Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supporting Information

Synthesis and cytotoxicity evaluation of regioisomeric substituted *N*-phenyl-3′-(chrom-4-one-3-yl)-isoxazolidines: induction of apoptosis through mitochondrial-dependent pathway

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Experimental

Synthesis

Materials and equipments: Starting materials and reagents were purchased from commercial suppliers and used after further purification (crystallization/ distillation). JEOL (300 MHz) NMR spectrometer, used to record ^{1}H and ^{13}C NMR (75 MHz) spectra and chemical shifts (δ) are reported in ppm from tetramethylsilane (TMS) used as the internal standard and coupling constant (*J*) values in Hertz. IR spectra were recorded on Shimadzu 8400S FT-IR spectrophotometer (KBr, cm^{-1}). Mass spectra, EI and ESI methods were recorded on Shimadzu GCMS-QP-2000A and Bruker Daltonics Esquire 300 mass spectrometers, respectively. Elemental Analyses were carried out on a Thermoelectron EA-112 elemental analyzer and are reported in percent atomic abundance. All melting points are uncorrected and measured in open glass- capillaries using Veego Precision Digital Melting Point Apparatus.

General method for synthesis of C-(chromon-3-yl)-N-phenylnitrone (5)

Substituted 3-formylchromone (3.0 g, 2.8mmol) was dissolved in dry benzene (30 ml) and to the clear solution, *N*–phenyl-hydroxylamine (4.08 g, 2.8mmol) was added and the contents were slightly heated and allowed to stand at room temperature. After 30 minutes, *N*-phenylnitrone (**9**) separated out as a light yellow solid, which was filtered. It was used immediately to prevent its rearrangement.

General procedure for reactions of N-phenylnitrone (5) with various dipolarphiles*:*

Reactions of *N*-phenylnitrones with monosubstituted dipolarophiles were carried out by mixing *N*-phenylnitrone (**5**, 0.75mmole) with dipolarophile (2.25mmole) in dry CH_2Cl_2 (40 ml) and the reaction mixture was stirred under the exclusion of moisture, until all the *N*-Phenylnitrone was consumed (TLC). The solvent was removed under vacuum and the residue was purified by column chromatography using 60-120 mesh silica and 1-8% EtOAc in hexane as eluent. The reactions with disubstituted (**9**)/cyclic dipolarophile (**11**) were carried out under same reaction conditions and products have been isolated by column chromatography. The reported yields are based on isolated pure products and products were characterized by spectroscopic techniques (^{1}H NMR, ^{13}C NMR, IR and Mass).

2',5'-diphenyl-3'-(6-flouro-chrom-4-one-3yl)-isoxazolidine (***7j***):

Yellow semisolid; yield (200 mg, 80%); **IR** ($CHCl_3$) ν_{max}: 1655.4, 1613, 1605, 1580, 1470, 1403, 1320, 1271, 1226, 1175, 1141, 1034, 987, 914, 857, 790, 700.6 cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.28 (s, 1H, C_2H), 7.74 (d, 1H, J = 6.3 Hz, C_5H), 7.35-6.83 (m, 7H, ArH), 5.04 (dist.d, 2H, J = 6 Hz,

$C_{3'}H$ and $C_{5'}H$), 3.33-3.24 (m, 1H, $C_{4'}Hb$), 2.16-2.03 (m, 1H, $C_{4'}H_a$); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 175.8(C_4), 161.1(C_6), 157.8(C_{8a}), 153.3(C_2), 151.4(q-Ar), 137.8(q-Ar), 129.1(CH-Ar), 128.5(CH-Ar), 128.2(CH-Ar), 126.7(CH-Ar), 125.1(C_{4a}), 125.0(C_3), 121.9(CH-Ar), 120.3(C_7), 120.2(C_8), 113.9(CH-Ar), 110.7(C_5), 80.1($C_{5'}$), 63.6($C_{3'}$), 45.3($C_{4'}$); **MS** (ESI) ***m/z***: 387 (M^+), Anal. calcd. For ($C_{24}H_{18}FNO_3$) C 74.4, H 4.68 and N 3.62% Found C 74.6, H 4.32 and N 3.33%.

2',5'-diphenyl-3'-(6-bromo-chrom-4-one-3yl)-isoxazolidine (***7k***):

Dark yellow semisolid; yield (212 mg, 85%); **IR** ($CHCl_3$) **ν_{max}** : 1653.7, 1615, 1607, 1585, 1495.5, 1470, 1410, 1350, 1320, 1271, 1226, 1200.7, 1175, 1149, 1102, 1034, 987, 956, 914, 859, 790, 764.7, 700.6 cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.29 (s, 1H, C_5H), 8.23 (d, 1H, J = 2.4 Hz, C_2H), 7.66 (d, 1H, J = 1.8 Hz, C_7H), 7.62 (d, 1H, J = 2.4 Hz, C_8H), 7.33-6.8 (m, Ar-H), 5.09-5.02 (m, 2H, $C_{3'}H$ and $C_{5'}H$), 3.30-3.26 (m, 1H, $C_{4'}Ha$), 2.14-2.09 (ddd, 1H, J_{gem} = 9 & J = 6.3, 3.3 Hz, $C_{4'}Hb$); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 175.0(C_4), 155.0(C_{8a}), 152.9(C_2), 151.0(q), 138.1(q), 137.0(C_7), 136.0(C_6), 128.7(CH), 128.1(CH), 127.8(CH), 126.3(CH), 125.3(C_{4a}), 125.0(C_5), 124.1(C_3),121.0(CH), 119.6(C_8), 113.5(CH), 79.7($C_{5'}$), 64.0($C_{3'}$), 45.0($C_{4'}$); **MS** (ESI) ***m/z*:** 447 (M^+), 449 (M^++2) Anal. calcd. For ($C_{24}H_{18}BrNO_3$) C 64.3, H 4.05 and N 3.12% Found C 64.6, H 4.21 and N 3.33%.

2',5'-diphenyl-3'-(6,8-chloro-chrom-4-one-3yl)-isoxazolidine (**7l**):

Pale yellow semi solid, yield (200 mg, 80%); **IR** ($CHCl_3$)- **ν_{max}** 3074, 1636,(CO), 1563.35, 1455.07, 1309.2, 722.7 cm^{-1}; **1H NMR** (300 MHz , $CDCl_3$): δ 8.19(s, 1H, C_2H), 8.04(d, 1H, J = 0.6 Hz, C_5H), 8.03(d, 1H, J = 0.9 Hz, C_7H), 7.6-7.4(m, Ar-Hs), 5.6(dd, 1H, J = 11.7, 5.1 Hz, $C_{5'}H$), 5.14(dd, 1H, J = 10.8 & 1.2 Hz, $C_{3'}H$), 3.19(ddd, 1H, J_{gem} = 12.2 & J = 11.7, 10.8 Hz, $C_{4'}Ha$), 2.39(ddd, 1H, J_{gem} = 12.2 & J = 5.1, 1.2 Hz, $C_{4'}Hb$), **^{13}C NMR** (75 MHz, $CDCl_3$): δ 189.37 (C_4), 171.9(C_{8a}), 157(C_2), 151.26(q), 147.8(C_7), 133.0(C_6),129.3(C_{4a}), 129.9(CH), 128.9(CH), 128.7(CH), 126.7(CH), 126.5(C_5), 125.5(C_3), 123.7(CH), 118.9(C_8), 115.04(CH), 99.3($C_{5'}$), 76.58(OCH_2), 63.92($C_{3'}$), 44.78($C_{4'}$); **MS** (ESI) *m/z*: 456(M+Na^+), 433(M^+), 431(M-2) Anal. calcd. For ($C_{24}H_{17}Cl_2NO_3$) C 65.77, H 3.91 and N 3.20% Found C 65.51, H 3.32 and N 3.01%.

5'-ethoxy-2'-phenyl-3'-(6-flouro-chrom-4-one-3yl)-isoxazolidine (***7m***):

Light yellow semisolid; yield (195 mg, 78%); **IR** ($CHCl_3$) ν_{max}: 1666.7, 1610.3, 1573.8, 1491.6, 1471.2, 1452, 1386, 1356, 1317, 1259, 1151, 1101 cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.22 (s, 1H, C_2H), 7.79 (d, 1H, J = 7.8 Hz, C_5H), 7.39 (dd, 1H, J = 9, 3.9 Hz, C_7H), 7.33-6.83 (m, Ar-H), 5.34 (dist.d, 1H, J = 5.4 Hz, $C_{5'}H$), 4.80 (dist.d, 1H, J = 9.6 Hz, $C_{3'}H$), 3.87 (dq, 1H, J = 16.8, 9.6 Hz,

OCH_2), 3.55 (dq, 1H, *J* = 16.8, 9.9 Hz, OCH_2), 2.80 (ddd, 1H, J_{gem} = 14 & *J* = 9.6, 5.4 Hz, C_4'Ha), 2.19 (d, 1H, J_{gem} = 14 Hz, C_4'Hb), 1.17 (t, 3H, *J* = 7.05 Hz, CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 176.0(C_4), 161.5(C_6), 157.1(C_{8a}), 155.1(C_2), 150.5(q-Ar), 128.8(CH-Ar), 126.0(C_{4a}), 124.2(C_3), 122.3(CH-Ar), 120.3(C_7), 120.2(C_8), 115.2(CH-Ar), 110.7(C_5), 101.9(C_5'), 63.7(OCH_2), 60.1(C_3'), 41.7(C_4'), 15.5(CH_3); **MS** (ESI) ***m/z***: 355 (M^+) Anal. calcd. For ($C_{20}H_{18}FNO_4$) C 67.6, H 5.11 and N 3.94% Found C 66.9, H 4.89 and N 3.39%.

5'-ethoxy-2'-phenyl-3'-(6-bromo-chrom-4-one-3yl)-isoxazolidine (***7n***):

Yellow semisolid; yield (200 mg, 80%); **IR** ($CHCl_3$) ν_{max}: 1645, 1600,1580, 1500, 1460, 1428, 1400, 1350, 1298, 1229, 1210, 1158, 1100, 1050, 970, 960 cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.26 (d, 1H, *J* = 1.8 Hz, C_2H), 8.21 (s, 1H, C_5H), 7.64 (dd, 1H, *J* = 8.8, 2.1 Hz, C_7H), 7.27 (d, 1H, *J* = 9 Hz, C_8H), 7.11-6.80 (m, Ar-H), 5.34 (d, 1H, *J* = 5.7 Hz, C_5'H), 4.79 (dist.dd, 1H, *J* = 8.5, 1.5 Hz, C_3'H), 3.81 (dist.dq, 1H, *J* = 15.4, 8.1 Hz, OCH_2), 3.49 (dist.dq, 1H, *J* = 15.3, 8.1 Hz, OCH_2), 2.76 (ddd, 1H, J_{gem} = 14.4 & *J* = 8.5, 5.7 Hz, C_4'Ha), 2.17 (dist.dd, 1H, J_{gem} = 14.4 & *J* =1.5 Hz, C_4'Hb), 1.14 (t, 3H, *J* = 7.2 Hz, CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 175.0(C_4), 155.2(C_{8a}), 155.0(C_2), 150.5(q), 136.3(C_7), 128.7(C_6), 128.6(CH), 125.4(C_5), 124.9(C_{4a}), 122.2(C_3), 120.0(CH), 118.5(C_8), 115.2(CH), 101.8(C_5'), 63.6(OCH_2-), 60.1(C_3'), 41.5(C_4'), 15.4(CH_3); **MS** (ESI) ***m/z***: 415 (M^+), 417 (M^++2) Anal. calcd. For ($C_{20}H_{18}BrNO_4$) C 57.71, H 4.36 and N 3.36% Found C 57.6, H 4.32 and N 2.78%.

5'-ethoxy-2'-phenyl-3'-(6,8-dichloro-chrom-4-one-3yl)-isoxazolidine (***7o***):

Light yellow semi solid, yield (216 mg , 86%); **IR** ($CHCl_3$) **ν_{max}**: 2924.6, 1635.3(CO), 1569.04, 1455.22, 1270.67, 1171.49, 1095.15, 756.2, 692.25cm^{-1}; **1H NMR** (300 MHz , $CDCl_3$): δ 8.21(s, 1H, C_2H), 8.12(d, 1H, *J* = 0.9 Hz, C_5H), 8.11(d, 1H, *J* = 0.9 Hz, C_7H), 7.60-7.1(m, Ar-Hs), 5.35(dd, 1H, *J* = 7.4, 3 Hz, C_5'H), 5.25(dd, 1H, *J* = 7.6, 2.2 Hz, C_3'H), 4.02(ddd, 1H, J_{gem} = 12.9 & *J* = 7.4, 7.6 Hz, C_4'Ha), 3.73 (dq, 1H, J_{gem} = 9.3 & *J* = 4.6 Hz, OCH_2), 3.52(dq, 1H, J_{gem} = 9.3 & *J* = 4.8 Hz, OCH_2), 3.19 (ddd, 1H, J_{gem} = 12.9 & *J* = 3, 2.2 Hz, C_4'Hb), 1.1(t, 3Hs, *J* = 6.9, CH_3); **^{13}C NMR** (75MHz, $CDCl_3$): δ 171.9(C_4), 157(C_{8a}), 131.6(C_7), 129.9(C_6), 129.5(C_5), 129.0(C_3), 126,8(C_2), 124.9(CH), 124.7(CH), 124.4(CH), 123.6(CH), 118.9(C_8), 107.3(C_5'), 63.8(OCH_2); **MS** (ESI) *m/z:* 424(M+Na^+) Anal. calcd. For ($C_{20}H_{17}Cl_2NO_4$) C 59.13, H 4.22 and N 3.45% Found C 58.6, H 4.02 and N 3.03%.

5'-(pyridin-4-yl)-2'-phenyl-3'-(chrom-4-one-3yl)-isoxazolidine (***7p***):

Off white solid, yield (215 mg , 85%); mp 72-74 ^{o}C**; IR** ($CHCl_3$)- ν_{max} 3382.86 , 3029.5, 2924.7 , 1739.06(CO) , 1643.7 , 1465.45 , 1405.6 , 1311.62 , 1170.2 , 1092.8 , 761.5 , 695.88 cm^{-1}; **^{1}H NMR** (300 MHz , $CDCl_3$): δ 8.34(s, 1H, C_2-H), 8.22(d, 1H, *J* =1.5 Hz, C_5-H), 7.69-7.65(m, 1H, C_7-H), 7.50-7.44(m, Ar-H), 5.20(dd, 1H, *J* = 8.8, 5.2, $C_{5'}$-H), 5.17(dd, 1H, *J* = 8.5, 5.7 Hz, $C_{3'}$-H), 3.43(ddd, 1H, J_{gem} = 12.6, *J* = 8.8, 8.5 Hz, $C_{4'}$-Ha), 2.24(ddd, 1H, J_{gem} = 12.6, *J* = 5.7, 5.2 Hz, $C_{4'}$-Hb); **^{13}C NMR** (75MHz, $CDCl_3$): δ 176.8(C_4), 156.6(C_{8a}), 153(C_2), 150.9(C_3), 147($C_{6''}$& $C_{2''}$ of pyridine ring), 133.7(C_7), 129.2($C_{3''}$ & $C_{5''}$ of pyridine ring), 125.72(C_6), 125.2(C_5), 124.99(CH), 123.86(CH), 122.37(CH), 121.09(CH), 118.3(C_8), 76.5($C_{5'}$), 63.5($C_{3'}$), 44.6($C_{4'}$); **MS** (ESI)***m/z:*** 392.9($M+Na^+$), 370(M^+) Anal. calcd. For ($C_{23}H_{18}N_2O_3$) C 74.58, H 4.90 and N 7.56% Found C 74.84, H 4.32 and N 7.13%.

5'-acetoxy-5'-methyl-2'-phenyl-3'-(6-flouro-chrom-4-one-3yl)-isoxazolidine ***(10a)****:*

Light yellow semisolid; yield (175 mg, 70%); **IR** ($CHCl_3$) ν_{max}: 1741, 1651.7, 1609, 1474.2, 1397.3, 1358.8, 1324.6, 1290.4, 1261, 1184, 1140, 1098, 1025, 970, 885, 870, 769 cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.20 (m, 1H, C_5H), 7.88 (m, 1H, C_7H), 7.49-6.86 (m, 7H, Ar-H), 5.12 (dist.d, 1H, *J* = 6.9 Hz, $C_{3'}$H), 3.62 (dd, 1H, J_{gem} = 12.9 & *J* = 8.4 Hz, $C_{4'}$Ha), 3.46 (s, 3H, OCH_3), 2.11 (dd, 1H, J_{gem} = 12.9 & *J* = 6.9 Hz, $C_{4'}$Hb), 1.58 (s, 3H, $C_{5'}CH_3$); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 173.2(C_4), 162.0(C_6), 157.2(C_{8a}), 154.2(C_2), 150.7(q-Ar), 129.1(CH-Ar), 127.3(C_{4a}), 125.3(C_3), 122.0(CH-Ar), 120.8(C_7), 120.7(C_8), 114.3(CH-Ar), 110.9(C_5), 84.2($C_{5'}$), 62.0($C_{3'}$), 52.6(OCH_3), 46.0($C_{4'}$), 23.2($C_{5'}CH_3$); **MS** (ESI) ***m/z***: 367 (M^+) Anal. calcd. For ($C_{21}H_{18}FNO_5$) C 65.79, H 4.73 and N 3.65% Found C 64.91, H 4.27 and N 3.07%.

5'-acetoxy-5'-methyl-2'-phenyl-3'-(6-bromo-chrom-4-one-3yl)-isoxazolidine (***10b***):

Dark yellow semisolid; yield (163 mg, 65%); **IR** ($CHCl_3$) ν_{max}: 1743.5, 1649.5, 1602.5, 1495.6, 1474.2, 1397.3, 1358.8, 1324.6, 1290.4, 1260.5, 1213.5, 1184, 1140, 1098, 1025, 970, 936, 897, 880, 858, 769 cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.35 (d, 1H, *J* = 2.4 Hz, C_5H), 8.19 (d, 1H, *J* = 0.9 Hz, C_2H), 7.75 (dd, 1H, *J* = 8.7, 2.7 Hz, C_7H), 7.35 (d, 1H, *J* = 5.7 Hz, C_8H), 7.30-6.86 (m, Ar-H), 5.11 (dist.t, 1H, *J* = 7.2 Hz, $C_{3'}$H), 3.61 (dd, 1H, J_{gem} = 12.9 & *J* = 8.1 Hz, $C_{4'}$Hb), 3.46 (s, 3H, OCH_3), 2.07 (dd, 1H, J_{gem} = 12.9 & *J* = 6.6 Hz, $C_{4'}$Ha), 1.56 (s, 3H, $C_{5'}CH_3$); **^{13}C NMR** (75 MHz,$CDCl_3$): δ 174.0(C_4), 172.3(-CO_2-), 155.9(C_{8a}), 153.6(C_2), 150.3(q), 136.0(C_7), 127.9(CH), 126.1(C_6), 125.6(C_5), 125.0(C_{4a}), 122.0(C_3), 120.9(CH), 118.5(C_8), 113.1(CH), 83.0($C_{5'}$), 61.7($C_{3'}$),

52.7(OCH_3), 45.9($C_{4'}$), 23.0($C_{5'}$-CH_3); **MS** (ESI) *m/z*: 427 (M^+), 429 (M^++2) Anal. calcd. For ($C_{21}H_{18}BrNO_5$) C 56.77, H 4.08 and N 3.15% Found C 55.96, H 3.73 and N 2.51%.

5'-acetoxy-5'-methyl-2'-phenyl-3'-(6-methyl-chrom-4-one-3yl)-isoxazolidine (***10c***):

Light yellow semisolid; yield (180 mg, 72%); **IR** ($CHCl_3$) ν_{max} : 1740, 1641.5, 1600, 1490, 1471, 1397, 1355.8, 1320.6, 1292, 1260, 1213.5, 1184, 1145, 1095, 1020, 975, 930, 890, 881, 858, 765 cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.16 (d, 1H, *J* = 1.2 Hz, C_2H), 7.9 (s, 1H, C_5H), 7.46 (dd, 1H, *J* = 8.4, 2.4 Hz, C_7H), 7.33 (d, 1H, *J* = 8.4 Hz, C_8H), 7.19-6.82 (m, 5H, Ar-H), 5.12 (dd, 1H, *J* = 7.5, 6.6 Hz, $C_{3'}$H), 3.61 (dd, 1H, J_{gem} = 12.9 & *J* = 7.5 Hz, $C_{4'}$-Hb), 3.46 (s, 3H, OCH_3), 2.49 (s, 3H, C_6-CH_3), 2.13 (dd, 1H, J_{gem} = 12.9 & *J* = 6.6 Hz, $C_{4'}$-Ha), 1.6 (s, 3H, $C_{5'}$-CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 175(C_4), 171.0(-CO_2-), 155.9(C_{8a}), 153.6(C_2), 150.3(q), 135.0(C_6), 134.1(C_7), 127.9(CH), 125.6(C_5), 125.0(C_{4a}), 122.0(C_3), 120.9(CH), 118.5(C_8), 113.1(CH), 83.0($C_{5'}$), 61.7($C_{3'}$), 52.7(-OCH_3), 45.9($C_{4'}$), 23.0($C_{5'}$-CH_3); **MS** (ESI) ***m/z***: 363 (M^+), 364 (M^++1) Anal. calcd. For ($C_{22}H_{21}NO_5$) C 69.64, H 5.58 and N 3.69% Found C 69.13, H 5.79 and N 3.97%.

3'-(6-Fluoro-chrom-4-one-3yl)-2,5-diphenyl-tetrahydro-pyrrolo[3,4-d]isoxazole-4,6 dione (***12a***):

Yellow solid; yield (190 mg, 76%); mp 172-174 ^{0}C; **IR** (KBr) ν_{max}: 1727, 1647.1, 1617, 1575, 1450.2, 1392, 1325, 1262, 1095, 1015, 862, 807, 755, 696.5, 615 cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.22 (s, 1H, C_2H), 7.81 (dd, 1H, *J* = 7.6, 3.3 Hz, C_7H), 7.44-6.74 (m, Ar-H), 5.85 (s, 1H, $C_{3'}$H), 4.88 (d, 1H, *J* = 7.5 Hz, $C_{5'}$H), 4.08 (d, 1H, *J* = 7.5 Hz, $C_{4'}$H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 176.9(C_4), 175.0(C=O), 174.9(C=O), 161.2(C_6), 156.9(C_{8a}), 156.0(C_2), 150.2(q-Ar), 136.7(q), 128.6(CH-Ar), 125.8(C_{4a}), 124.1(C_3), 122.6(CH-Ar), 121.0(C_7), 120.2(C_8), 115.6(CH-Ar), 110.1(C_5), 76.2($C_{5'}$), 70.7($C_{3'}$), 56.6($C_{4'}$); **MS** (ESI) *m/z*: 456 (M^+) Anal. calcd. For ($C_{26}H_{17}FN_2O_5$) C 68.42, H 3.75 and N 6.14% Found C 67.89, H 3.29 and N 5.67%.

3'-(6-Bromo-chrom-4-one-3yl)-2,5-diphenyl-tetrahydro-pyrrolo[3,4-d]isoxazole-4,6-dione (***12b***):

Yellow solid; yield (195 mg, 78%); mp 176-178 ^{0}C; **IR** (KBr) ν_{max} : 1724, 1643.2, 1614, 1595, 1454.2, 1392, 1315, 1261, 1095, 1018, 862, 800, 757.9, 696.5, 619 cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.27 (dd, 1H, *J* = 4.8, 2.1 Hz, C_7H), 8.21 (s, 1H, C_2H), 7.70-6.41 (m, Ar-H), 5.21 (s, 1H, $C_{3'}$H), 4.96 (d, 1H, *J* = 7.8 Hz, $C_{5'}$H), 4.11 (d, 1H, *J* = 7.8 Hz, $C_{4'}$H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 175.0(C_4), 174.1(C=O), 173.7(C=O), 154.9(C_{8a}), 152.6(C_2), 150.2(q), 134.7(C_6), 133.9(q), 133.1(C_7), 127.6(CH), 125.4(C_5), 124.0(C_{4a}), 121.7(C_3), 120.6(CH), 118.0(C_8), 113.1(CH), 75.6($C_{5'}$), 71.8($C_{3'}$), 54.1($C_{4'}$); **MS** (ESI) *m/z*: 517 (M^+), 519 (M^+ + 2) Anal. calcd. For ($C_{26}H_{17}BrN_2O_5$) C 60.36, H 3.31 and N 5.42% Found C 59.6, H 2.91 and N 4.73%.

3'-(6-Methyl-chrom-4-one-3yl)-2,5-diphenyl-tetrahydro-pyrrolo[3,4-d]isoxazole-4,6-dione (***12c***): Yellow solid; yield (200 mg, 80%); mp 170-172 ^{0}C; **IR** (KBr) ν_{max} : 1724, 1643.2, 1614, 1595, 1454.2, 1392, 1315, 1261, 1095, 1018, 862, 800, 757.9, 696.5, 619**;** **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.17 (s, 1H, C_2H), 7.91 (s, 1H, C_5H), 7.39-6.74 (m, Ar-H), 5.85 (s, 1H, $C_{3'}$H), 4.90 (d, 1H, *J* = 7.2 Hz, $C_{5'}$H), 4.11 (d, 1H, *J* = 7.2 Hz, $C_{4'}$H), 2.42 (s, 3H, C_6-CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 176.0(C_4), 173.4(C=O), 172.0(C=O), 154.9(C_{8a}), 152.6(C_2), 150.2(q), 135.1(C_6), 134.9(q), 134.5(C_7), 127.6(CH), 125.4(C_5), 124.0(C_{4a}), 121.7(C_3), 120.6(CH), 118.0(C_8), 113.1(CH), 76.6($C_{5'}$), 71.0($C_{3'}$), 56.1($C_{4'}$); **MS** (ESI) *m/z*: 452 (M^+) Anal. calcd. For ($C_{27}H_{20}N_2O_5$) C 71.67, H 4.46 and N 6.19% Found C 70.67, H 4.01 and N 5.33%.

Biological activity evaluation

***In Vitro* Cytotoxic activity**

For evaluating cytotoxicity the compounds were dissolved in DMSO and stock solution of 2×10^4 μM was prepared. Stock solutions were further diluted with complete growth medium supplemented with 50 μg/ml gentamicin to obtain required test concentrations. 5-Fluorouracil and paclitaxel were dissolved in DMSO and stock solution of 2×10^3μM was prepared. Mitomycin-C was dissolved in double distilled water and stock solution of 2×10^3 μM was prepared. Stock solutions were further diluted with complete growth medium supplemented with 50μg/ml gentamicin to obtain desired concentrations. All the cells were maintained in RPMI-1640 medium, supplemented with fetal bovine serum (10%), 100 units/ml penicillin and 100μg/ml streptomycin (complete medium). Viability of cells was evaluated by trypan blue exclusion method immediately before setting up the experiment for cytotoxicity determination. Cells with >98% viability were used in the assay. The cells were seeded into 96 well cell culture plates (1×10^4 cells/100 μl/well) and incubated in CO_2 incubator (37ºC, 5% CO_2, 95% relative humidity) for 24 hours. After 24 hours, compounds **7a-p**, **10a-c**, **12a-c** and positive controls (100 μl/well) were added in quadruplets and the plates were further incubated in CO_2 incubator for 48 hours. Suitable controls were also included in each experiment. After 48 hours chilled trichloroacetic acid (50% w/v, 50 μl/well) was laid gently on top of the medium in all the wells. The plates were incubated at 4^{o}C for one hour to fix the cells. All the contents of the wells were gently pipette out and discarded. The plates were washed five times with distilled water to remove trichloroacetic acid, growth medium, low molecular weight metabolites and serum proteins etc. The plates were air-dried. Sulphorhodamine-B (0.4% SRB in 1% acetic acid, 100 μl/well) was added to each well of the 96 well plates and plates were incubated at room

temperature for 30 min. Excess of the dye was washed off using 1% acetic acid and the plates were air-dried. Tris buffer (10mM, pH 10.5, 100 µl/well) was added to each well and plates were shaken on a mechanical stirrer for 10 min and O. D. was recorded on ELISA reader at 540 nm.

Fluorescence microscopic studies:

The use of fluorescence microscope has been advocated for the accurate assessment of mechanism of cell death in human cancer cells. To ascertain the mechanism of cell death, human promyelocytic leukemia cell line HL-60 was used. Standard culture conditions were used. The cell cultures were grown in a CO_2 incubator at 37° C with 90% humidity and 5% CO_2 gas environment. HL-60 cells were seeded in 6-well tissue culture plates at the density of 2×10^5 cells per ml in complete medium supplemented with 10% FCS in the presence and absence (as controls) of **10c** at 30, 50 and 100 µM concentrations for 24 h. Camptothecin 1 µM for 6 h was used as a positive control. To assess the mechanism of cell death, after incubation for specified time at 37°C, the cells were processed for fluorescence microscopic studies. For fluorescent DAPI staining, air dried smears of HL-60 cells were fixed in methanol at-20°C for 20 min, air dried and stained with DAPI at 1µg/ml in PBS at room temperature for 20 min in the dark and the slides were mounted in glycerol–PBS (1:1) and examined in an inverted fluorescence microscope (Olympus, 1X81).

DNA fragmentation test:

DNA fragmentation, which is a typical hallmark of apoptosis, was assessed by electrophoresis of extracted genomic DNA from HL-60 cells as described previously with some modifications.[21] HL-60 cells in the log phase were cultured (37°C, 5% CO_2) in RPMI medium (pH 7.2) in a CO_2 incubator. Overall, 2×10^6 cells after various treatments (10, 30, 50 and 100µM) were incubated for 24 hours and then washed in PBS containing 10 mM EDTA. The pellet was lysed in 250 µL of lysis buffer (100 mM NaCl, 5 mM EDTA, 10 mM Tris–HCl, pH 8.0, 5% Triton X-100, 0.25% SDS) containing 400 µg/mL DNase-free RNase and incubated at 37°C for 90 minutes followed by 1-hour incubation with proteinase-K (200 µg/mL) at 50°C for 1 hour. The DNA was extracted with 200 µL of phenol:chloroform:isoamyl alcohol (25:24:1) mixture for 1 minute and centrifuged at 13000 × *g* for 3 minutes. The aqueous phase was further extracted with chloroform and centrifuged. DNA was precipitated from aqueous phase with 3 volumes of chilled alcohol containing 0.3M sodium acetate at 4°C overnight. The precipitate was centrifuged at 13000 × *g* for 10 minutes. The DNA pellet was washed in 80% alcohol, dried, dissolved in 50µL TE buffer, and electrophoresed in 1.6% agarose gel at 50 V, stained with ethidium bromide, and visualized in Bio-Rad gel documentation system.

Cell Cycle Phase Distribution:

Cell cycle phase distribution was done as described earlier.[22] Briefly, HL-60 cells (1×10^6/mL) in exponential phase of growth were seeded in 6-well plates and allowed to adhere for 24 hours. The old medium was replaced by fresh medium without and with compound **10c** in different concentrations (10, 30, 50 and 100 μM) and further incubated for 24 hours. Cells were trypsinised, centrifuged, and washed with PBS. Cells were fixed in 70% ethanol, washed with PBS, subjected to proteinase and RNase digestion (37°C for 30 minutes), followed by staining of clean nuclear materials (nuclei) with PI (25 μg/mL) using procedures and reagents as described by manufacturer in the Cycle Test plus DNA reagent kit (Becton-Dickinson, Franklin Lakes, NJ). The preparations were analyzed for DNA content using BD-FACS Caliber flow cytometer. Data were collected in list mode on 10000 events for FL2-A versus FL2.

Loss of Mitochondrial Membrane Potential:

Changes in mitochondrial transmembrane potential ($\Delta\Psi m$) as a result of mitochondrial perturbation were measured after staining with Rh-123. Cells (1×10^6/1.5 mL/12-well plates) were treated with the test compound **10c** and incubated for 24 hours. Rh-123 (5 μM) was added 1 hour before the termination of the experiment. Cells were collected and washed in PBS. The decrease in fluorescence intensity because of mitochondrial membrane potential loss was analyzed in FL-1 channel on flow cytometer. Intensity of Rh-123 is directly related to mitochondrial membrane potential.