**Synthesis and Characterization of Novel 2-Amino-Chromene-Nitriles that Target Bcl-2 in Acute Myeloid Leukemia Cell lines**

Hosadurga K. Keerthy, Manoj Garg, Chakrabhavi D. Mohan, Vikas Madan,Deepika Kanojia, Rangappa Shobith , Shivananju Nanjundaswamy, Daniel J. Mason, Andreas Bender,Basappa, Kanchugarakoppal S. Rangappa, H. Phillip Koeffler

**Data S1**

**General procedure for one pot synthesis of 2-amino chromene-3-carbonitriles:** To a solution of primary alcohol (1.0 mmol) in a mixture of solvents Ethyl acetate: DMSO (2: 1 ratio) was added T3P® (2.5 mmol, 50% solution in ethyl acetate) at RT. This was followed by the addition of malanonitrile (1.2 mmol) and β-naphthol/resorcinol/4-hydroxy coumarin (1.0 mmol). Reaction was monitored by TLC (Hexane:EtOAc 7:3). Reaction mixture was stirred stipulated time at room temperature. After completion of the reaction, the mixture was diluted with water. The product was extracted with ethyl acetate, and the combined organic layers were washed with water followed by brine solution. The organic phase was dried over anhydrous Na2SO4. The solvent was removed under reduced pressure to afford a crude product which was purified by column chromatography using hexane: ethyl acetate mixture (7:3) as an eluent. Spectral properties are consistent with the assigned structures of novel 2-amino chromene-3-carbonitriles and the reported structural analysis of molecules matched those that were synthesized in our laboratory.

**2-amino-4-(4-oxo-4H-chromene-3-yl)-4H-benzo[g]chromene-3-carbonitrile (4d):** Brown Solid, IR νmax: 3352 cm-1 ν(NH2), 2189 cm-1 ν(CN), 1736 cm-1 ν(C=O), 1655 cm-1 ν(vinyl nitrile) ;1H NMR (DMSO, 400 MHz) δ 2.083 (2H, -NH2, s), δ 4.568 (1H, Methine, s), δ 6.932 (1H, Ar-H, s), δ 7.088-7.180 (2H, Ar-H, m), δ 7.192-7.291 (2H, Ar-H, m), δ 7.302-7.392 (1H, Ar-H, m), δ 7.401-7.531 (1H, Ar-H, m), δ 7.570-7.706 (2H, Ar-H, m), δ 7.772-7.832 12H, Ar-H, m); 13C NMR (CDCl3, 300MHz) δ 30.13, δ 57.95, δ 108.83, δ 115.60, δ 117.66, δ 119.48, δ 122.53, δ 123.46, δ 123.57, δ 125.17, δ 125.75, δ 127.35, δ 128.46, δ 128.95, δ 129.44, δ 131.08, δ 132.53, δ 135.57, δ 151.13, δ 155.79, δ 157.22, δ 175.57, δ 182.53; LCMS (MM:ES+APCI) (M+H)+ 367.

**2-amino-4-(2-butyl-4-chloro-1H-imidazol-5-yl)-4H-benzo[g]chromene-3-carbonitrile (4e):** Brown Solid, IR νmax: 3389 cm-1 ν(NH2), 2152 cm-1 ν(CN), 1671 cm-1 ν(vinyl nitrile) , 725 cm-1 ν(C-Cl); 1H NMR (DMSO, 400 MHz) δ 0.799-0.903 (3H, -CH3, m), δ 1.235-1.299 (2H, -CH2-, m), δ 1.416-1.518 (2H, -CH2, m), δ 2.006-2.043 (2H, -CH2-, m), δ 2.083 (2H, -NH2, s), δ 4.486 (1H, Methine, s), δ 7.138-7.237 (2H, Ar-H, m), δ 7.405-7.578 (2H, Ar-H, m), δ 7.696-7.726 (2H, Ar-H, m), δ 9.819 (1H, -NH-, s); 13C NMR (CDCl3, 300MHz) δ 14.52, δ 21.77, δ 23.04, δ 31.79, δ 33.48, δ 58.33, δ 107.32, δ 115.83, δ 123.07, δ 124.20, δ 125.14, δ 125.69, δ 126.12, δ 127.43, δ 128.62, δ 129.77, δ 131.81, δ 133.04, δ 147.95, δ 155.57, δ 177.14; LCMS (MM:ES+APCI) (M+H)+ 379.

**2-amino-4-(2,6-dichlorophenyl)-4H-benzo[g]chromene-3-carbonitrile (4g):** Brown Solid; IR νmax: 3362 cm-1 ν(NH2), 2171 cm-1 ν(CN), 1707 cm-1 ν(vinyl nitrile) , 725 cm-1 ν(C-Cl); 1H NMR (DMSO, 400 MHz) δ 2.109 (2H, -NH2, s), δ 4.900 (1H, Methine, s), δ 7.056-7.120 (1H, Ar-H, m), δ 7.201-7.273 (1H, Ar-H, m), δ 7.365-7.421 (2H, Ar-H, m), δ 7.506-7.592 (2H, Ar-H, m), δ 7.607 (1H, Ar-H, s), δ 7.674-7.782 (1H, Ar-H, m), δ 7.803-7.910 (1H, Ar-H, m); 13C NMR (CDCl3, 300 MHz) δ 13.22, δ 56.15, δ 104.56, δ 118.24, δ 123.64, δ 124.85, δ 125.34, δ 126.08, δ 127.29, δ 127.74, δ 128.05, δ 128.99, δ 129.47, δ 130.10, δ 133.05, δ 135.00, δ 135.08, δ 143.89, δ 154.49, δ 177.63; LCMS (MM:ES+APCI) (M+H)+ 368.

**2-amino-4-(4-oxo-4H-chromene-3-yl)-7-hydroxy-chromene-3-carbonitrile (4k):** Pale red Solid; IR νmax: 3313 cm-1 ν(NH2), 2228 cm-1 ν(CN), 1707 cm-1 ν(C=O), 1655 cm-1 ν(vinyl nitrile); 1H NMR (DMSO, 400 MHz) δ δ 2.003 (2H, -NH2, s), δ 4.115 (1H, Methine, s), δ 5.184 (1H, -OH, s), δ 6.968-7.034 (1H, Ar-H, m), δ 7.071-7.183 (2H, Ar-H, m), δ 7.206-7.273 (2H, Ar-H, m), δ 7.309-7.354 (1H, Ar-H, m), δ 7.380-7.462 (1H, Ar-H, s), δ 7.491-7.565 (1H, Ar-H, m); 13C NMR (CDCl3, 300 MHz) δ 35.10, δ 56.61, δ 104.77, δ 114.23, δ 125.82, δ 126.26, δ 126.93, δ 127.43, δ 128.44, δ 130.32, δ 133.09, δ 135.10, δ 138.03, δ 140.36, δ 152.22, δ 153.85, δ 155.53, δ 177.23, δ 182.36; LCMS (MM:ES+APCI) (M+H)+ 333.

**2-amino-4-(2-butyl-4-chloro-1H-imidazol-5-yl)-7-hydroxy-chromene-3-carbonitrile (4l):**Brown Solid; IR νmax: 3506 cm-1 ν(OH), 3258 cm-1 ν(NH2), 2180 cm-1 ν(CN), 1646 cm-1 ν(vinyl nitrile) , 722 cm-1 ν(C-Cl); 1H NMR (DMSO, 400 MHz) δ 0.873-0.902 (3H, -CH3, m), δ 1.226-1.352 (2H, -CH2-, m), δ 1.499-1.555 (2H, -CH2, m), δ 2.000 (2H, -NH2, s), δ 2.451-2.470 (2H, -CH2-, m), δ 4.744 (1H, Methine, s), δ 5.672 ( 1H, -OH, s), δ 6.376 (1H, Ar-H, m), δ 6.497-6.535 (1H, Ar-H, m), δ 6.711-6.732 (1H, Ar-H, m), δ 11.876 (1H, -NH-, s); 13C NMR (CDCl3, 300MHz) δ 17.20, δ 24.94, δ 25.54, δ 31.22, δ 33.15, δ 58.81, δ 104.32, δ 106.95, δ 110.26, δ 118.50, δ 126.54, δ 130.05, δ 133.54, δ 143.56, δ 152.85, δ 153.63, δ 177.05; LCMS (MM:ES+APCI) (M+H)+ 345.

**2-amino-4-(4-oxo-4H-chromene-3-yl)-5-oxo-4,5-dihydropyrano [3,2-c]chromene-3-carbonitrile (4o):** Orange Solid, IR νmax: 2962 cm-1 ν(NH2), 2196 cm-1 ν(CN), 1673 cm-1 ν(C=O) ,1606cm-1 ν(vinyl nitrile); 1H NMR DMSO, 400 MHz:- δ 3.155 (1H, Methine, s), δ 6.836 (1H, Ar-H, s), δ 7.178-7.239 (1H, Ar-H, m), δ 7.381-7.421 (1H, Ar-H, m), δ 7.452-7.456 (1H, Ar-H, m), δ 7.470-7.473 (1H, Ar-H, m), δ 7.490-7.494 (1H, Ar-H, m), δ 7.570-7.591 (1H, Ar-H, m), δ 7.713-7.718 (1H, Ar-H, m), δ 7.731-7.735 (1H, Ar-H, m); 13C NMR (CDCl3, 300MHz) δ 18.11, δ 56.22, δ 95.24, δ 114.37, δ 116.29, δ 116.51, δ 116.96, δ 120.54, δ 122.15, δ 123.42, δ 125.49, δ 127.32, δ 129.85, δ 134.21, δ 148.33, δ 150.51, δ 156.28, δ 158.77, δ 159.01, δ 160.20, δ 182.11; LCMS (MM:ES+APCI) (M+H)+ 385.

**2-amino-4-(2-butyl-4-chloro-1H-imidazol-5-yl)-5-oxo-4,5-dihydropyrano [3,2-c]chromene-3-carbonitrile (4p):** Pale Yellow Solid, IR νmax: 3228 cm-1 ν(NH2), 2193 cm-1 ν(CN), 1718 cm-1 ν(C=O) ,1670cm-1 ν(vinyl nitrile); 1H NMR DMSO, 400 MHz:- δ 0.871-0.952 (3H, -CH3, m), δ 1.215-1.307 (2H, -CH2-, m), δ 1.506-1.581 (2H, -CH2-, m), δ 2.292-2.324 (2H, -CH2-, m), δ 4.570 (1H, Methine, s), δ 7.398-7.435 (1H, Ar-H, m), δ 7.447-7.523 (1H, Ar-H, m), δ 7.708-7.751 (1H, Ar-H, m), δ 7.883-7.903 (1H, -CH2-, m); 13C NMR (CDCl3, 300MHz) δ 15.32, δ 18.51, δ 23.45, δ 32.92, δ 34.27, δ 59.11, δ 106.33, δ 118.36, δ 118.97, δ 122.49, δ 124.08, δ 126.59, δ 127.82, δ 128.45, δ 136.14, δ 149.07, δ 151.22, δ 160.31, δ 161.25, δ 162.00; LCMS (MM:ES+APCI) (M+H)+ 397.

**2-amino-4-(2-methyl-1H-indol-3yl)-5-oxo-4,5-dihydropyrano [3,2-c]chromene-3-carbonitrile (4r):** Yellow Solid; IR νmax: 3352 cm-1 ν(NH2), 2217 cm-1 ν(CN), 1649 cm-1 ν(C=O) ,1567cm-1 ν(vinyl nitrile); 1H NMR DMSO, 300 MHz:- δ 3.35 (3H, -CH3, s), δ 4.15 (1H, Methine, s), δ 7.21-7.27 (2H, Ar-H, m), δ 7.29 (1H, Ar-H, m), δ 7.44-7.47 (1H, Ar-H, m), δ 7.78-7.90 (1H, Ar-H, m), δ 8.01-8.09 (1H, Ar-H, m), δ 8.10-8.31 (2H, Ar-H, m); 13C NMR (CDCl3, 300MHz) δ 12.52, δ 30.67, δ 57.65, δ 108.93, δ 109.77, δ 112.26, δ 116.61, δ 117.09, δ 117.12, δ 121.89, δ 122.02, δ 123.54, δ 124.33, δ 126.07, δ 127.4, δ 130.20, δ 132.85, δ 136.71, δ 151.29, δ 152.97, δ 161.36, δ 163.52; LCMS (MM:ES+APCI) (M-H)- 368.

**2-amino-4-(3,4-dimethoxyphenyl)-8,9-dimethyl-5-oxo-4,5-dihydropyrano [3,2-c]chromene-3-carbonitrile (4s):** White Solid; IR νmax:3323 cm-1 ν(NH2), 2195 cm-1 ν(CN), 1703 cm-1 ν(C=O) ,1668cm-1 ν(vinyl nitrile); 1H NMR DMSO, 300 MHz:- δ 2.33 (6H, -CH3, s), δ 3.71 (6H, -CH3, s), δ 4.37 (1H, Methine, s), δ 6.70-6.89 (2H, Ar-H, m), δ 7.28-7.32 (2H, Ar-H, m), δ 7.64 (1H, Ar-H, m); 13C NMR (CDCl3, 300MHz) δ 18.97, δ 19.77, δ 38.66, δ 56.47, δ 56.51, δ 57.22, δ 106.21, δ 115.25, δ 115.91, δ 116.93, δ 117.38, δ 120.55, δ 123.48, δ 126.34, δ 132.42, δ 136.21, δ 137.11, δ 145.21, δ 146.11, δ 148.71, δ 158.32, δ 160.13, δ 162.27; LCMS (MM:ES+APCI) (M+H)+ 405.

**2-amino-4-(4-(trifluoromethyl)phenyl)-8,9-dimethyl-5-oxo--4,5-dihydropyrano [3,2-c]chromene-3-carbonitrile (4t):** White Solid; IR νmax: 3324 cm-1 ν(NH2), 2215 cm-1 ν(CN), 1707 cm-1 ν(C=O) ,1668cm-1 ν(vinyl nitrile); 1H NMR DMSO, 300 MHz:- δ 3.34 (6H, -CH3, s), δ 4.37 (1H, Methine, s), δ 6.70 (1H, Ar-H, m), δ 6.72-6.73 (1H, Ar-H, m), δ 6.81 (1H, Ar-H, m), δ 7.21-7.24 (1H, Ar-H, m), δ 7.28-.7.32 (1H, Ar-H, m), δ 7.64 (1H, Ar-H, m); 13C NMR (CDCl3, 300MHz) δ 18.97, δ 19.77, δ 36.43, δ 55.46, δ 58.13, δ 103.06, δ 110.41, δ 111.45, δ 111.84, δ 116.88, δ 119.30, δ 119.53, δ 122.16, δ 133.17, δ 135.97, δ 142.76, δ 147.87, δ 148.87, δ 150.54, δ 153.27, δ 157.95, δ 159.79;LCMS (MM:ES+APCI) (M+H)+ 413.