.80 (d, \(J = 8.5\) Hz, 1H, indolyl), 7.83 (1H, \(J = 8.6\) Hz, d, indolyl), 8.09 (s, 1H, H-5
pyridine), 8.41 (d, 1H, indolyl), 11.82 (1H, d, NH indolyl, D$_2$O exchangeable). $^{13}$C-(DMSO-d$_6$) $\sigma$
50.67 (CH$_2$), 54.53 (CH$_2$); 107.42 (CN), 112.76, 115.03, 121.68, 121.98, 122.94, 125.50, 128.98,
129.14, 129.55, 129.74, 131.28, 133.31, 137.53, 138.47, 145.80, 153.42, 160.22 (aromatic
carbons). MS (m/z, %) calcd. for C$_{20}$H$_{17}$N$_5$S: 359.12; found 359.00 [M$^+$, 100].

2-((2-Aminoethyl)amino)-6-(1H-indol-3-yl)-4-(4-methoxyphenyl)nicotinonitrile (15b). Yield
(96%); mp: 205-209 °C; IR (KBr) cm$^{-1}$: 3366 (NH & NH$_2$), 2201 (CN). $^1$H-NMR (DMSO-d$_6$): $\sigma$
2.86 (t, $J = 6.1$ Hz, 2H, C$_2$H$_2$); 3.31 (broad, 1H, NH, D$_2$O exchangeable), 3.66 (m, 2H, CH$_2$), 3.82
(s, 3H, OCH$_3$), 4.09 (broad, 1H, NH); 7.07 (d, $J = 8.4$ Hz, 2H, aromatic), 7.14 (t, $J = 77$ Hz, 2H,
indolyl), 7.33 (d, $J = 6.1$ Hz, 1H, indolyl), 7.41 (d, $J = 6.1$ Hz, 1H, indolyl), 7.60 (d, $J = 8.4$ Hz,
2H, aromatic), 8.31 (s, 1H, H-5 pyridine), 8.47 (d, 1H, indolyl), 11.73 (d, 1H, NH indolyl, D$_2$O
exchangeable). $^{13}$C-NMR (DMSO-d$_6$) $\sigma$ 40.16 (CH$_2$), 40.33 (CH$_2$), 55.87 (OCH$_3$), 108.11 (CN);
112.51, 114.63, 121.17, 122.39, 122.55, 125.97, 128.86, 129.39, 130.24, 137.62, 153.44, 158.18,
158.44, 160.16, 160.70 (aromatic carbons). MS (m/z, %) calcd. for C$_{23}$H$_{21}$N$_5$O: 383.17; found
383.00 [M$^+$, 10].

2-((2-Aminoethyl)amino)-4-(4-fluorophenyl)-6-(1H-indol-3-yl)nicotinonitrile (15c). Yield
(89%); mp: 193-196 °C; IR (KBr) cm$^{-1}$: 3381 (NH), 3049 (NH$_2$), and 2195 (CN). $^1$H-NMR
(DMSO-d$_6$): $\sigma$ 2.98 (t, $J = 6.2$ Hz, 2H, CH$_2$), 3.13 (broad, 1H, NH, D$_2$O exchangeable), 3.42. (m,
2H, CH$_2$), 4.10 (broad, 1H, NH, D$_2$O exchangeable), 7.19 (t, $J = 7.6$ Hz, 2H, indole), 7.25 (d, $J =
7.1$ Hz, 1H, indolyl), 7.13 (d, $J = 8.5$ Hz, 2H, aromatic), 7.34 (d, $J = 8.5$ Hz, 2H, aromatic), 8.01
(d, $J = 7.3$ Hz, 1H, indolyl), 8.21 (s, 1H, H-5 pyridine), 8.31 (d, 1H, indolyl), 11.59 (d, 1H, NH
indolyl, D$_2$O exchangeable). $^{13}$C-NMR (DMSO-d$_6$) $\sigma$ 40.88 (CH$_2$), 43.87 (CH$_2$), 107.76 (CN), 112.36, 112.67, 115.66, 119.24, 121.29, 122.12, 122.62, 124.64, 125.87, 128.88, 129.79, 137.68, 150.45, 154.30, 157.72, 160.31, 162.82 (aromatic carbons). MS (m/z, %) calcd. for C$_{22}$H$_{18}$FN$_5$: 371.15; found 371.00 [M$^+$, 100].

2-((2-Aminoethyl)amino)-4-(4-chlorophenyl)-6-(1H-indol-3-yl)nicotinonitrile (15d). Yield (93%); mp: 204-207 °C; IR (KBr) cm$^{-1}$: 3381, 3049 (NH and NH$_2$), and 2195 (CN). $^1$H-NMR (DMSO-d$_6$) $\sigma$ 2.91 (t, $J$ = 6.4 Hz, 2H, CH$_2$), 3.42 (broad, 2H, NH$_2$, D$_2$O exchangeable), 3.62 (m, 2H, CH$_2$), 4.09 (broad, 1H, NH, D$_2$O exchangeable), 7.19 (t, $J$ = 7.7 Hz, 2H, indolyl), 7.22 (d, J = 6.1 Hz, 1H, indolyl), 7.46 (d, $J$ = 6.1 Hz, 1H, indolyl); 7.60 (d, $J$ = 8.4 Hz, 2H, aromatic); 7.65 (2H, J = 8.4 Hz, d, aromatic); 8.36 (s,1H, H-5 pyridine); 8.48 (d, 1H, indolyl), 11.79 (1H, d, NH indolyl, D$_2$O exchangeable). $^{13}$C (DMSO-d$_6$) $\sigma$ 39.99 (CH$_2$), 40.16 (CH$_2$), 108.20 (CN), 112.36, 112.66, 115.66, 119.23, 121.29, 122.10, 122.62, 124.65, 125.87, 128.88, 129.79, 137.68, 150.45, 154.30, 157.71, 160.31, 162.82 (aromatic carbons). MS (m/z, %) calcd. for C$_{22}$H$_{18}$ClN$_5$: 387.13; found 387.00 [M$^+$, 9].

2-((2-Aminoethyl)amino)-4-(4-bromophenyl)-6-(1H-indol-3-yl)nicotinonitrile (15e). Yield (93%); mp: 198-201 °C; IR (KBr) cm$^{-1}$: 3377 (NH), 3059 (NH$_2$), and 2202 (CN). $^1$H NMR (DMSO-d$_6$): $\sigma$ 2.69 (2H, $J$ = 6.1 Hz, CH$_2$), 3.35 (2H, broad, NH$_2$, D$_2$O exchangeable), 3.59 (m, 2H, CH$_2$), 4.07 (broad, 1H, NH, D$_2$O exchangeable), 7.14 (t, $J$ = 6.9 Hz, 2H, indolyl), 7.18 (d, J = 9.9 Hz, 1H, indolyl), 7.42 (1H, $J$ = 8.5 Hz, d, indolyl), 7.58 (2H, $J$ = 8.5 Hz, d, aromatic), 7.73 (d, J = 8.5 Hz, 2H, aromatic), 8.31(s, 1H, H-5 pyridine), 8.48 (d, 1H, indolyl), 11.84 (d, 1H, NH
indolyl, D$_2$O exchangeable). $^{13}$C (DMSO-d$_6$): $\sigma$ 40.48 (CH$_2$), 40.74 (CH$_2$), 108.71 (CN), 112.50, 115.32, 115.45, 117.91, 122.00, 122.51, 123.52, 125.73, 129.85, 129.85, 130.55, 132.25, 137.00, 137.55, 152.56, 158.34, 159.77 (aromatic carbons). MS (m/z, %) calcd. for C$_{22}$H$_{18}$BrN$_5$: 432.07; found 432.00 [M$^+$, 55].

**Cell culture**

**Cell culture.** Human cervix adenocarcinoma HeLa (ATCC no. CCL-2), human ovarian adenocarcinoma cell line SK-OV-3 (ATCC no. HTB-77), and human breast adenocarcinoma MCF-7 (ATCC no. HTB-22) were obtained from American Type Culture Collection. Cells were grown on 75 cm$^2$ cell culture flasks with EMEM (Eagle’s minimum essential medium), supplemented with 10% fetal bovine serum, and 1% penicillin/streptomycin solution (10,000 units of penicillin and 10 mg of streptomycin in 0.9% NaCl) in a humidified atmosphere of 5% CO$_2$, 95% air at 37 ºC.

**Cell proliferation assay.** Cell proliferation assay was carried out using CellTiter 96 aqueous one solution cell proliferation assay kit (Promega, USA). Briefly, upon reaching about 75-80% confluency, 5000 cells/well were plated in 96-well microplate in 100 µL media. After seeding for 24 h, the cells were treated with compounds (50 µM) in triplicate. Doxorubicin (10 µM) was used as the positive control. At the end of the sample exposure period (72 h), 20 µL CellTiter 96 aqueous solution was added. The plate was returned to the incubator for 1 h in a humidified atmosphere at 37 ºC. The absorbance of the formazan product was measured at 490 nm using microplate reader. The blank control was recorded by measuring the absorbance at 490 nm with
wells containing medium mixed with CellTiter 96 aqueous solution but no cells. Results were expressed as the percentage of the control (without compound set at 100%).

**Partition Coefficient:** Calculated partition coefficient values of all the compounds were determined by using ChemDraw 10.0 software from CambridgeSoft (Table S1).

<table>
<thead>
<tr>
<th>Compound No,</th>
<th>Log P*</th>
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<td>13a</td>
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<td>Dox</td>
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</table>

*aCalculated partition coefficient determined by using ChemDraw 10.0 software from CambridgeSoft.
**IC\textsubscript{50} determination assay.** IC\textsubscript{50} determination assay was carried out using CellTiter 96 aqueous one solution cell proliferation assay kit (Promega, USA). Briefly, SK-OV-3, HeLa, and MCF-7 cells (5000 cells/well) were plated in 96-well microplate in 100 µL media. After seeding for 24 h, the cells were treated with different concentrations of compounds (1 µM- 50 µM) in triplicate. After 72 h of incubation, 20 µL CellTiter 96 aqueous solution was added. The plate was returned to the incubator for 1 h in a humidified atmosphere at 37 °C. The absorbance of the formazan product was measured at 490 nm using microplate reader. The blank control was recorded by measuring the absorbance at 490 nm with wells containing medium mixed with CellTiter 96 aqueous solution but no cells. The IC\textsubscript{50} values were extrapolated from concentration–effect curves using linear regression analysis in GraphPad Prism®, version 5.03.
Figure S1. IC₅₀ determination for compounds 15a, 15b, 15d, and 15e against SKOV-3, MCF-7 and Hela cells.