Supporting information

Synthesis and biological screening of pyrano[3,2-c]quinoline analogues as anti-inflammatory and anticancer agents

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Experimental

All un(substituted) aldehydes and malononitrile were procured from commercially available sources and used directly without any further purification. The complete course of reaction was performed in an open reaction flask and monitored by thin layer chromatography (TLC) on pre-coated silica gel 60F 254 aluminum plates using ethyl acetate:hexane(1:1) as mobile phase. UV light and iodine or aqueous ceric ammonium molybdate solution or potassium permanganate stain followed by charring on a hot-plate were used as visualizing agents. Melting points of the synthesized compounds were recorded by open capillary method on controlled temperature (using standard Zeal's calibrated thermometer) and are uncorrected. 1H and 13C-NMR spectra were recorded on Bruker AC 300 MHz FT-NMR in DMSO-d6 using Trimethyl silane(TMS) as internal standard. Chemical shifts (δ) are expressed as ppm relative to TMS; whereas coupling constants (J) are in Hz. All IR spectra were recorded on Shimadzu FT-IR 8400 spectrometer using KBr pellet method. All FAB-MS spectra were recorded on JEOL SX 102/DA-6000.

General procedure for the synthesis of pyrano-[3,2-c]-quinolones 4(a-u) (1)

A reaction mixture of equal molar proportion of 2,4-dihydroxy-1-methylquinolin (1, scheme-1) with un(substituted) aromatic aldehydes (2, scheme-1) and malononitrile (3, scheme-1) along with catalytic amount of triethyl amine was refluxed in 10 part of absolute alcohol for 50 minutes. The reaction mixture was allowed to cool to room temperature; the precipitated product was collected by filtration and washed with a small quantity of absolute alcohol.

Anti-inflammatory and Cytotoxicity assay

THP-1 cell assay

The analogue library was screened for anti-inflammatory activity for inhibition of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) inhibitors in lipopolysaccharide stimulated THP-1 cells according to method described by Hwang C et. al (2). The THP-1 is a mature monocytic cell line which expresses all features of human monocyte/macrophage e.g., induction of proinflammatory cytokines via induction of CD14 receptor. These cells on stimulation with lipopolysaccharide express proinflammatory cytokines like TNF-α and IL-6. The inhibition of these inflammatory parameters is a strategy for therapy of several inflammatory disorders like rheumatoid arthritis, inflammatory bowel syndrome, psoriasis, etc. This assay is therefore used as a screening model for identifying small molecule inhibitors from natural resources.

The THP-1 cells were cultured in RPMI 1640 culture medium having 100 U/ml penicillin, 100µg/ml streptomycin and 10% fetal bovine serum. The cells were differentiated with phorbol myristate acetate. Following cell plating, the cells are treated with test compounds. At a final concentration of 1 µg/ml, LPS was added. Plates were incubated for 24h and 5% CO_2 at 37^{o}C. Supernatants were assayed for TNF- α and IL-6 by ELISA as instructed by the manufacturer (BD, Biosciences). The cells were also evaluated for cytotoxicity. Percent inhibition of cytokine release was calculated compared to the control. The 50% inhibitory concentration (IC50) values were calculated by a nonlinear regression method.

Human peripheral blood mononuclear cell (hPBMC) assay

TNF-α production by LPS in human peripheral blood mononuclear cells (hPBMC) was measured according to the method described by Henry et al.(3). Briefly, blood from healthy human donors was collected into Potassium EDTA vacutainer tubes. hPBMC were isolated using gradient centrifugation in Ficoll-Paque solution (Pharmacia). Isolated hPBMC were cultured in RPMI 1640 culture medium (Gibco BRL, Pasley, UK) containing 10% fetal bovine serum (FBS) (Hyclone, Utah, USA), 100 U/ml penicillin (Sigma Chemical Co. St Louis, Mo.) and 100 µg/ml streptomycin (Sigma Chemical Co. St Louis, Mo.). Concentration of the cell was adjusted to 1×10^6 cells/ml. The viability was ≧98% uniform as determined by trypan blue dye exclusion. 100 µg cell suspension was added to the wells of a 96-well culture plate. Followed cell plating, 79 µl of the culture medium and 1 µl of eight different concentrations of the test compounds (final concentration 0.03, 0.1, 0.3, 1, 3, 10, 30, 100 µM) dissolved in DMSO (dimethylsulfoxide, Sigma, Mo., USA) were added to the cells. The final concentration of DMSO was adjusted to 0.5%. The appropriate DMSO concentration was used as control. Rolipram (30 µM) was used as a standard compound. The plates were incubated for 30 min at 37° C. in an atmosphere of 5% CO2. Finally, 20 µl (10 µg/ml) per well of LPS, (Escherchia coli 0127:B8, Sigma Chemical Co., St. Louis, Mo.) was added, for a final concentration of 1 µg/ml. The plates were incubated at 37° C. for 4.5 h in an atmosphere of 5% CO2. To determine the cytotoxicity of the test compounds, the cell viability was assessed using MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfonyl)-2H-tetrazolium) reagent (Promega) after 4.5 h of incubation. Supernatants were harvested and assayed for TNF-α by ELISA as described by the manufacturer (R&D Systems, MN, BD Biosciences Pharmingen) or by cytotoxicity bioassay in L929 cells. The 50% inhibitory concentration (IC_{50}) values were calculated by a nonlinear regression method using GraphPad software (Prism 3.03).

Anticancer Activity assay

Anticancer screening for isolation of small molecules from natural resources with antiproliferative potential is conducted using cell lines from multiple cancer origins according to the method described by Dengler WA et. al (4). The cell lines we have used for these screens include Panc1 (pancreas), H460 (non-small cell lung carcinoma), Siha (cervix), ACHN (renal) and Calu-1 (lung).

Seed cells (at density, from 3000-7500 cells/well, depending on cell type) in 2000 µl in tissue culture grade 96 well plate and allow them to recover for 24 h in humidified 5% CO_2 incubator at 37°C (Note: If cells to be used grow in suspension, add compound immediately after cell seeding). Compound treatment is done with (dissolved first in DMSO) final DMSO concentration should not exceed 0.5% in wells and incubate plate for 48 h in humidified 5% CO_2 incubator at 37°C. The medium was removed after 48 h from the wells and added 25 µl of Propidium Iodide (50 µg/ml in water/medium) per well. The same plate was freezed at –80°C for 24 h. The plate was allowed to thawed at room temperature. The plate was read on Fluorometer (Polar-Star BMG Tech), using 530 nm Excitation and 620 nm Emission wavelength. (First Reading). The percent cytotoxicity was calculated by using the following formula:

$$\%\ \text{Cytotoxicity} = \frac{\text{Control} - \text{Treated cells}}{\text{Control}} \times 100$$

Spectral characterization of the pyrano-[3,2-c]-quinolones 4(a-u)

2-amino-6-methyl-5-oxo-4-phenyl-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile (4a)
m.p. 153-155°C; 1H NMR (DMSO-d6) δ 8.05(d, J=7.5Hz, 1H), 7.82(t, J=7.4 & 7.3Hz, 1H), 7.62(d, J=7.5Hz, 1H), 7.28(t, J=7.4 & 7.6Hz, 1H), 7.18-7.32(m, 5H), 4.62(s, 2H), 3.61(s, 3H), 2.28(s, 1H) 13C NMR (DMSO-d6) δ 161.2, 159.8, 153.1, 143.7, 140.5, 130.5, 128.6, 127.7, 123.2, 122.9, 120.8, 119.9, 116.8, 114.5, 56.6, 44.6, 140.5. IR (KBr pallet, cm^{-1}) 3392, 3153, 2900, 2204, 1678, 1629, 1598, 1579, 1452, 1380, 749. m/z $C_{20}H_{15}N_3O_2$ calculated 329.35 found (M+) 330.

2-amino-6-methyl-5-oxo-4-(2-chlorophenyl)-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile (4b)
m.p. 248-251°C; 1H NMR (DMSO-d6) δ 8.15(d, J=7.6Hz, 1H), 7.85(t, J=7.5 & 7.4Hz, 1H), 7.61(d, J=7.4Hz, 1H), 7.41(t, J=7.5 & 7.4Hz, 1H), 7.17-7.38(m, 4H), 4.41(s, 2H), 3.53(s, 3H), 2.39(s, 1H) 13C NMR (DMSO-d6) δ 161.2, 159.7, 152.7, 140.5, 133.4, 130.8, 130.5, 129.3, 128.9, 127.7, 123.2, 122.9, 119.8, 116.8, 114.5, 56.8, 40.6, 29.2. IR (KBr pallet, cm^{-1}) 3357, 3114, 2858, 2192, 1687, 1643, 1600, 1575, 1465, 1377, 758, 747. m/z $C_{20}H_{14}ClN_3O_2$ calculated 363.80 found (M+) 364.

2-amino-6-methyl-5-oxo-4-(3-chlorophenyl)-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile (4c)
m.p. 267-270°C; 1H NMR (DMSO-d6) δ 8.10(d, J=7.4Hz, 1H), 7.81(t, J=7.4 & 7.3Hz, 1H), 7.67(d, J=1.5Hz, 1H), 7.63(d, J=7.5Hz, 1H), 7.27(t, J=7.4 & 7.2Hz, 1H), 7.10-7.24(m, 3H), 4.43(s, 2H), 3.45(s, 3H), 2.28(s, 1H) 13C NMR (DMSO-d6) δ 161.2, 159.8, 153.1, 145.5, 140.5, 132.9, 130.5, 129.2, 128.3, 127.7, 126.4, 123.2, 122.9, 120.7, 119.9, 116.8, 114.5, 56.8, 44.1, 29.2. IR (KBr pallet, cm^{-1}) 3409, 3188, 2947, 2194, 1674, 1629, 1598, 1502, 1380, 889, 767. m/z $C_{20}H_{14}ClN_3O_2$ calculated 363.80 found (M+) 364.

2-amino-6-methyl-5-oxo-4-(4-chlorophenyl)-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile (4d)
m.p. 138-140°C; 1H NMR (DMSO-d6) δ 8.16(d, J=7.5Hz, 1H), 7.79(t, J=7.3 & 7.2Hz, 1H), 7.61(d, J=7.4Hz, 1H), 7.29(t, J=7.3 & 7.1Hz, 1H), 7.28(dd, J=7.3 & 1.3Hz, 2H), 7.13(dd, J=7.1 & 1.2Hz, 2H), 4.39(s, 2H), 3.37(s, 3H), δ 2.27(s, 1H). 13C NMR (DMSO-d6) δ 161.2,

159.8, 153.1, 141.7, 140.5, 131.6, 130.5, 128.8, 128.5, 123.2, 122.9, 120.8, 119.9, 116.8, 114.5, 56.6, 44.3, 29.2. IR (KBr pallet, cm^{-1}) 3313, 3188, 2942, 2185, 1674, 1625, 1590, 1503, 1460, 1379, 840, 767. m/z $C_{20}H_{14}ClN_3O_2$ calculated 363.80 found (M+) 364.

2-amino-6-methyl-5-oxo-4-(2-nitrophenyl)-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile (4e)
m.p. 262-264^oC; 1H NMR (DMSO-d6) δ 8.47(d, J=7.4Hz, 1H), 7.92(t, J=7.5 & 7.2Hz, 1H), 7.76(d, J=7.5Hz, 1H), 7.67(t, J=7.4 & 7.1Hz, 1H), 7.54-7.68(m, 4H), 4.59(s, 2H), 3.39(s, 3H), 2.31(s, 1H). 13C NMR (DMSO-d6) δ 13C 161.2, 159.7, 152.7, 148.4, 140.5, 137.3, 131.4, 130.5, 129.2, 128.6, 123.8, 123.2, 122.9, 119.5, 119.0, 116.8, 114.5, 56.3, 38.7, 29.2. IR (KBr pallet, cm^{-1}) 3433, 3190, 2885, 2200, 1685, 1637, 1600, 1577, 1379, 1355, 786. m/z $C_{20}H_{14}N_4O_4$ calculated 374.35 found (M+) 375.

2-amino-6-methyl-5-oxo-4-(3-nitrophenyl)-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile (4f)
m.p. 190-193^oC; 1H NMR (DMSO-d6) δ 8.63(d, J=1.5Hz, 1H), 8.43(d, J=7.5Hz, 1H), 7.87(t, J=7.4 & 7.3Hz, 1H), 7.63(d, J=7.5Hz, 1H), 7.35(t, J=7.4 & 7.2Hz, 1H), 7.27-7.24(m, 3H), 4.52(s, 2H), 3.47(s, 3H), 2.29(s, 1H) 13C NMR (DMSO-d6) δ 161.2, 159.8, 153.1, 148.0, 143.7, 140.5, 133.6, 130.5, 129.9, 123.3, 123.2, 122.9, 121.6, 120.7, 119.9, 116.8, 114.5, 56.5, 43.6, 29.2. IR (KBr pallet, cm^{-1}) 3328, 3172, 2868, 2191, 1676, 1611, 1596, 1525, 1463, 1386, 1350, 964. m/z $C_{20}H_{14}N_4O_4$ calculated 374.35 found (M+) 375.

2-amino-6-methyl-5-oxo-4-(4-nitrophenyl)-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile (4g)
m.p. 259-260^oC; 1H NMR (DMSO-d6) δ 8.52(d, J=7.4Hz, 1H), 7.81(t, J=7.4 & 7.2Hz, 1H), 7.79(d, J=7.1Hz, 1H), 7.59(dd, J=7.3 & 1.5Hz, 2H), 7.51(t, J=7.4 & 7.3Hz, 1H), 7.29(dd, J=7.2 & 1.1Hz, 2H), 4.65(s, 2H), 3.39(s, 3H), 2.29(s, 1H). 13C NMR (DMSO-d6) δ 161.2, 159.8, 153.1, 149.3, 146.7, 140.5, 130.5, 128.3, 123.8, 123.2, 122.9, 120.8, 119.9, 116.8, 114.5, 56.6, 44.6, 29.2. IR (KBr pallet, cm^{-1}) 3404, 3188, 2952, 2185, 1674, 1632, 1593, 1514, 1461, 1379, 1346, 821. m/z $C_{20}H_{14}N_4O_4$ calculated 374.35 found (M+) 375.

2-amino-6-methyl-5-oxo-4-(4-fluorophenyl)-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile (4h)
m.p. 88-89^oC; 1H NMR (DMSO-d6) δ 8.19(d, J=7.5Hz, 1H), 7.83(t, J=7.3 & 7.2Hz, 1H), 7.59(d, J=7.4Hz, 1H), 7.31(t, J=7.3 & 7.1Hz, 1H), 7.29(dd, J=7.3 & 1.3Hz, 2H), 7.11(dd, J=7.1 & 1.2Hz, 2H), 4.36(s, 2H), 3.38(s, 3H), 2.25(s, 1H). 13C NMR (DMSO-d6) δ 162.6, 161.2, 159.8, 153.1, 140.5, 139.0, 130.5, 129.3, 123.2, 122.9, 120.8, 119.9, 116.8, 115.7, 114.5, 56.6, 44.6, 29.2. IR (KBr pallet, cm^{-1}) 3313, 3183, 2895, 2185, 1674, 1629, 1596, 1502, 1380, 1126, 848. m/z $C_{20}H_{14}FN_3O_2$ calculated 347.34 found (M+) 348.

2-amino-6-methyl-5-oxo-4-(3-bromophenyl)-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile (4i)
m.p. 275-277^oC; 1H NMR (DMSO-d6) δ 8.10(d, J=7.4Hz, 1H), 7.81(t, J=7.4 & 7.3Hz, 1H), 7.67(d, J=1.5Hz, 1H), 7.63(d, J=7.5Hz, 1H), 7.27(t, J=7.4 & 7.2Hz, 1H), 7.10-7.24(m, 3H), 4.43(s, 2H), 3.45(s, 3H), 2.28(s, 1H). 13C NMR (DMSO-d6) δ 161.2, 159.8, 153.1, 146.3, 140.5, 132.0, 130.5, 129.7, 126.8, 123.2, 122.9, 122.7, 120.7, 119.9, 116.8, 114.5, 56.8, 43.8, 29.2. IR (KBr pallet, cm^{-1}) 3357, 3199, 2915, 2196, 1672, 1624, 1595, 1504, 1463, 1380, 848, 717. m/z $C_{20}H_{14}BrN_3O_2$ calculated 408.25 found (M+) 409.

2-amino-6-methyl-5-oxo-4-(3-phenoxyphenyl)-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile (4j)
m.p. 263-264^oC; 1H NMR (DMSO-d6) δ 8.01(d, J=7.8Hz, 1H), 7.12(t, J=7.5 & 7.2Hz, 1H), 7.58(d, J=8.7Hz, 1H), 7.36(t, J=7.5 & 8.1Hz, 1H), 7.42-7.24(m, 4H), 7.00-6.89(m, 5H), 4.53(s, 2H), 3.56(s, 3H), 2.38(s, 1H) 13C NMR (DMSO-d6) δ 161.2, 159.8, 156.7, 156.6, 153.1, 144.3, 140.5, 130.5, 129.9, 129.4, 124.2, 123.2, 122.9, 120.7, 119.9, 119.2, 118.4, 116.8, 114.5, 56.5, 44.1, 29.2. IR (KBr pallet, cm^{-1}) 3313, 3190, 2931, 2185, 1674, 1623, 1596, 1483, 1446, 1377, 1238, 829. m/z $C_{26}H_{19}N_3O_3$ calculated 421.45 found (M+) 422.

2-amino-6-methyl-5-oxo-4-(4-methylthiophenyl)-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile (4k)
m.p. 249-252^oC; 1H NMR (DMSO-d6) δ8.05(d, J=7.4Hz, 1H), 7.63(t, J=7.3 & 7.2Hz, 1H), 7.61(d, J=7.4Hz, 1H), 7.27(t, J=7.3 & 7.1Hz, 1H), 7.19(dd, J=7.3 & 1.3Hz, 2H), 7.11(dd, J=7.1 & 1.2Hz, 2H), 4.39(s, 2H), 3.37(s, 3H), 2.57(s, 3H), 2.27(s, 1H). 13C NMR (DMSO-d6) δ 161.2, 159.8, 153.1, 140.5, 139.1, 138.8, 130.5, 127.8, 127.5, 123.2, 122.9, 120.8, 119.8, 116.8, 114.5, 56.6, 44.8, 29.2. IR (KBr pallet, cm^{-1}) 3357, 3161, 2977, 2835, 2187, 1676, 1629, 1598, 1579, 1463, 1477, 833. 697. m/z $C_{21}H_{17}N_3O_2S$ calculated 375.44 found (M+) 376.

2-amino-6-methyl-5-oxo-4-(3-hydroxyphenyl)-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile (4l)
m.p. 283-285^oC; 1H NMR (DMSO-d6) δ 8.27(bs, 1H), 8.01(d, J=7.2Hz, 1H), 7.62(t, J=7.2 & 7.3Hz, 1H), 7.61(d, J=1.5Hz, 1H), 7.58(d, J=7.4Hz, 1H), 7.21(t, J=7.1 & 7.0Hz, 1H), 6.51-6.81(m, 3H), 4.31(s, 2H), 3.31(s, 3H), 2.28(s, 1H) 13C NMR (DMSO-d6) δ 161.2, 159.8, 157.2, 153.1, 145.3, 140.5, 130.5, 129.9, 123.2, 122.9, 120.7, 119.9, 119.4, 116.8, 115.6, 114.9, 114.5, 56.5, 44.2, 29.2. IR (KBr pallet, cm^{-1}) 3309, 3178, 2916, 2210, 1670, 1629, 1600, 1492, 1461, 1380, 1259, 854. m/z $C_{20}H_{15}N_3O_3$ calculated 345.35 found (M-) 345.

2-amino-6-methyl-5-oxo-4-(4-hydroxyphenyl)-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile (4m)
m.p. 129-132^oC; 1H NMR (DMSO-d6) δ 8.31(bs, 1H), 8.13(d, J=7.4Hz, 1H), 7.67(t, J=7.3 & 7.2Hz, 1H), 7.63(d, J=7.4Hz, 1H), 7.11(t, J=7.3 & 7.1Hz, 1H), 6.95(dd, J=7.3 & 1.3Hz, 2H), 6.67(dd, J=7.1 & 1.2Hz, 2H), 4.37(s, 2H), 3.33(s, 3H), 2.23(s, 1H). 13C NMR (DMSO-d6) δ 161.2, 159.8, 156.4, 153.1, 140.5, 133.2, 130.5, 129.9, 123.2, 122.9, 120.8, 119.9, 116.8, 115.5, 114.5, 56.6, 44.7, 29.2. IR (KBr pallet, cm^{-1}) 3328, 3139, 2916, 2191, 1668, 1611, 1595, 1512, 1400, 1382, 1255, 763. m/z $C_{20}H_{15}N_3O_3$ calculated 345.35 found (M-) 345.

2-amino-6-methyl-5-oxo-4-(4-N,N-dimethylphenyl)-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile (4n)
m.p. 278-280^oC; 1H NMR (DMSO-d6) δ 8.13(d, J=7.4Hz, 1H), 7.67(t, J=7.3 & 7.2Hz, 1H), 7.63(d, J=7.4Hz, 1H), 7.11(t, J=7.3 & 7.1Hz, 1H), 6.95(dd, J=7.3 & 1.3Hz, 2H), 6.67(dd, J=7.1 & 1.2Hz, 2H), 4.37(s, 2H), 3.33(s, 3H), 3.01(s, 6H), 2.23(s, 1H). 13C NMR (DMSO-d6) δ 161.2, 159.8, 153.1, 150.2, 140.5, 131.3, 130.5, 128.3, 123.2, 122.9, 120.8, 119.9, 116.8, 114.5, 111.9, 56.6, 43.7, 40.3, 29.2. IR (KBr pallet, cm^{-1}) 3358, 3127, 2922, 2852, 2190, 1675, 1591, 1565, 1520, 1399, 1363, 812. m/z $C_{22}H_{20}N_4O_2$ calculated 372.42 found (M+) 373.

2-Amino-6-methyl-5-oxo-4-(2-methoxyphenyl)-5,6-dihydro-4Hpyrano[3,2-c]quinoline-3-carbonitrile (4o)
m.p. 160-161^oC; 1H NMR (DMSO-d6) δ 8.01(d, J=7.8Hz, 1H), 7.71(t, J=7.8 & 7.2Hz, 1H), 7.58(d, J=8.4Hz, 1H), 7.39(t, J=7.5 & 8.1Hz, 1H), 7.29-6.75(m, 4H), 4.49(s, 2H), 3.70(s, 3H), 2.88(s, 3H), 2.24(s, 1H). 13C NMR (DMSO-d6) δ 161.2, 159.7, 157.8, 152.7, 140.5,

133.4, 131.5, 130.5, 129.6, 128.9, 123.2, 122.9, 121.6, 119.8, 119.0, 116.8, 114.5, 112.1, 56.9, 55.6, 38.4, 29.2. IR (KBr pallet, cm^{-1}) 3419, 3184, 2885, 2200, 1678, 1629, 1596, 1494, 1468, 1380, 1253, 748. m/z $C_{21}H_{17}N_3O_3$ calculated 359.38 found (M+) 360.

2-Amino-6-methyl-5-oxo-4-(4-methoxyphenyl)-5,6-dihydro-4Hpyrano[3,2-c]quinoline-3-carbonitrile (4p)
m.p. 239-242^oC; 1H NMR (DMSO-d6) δ 8.05(d, J=7.4Hz, 1H), 7.61(t, J=7.3 & 7.2Hz, 1H), 7.59(d, J=7.4Hz, 1H), 7.28(t, J=7.3 & 7.1Hz, 1H), 7.21(dd, J=7.3 & 1.3Hz, 2H), 7.13(dd, J=7.1 & 1.2Hz, 2H), 4.37(s, 2H), 3.67(s, 3H), 3.57(s, 3H), 2.26(s, 1H). 13C NMR (DMSO-d6) δ 161.2, 159.8, 157.2, 153.1, 140.5, 134.6, 130.5, 128.7, 123.2, 122.9, 120.8, 119.9, 116.8, 114.5, 113.8, 56.6, 55.3, 44.6, 29.2. IR (KBr pallet, cm^{-1}) 3352, 3163, 2953, 2853, 2185, 1672, 1629, 1598, 1508, 1436, 1379, 1251, 835. m/z $C_{21}H_{17}N_3O_3$ calculated 359.38 found (M+)360.

2-Amino-6-methyl-5-oxo-4-(3,4-dimethoxyphenyl)-5,6-dihydro-4Hpyrano[3,2-c]quinoline-3-carbonitrile (4q)
m.p. 251-253^oC; 1H NMR (DMSO-d6) δ 8.07(d, J=7.4Hz, 1H), 7.76(t, J=7.4 & 7.1Hz, 1H), 7.63(d, J=7.5Hz, 1H), 7.29(t, J=7.4 & 7.1Hz, 1H), 6.99(t, J=7.2 & 1.3Hz, 1H), 6.89(d, J=7.1, 1H), 6.80(d, J=1.1, 1H), 4.43(s, 2H), 3.71(s, 3H), 3.69(s, 3H), 3.57(s, 3H), 2.26(s, 1H). 13C NMR (DMSO-d6) δ 161.2, 159.8, 153.1, 149.1, 148.5, 140.5, 135.8, 130.5, 123.2, 122.9, 121.2, 120.7, 119.9, 116.8, 114.5, 112.1, 111.7, 56.5, 55.6, 44.1, 29.2. IR (KBr pallet, cm^{-1}) 3411, 3166, 2932, 2833, 2194, 1670, 1624, 1589, 1512, 1450, 1407, 1245, 1201, 858. m/z $C_{22}H_{19}N_3O_4$ calculated 389.40 found (M+) 390.

2-amino-4-(anthracen-9-yl)-6-methyl-5-oxo-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile (4r)
m.p. 236-239^oC; 1H NMR (DMSO-d6) δ 8.32(s, 1H), 8.05(d, J=7.5Hz, 1H), 7.82(t, J=7.4 & 7.3Hz, 1H), 7.62(d, J=7.5Hz, 1H), 7.28(t, J=7.4 & 7.6Hz, 1H), 7.18-7.40(m, 8H), 4.62(s, 2H), 3.61(s, 3H), 2.28(s, 1H). 13C NMR (DMSO-d6) δ 161.2, 159.5, 153.0, 140.5, 132.8, 132.0, 131.3, 130.5, 129.0, 128.4, 126.5, 126.1, 124.5, 123.2, 122.9, 119.9, 116.8, 114.5, 56.8, 44.5, 29.2. IR (KBr pallet, cm^{-1}) 3436, 3168, 2956, 2191, 1670, 1627, 1595, 1502, 1460, 1375, 756. m/z $C_{28}H_{19}N_3O_2$ calculated 429.47 found (M+) 430.

2-amino-4-(3-ethoxy-4-hydroxyphenyl)-6-methyl-5-oxo-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile (4s)
m.p. 203-205^oC; 1H NMR (DMSO-d6) δ 8.15(bs, 1H), 8.02(d, J=7.4Hz, 1H), 7.67(t, J=7.3 & 7.2Hz, 1H), 7.63(d, J=7.4Hz, 1H), 7.11(t, J=7.3 & 7.1Hz, 1H), 6.95(dd, J=7.3 & 1.3Hz, 2H), 6.67(d, J=1.2Hz, 1H), 4.37(s, 2H), 3.97(d, J=8.0Hz, 2H), 3.33(s, 3H), 2.23(s, 1H), 1.67(t, J=7.3 & 7.1Hz, 3H). 13C NMR (DMSO-d6) δ 161.2, 159.8, 153.1, 147.2, 146.9, 140.5, 133.5, 130.5, 123.2, 122.9, 121.2, 120.7, 119.9, 116.8, 115.1, 114.5, 112.7, 64.3, 56.5, 44.2, 29.2, 14.7. IR (KBr pallet, cm^{-1}) 3404, 3153, 2962, 2192, 1672, 1631, 1585, 1512, 1460, 1380, 1257, 1039, 947. m/z $C_{22}H_{19}N_3O_4$ calculated 389.40 found (M+) 390.

2-amino-4-(1H-indol-3-yl)-6-methyl-5-oxo-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile (4t)
m.p. 192-195^oC; 1H NMR (DMSO-d6) δ 8.69(s, 1H), 8.23(s, 1H), 8.19(d, J=7.5Hz, 1H), 7.67(t, J=7.4 & 7.3Hz, 1H), 7.63(d, J=7.5Hz, 1H), 7.35(t, J=7.4 & 7.2Hz, 1H), 7.06-7.31(m, 4H), 4.53(s, 2H), 3.36(s, 3H), 2.28(s, 1H). 13C NMR (DMSO-d6) δ 160.7, 159.6, 151.4, 140.5, 135.7, 130.5, 126.9, 123.2, 122.9, 122.4, 121.4, 120.2, 119.4, 119.0, 116.8, 114.5, 113.4, 112.1, 55.7, 38.6, 29.2. IR (KBr pallet, cm^{-1}) 3420, 3317, 3172, 2965, 1458, 2191, 1676, 1631, 1596, 1577, 1377, 1255, 754. m/z $C_{22}H_{16}N_4O_2$ calculated 368.39 found (M+) 369.

2-Amino-6-methyl-5-oxo-4-(3-methoxyphenyl)-5,6-dihydro-4Hpyrano[3,2-c]quinoline-3-carbonitrile (4u)
m.p. 216-218^oC; ^1H NMR (DMSO-d6) δ 8.02(d, J=7.8Hz, 1H), 7.71(t, J=7.8 & 7.2Hz, 1H), 7.58(d, J=8.4Hz, 1H), 7.39(t, J=7.5 & 8.1Hz, 1H), 7.29-6.75(m, 4H), 4.49(s, 2H), 3.70(s, 3H), 2.88(s, 3H), 2.32(s, 1H). ^{13}C NMR (DMSO-d6) δ 161.2, 159.8, 159.6, 153.1, 145.1, 140.5, 130.5, 129.2, 123.2, 122.9, 120.7, 120.6, 119.9, 116.8, 114.5, 113.0, 56.5, 55.3, 44.1, 29.2. IR (KBr pallet, cm^{-1}) 3344, 3172, 2945, 2200, 1691, 1637, 1601, 1581, 1436, 1380, 1093, 896. m/z $C_{21}H_{17}N_3O_3$ calculated 359.38 found (M+) 360.

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