Supporting Information

An efficient synthesis of 1,3-dimethyl-5-(2-phenyl-4H-chromen-4-ylidene)pyrimidine-2,4,6(1H,3H,5H)-triones and investigation of their interactions with β-lactoglobulin

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Instrumentation

Standard literature procedures were used to dry the solvents used in the experiments. An oven-dried round-bottomed flask was used to perform the reactions and they were conducted under the atmospheric oxygen. Thin-layer chromatography plates (Silica gel G) were visualized by exposure to ultraviolet light and/or iodine vapor. IR spectra were recorded on a Perkin Elmer FT-IR Spectrophotometer (Spectrum BX II) as KBr pellets. $^1$H NMR and $^{13}$C NMR spectra were recorded in CDCl$_3$ on a Bruker 300MHz or a Bruker 500MHz NMR spectrometer. Chemical shifts (δ) were reported in parts per million (ppm) taking CHCl$_3$ peak at δ 7.26. Coupling constants (J) are quoted in hertz (Hz). Mass spectra were recorded on a Jeol MS Station 700 mass spectrometer by the electron spray ionization (ESI). Elemental analyses were done using two Perkin-Elmer 2400 Series II C, H, N analyzers.

Materials

2-Hydroxychalcones and 1,3-dimethylbarbituric acid were used as starting materials for the synthesis of the title compounds. 2-Hydroxychalcones were synthesized by base catalyzed condensation of 2-hydroxybenzaldehydes and acetophenones$^1$, and 1,3-dimethylbarbituric acid was commercially available (TCI chemicals). TLC was done with silica gel G. Starting materials used in the reaction were commercially available.

General Procedures for Synthesis of 1,3-Dimethyl-5-(2-phenyl-4H-chromen-4-ylidene)pyrimidine-2,4,6(1H,3H,5H)-triones (6)

Solution of 2-hydroxychalcone (1) (1 mmol) and 1,3-dimethyl-barbituric acid (1 mmol) in anhydrous toluene (10 mL) was added amberlyst-15 (40 mg) at room temperature. The resulting mixture was refluxed with stirring under atmospheric oxygen for 8 h. After completion of reaction, sufficient amount of dichloromethane was added to dissolve the product and then amberlyst-15 was filtered off. The filtrate was concentrated by removal of solvent and the resulting crude product was purified by crystallization from DCM hexane solvent system.

Physical and Spectral data of 1,3-Dimethyl-5-(2-phenyl-4H-chromen-4-ylidene)pyrimidine-2,4,6(1H,3H,5H)-triones (6) and related compounds

1,3-Dimethyl-5-(2-phenyl-4H-chromen-4-ylidene)pyrimidine-2,4,6(1H,3H,5H)-trione (6a):

Red crystals (yield: 75 %), m.p. 171-172 °C, IR (KBr pellet): 1700, 1630, 1544, 1467, 1440, 1401, 1358, 1129, 1060, 861, 765 cm⁻¹, ¹H NMR (300 MHz, CDCl₃): δ 3.43 (6H, s, 2 × >NCH₃), 7.43 ( 1H, t, J=8.2 Hz), 7.56-7.61 (3H, m, Ar-H), 7.67 (1H, d, J=8.4 Hz), 7.78 (1H, t, J=8.4 Hz), 8.05-8.11 (3H, m, Ar-H), 9.13 (1H, s, H-3 of 2-chromene moiety). ¹³C NMR (75 MHz, CDCl₃): δ 28.4, 99.6, 111.9, 118.0, 121.4, 124.8, 127.2, 129.4, 131.0, 132.7, 135.1, 152.0, 154.8, 161.6, 162.6, 162.8. ESMS: m/z calculated for C₂₁H₁₇N₂O₄ [M + H]⁺: 361.1188, found 361.1180. Anal. Calcd for C₂₁H₁₆N₂O₄: C, 69.99; H, 4.48; N, 7.77. Found: C, 70.13; H, 4.67; N, 7.52.

1,3-Dimethyl-5-(2-p-tolyl-4H-chromen-4-ylidene)pyrimidine-2,4,6(1H,3H,5H)-trione (6b):

Red crystals (yield: 63 %), m.p. 175-176 °C, IR (KBr pellet): 1699, 1630, 1544, 1509, 1463, 1440, 1356, 1279, 1192, 1128, 1057, 763, 721, 615; ¹H NMR (300 MHz, CDCl₃): δ 2.47 (3H, s, CH₃), 3.43 (6H, s, 2 × >NCH₃), 7.37 (2H, d, J = 8.1Hz, Ar-H), 7.42 (1H, t, J = 7.5Hz, Ar-H), 7.66 (1H, d, J = 8.7Hz, Ar-H), 7.78 (1H, br. t, J=7.2 Hz Ar-H), 8.00 (2H, d, J =8.2 Hz, Ar-H), 8.05 (1H, br. d, J = 8.4 Hz, Ar-H), 9.07 (1H, s, H-3 of 2-chromene moiety). ¹³C NMR (75 MHz, CDCl₃): δ 21.7, 28.3, 98.9, 111.8, 118.0, 121.5, 124.8, 127.3, 128.1, 130.2, 131.8, 135.0, 144.0, 152.1, 154.7, 162.2, 162.9; Anal. Calcd for C₂₂H₁₈N₂O₄: C, 70.58; H, 4.85; N, 7.48. Found: C, 70.72; H, 5.02; N, 7.61.

5-(2-(4-Methoxyphenyl)-4H-chromen-4-ylidene)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione (6c)

Red crystals (yield: 81 %), m.p. 199-200 °C ¹H NMR (500 MHz, CDCl₃): δ 3.42 (6H, s, 2 × >NCH₃), 3.92 (3H, s, OCH₃), 7.05 (2H,d, J=8.5 Hz, H-3', 5'), 7.42 (1H, t, J=8.0 Hz, H-6), 7.65 (1H, d, J=8.5 Hz, H-8), 7.76 (1H, t, J=7.5 Hz, H-7), 8.05 (1H, d, J=8.5 Hz, H-5), 8.08 (2H,d, J=8.5 Hz, H-2', 6'), 9.00 (1H, s, H-3 of 2-chromene moiety). ¹³C NMR (75 MHz, CDCl₃): δ 28.3, 55.7, 98.2, 111.5, 115.0, 117.9, 121.6, 123.1, 124.8, 129.5, 131.8, 134.8, 152.2, 154.6, 162.3, 162.9, 163.8; ESMS: m/z calculated for C₂₂H₁₉N₂O₅ [M + H]⁺: 391.1294, found 391.1214. Anal. Calcd for C₂₂H₁₉N₂O₅: C, 67.69; H, 4.65; N, 7.18. Found: C, 67.49; H, 4.79; N, 7.03.

5-(2-(4-Chlorophenyl)-4H-chromen-4-ylidene)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione (6d):

Red crystals (yield:60%), m.p. 217-218 °C, ¹H NMR (300 MHz, CDCl₃): δ 3.42 (6H, s, 2 × >NCH₃), 7.42 (1H, t, J=6.9 Hz), 7.53 (2H, d, J=8.7 Hz, H-3' & H-5'), 7.64 (1H, d, J=8.1Hz, Ar-H), 7.78 (1H, t, J=7.5 Hz), 8.01-8.05 (3H, m, Ar-H), 9.12 (1H, s, H-3 of 2-chromene moiety). ¹³C NMR (75 MHz, CDCl₃): δ 28.5, 100.4, 111.6, 119.5, 122.3, 127.2, 129.4, 130.4, 130.7, 130.9, 132.9, 134.9, 151.8, 153.1, 160.8, 161.5, 162.7. Anal. Calcd for C₂₁H₁₅ClN₂O₄: C, 63.89; H, 3.83; N, 7.10. Found: C, 64.03; H, 3.76; N, 6.81.
5-(6-Chloro-2-phenyl-4H-chromen-4-ylidene)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione (6e):

Red crystals (yield: 72%), m.p. 215-206 °C, IR (KBr pellet): 2920, 2850, 1701, 1649, 1627,1515, 1467, 1366, 1262, 1192, 1120, 1022, 836, 753, 702, 641 cm⁻¹; 
¹H NMR (300 MHz, CDCl₃): δ 3.42 (6H, s, 2 × >NCH₃), 7.54-7.61 (4H, m, Ar-H), 7.69 (1H, dd, J=8.0 & 2.4 Hz, H-7), 8.01 (1H, d, J=2.4 Hz, H-5), 8.06 (2H, dd, J=8.2 & 2 Hz, H-2’& H-6’), 9.16 (1H, s, H-3 of 2-chromene moiety). 
¹³C NMR (75 MHz, CDCl₃): δ 28.5, 99.6, 111.8, 118.0, 119.4, 125.0, 128.3, 129.5, 129.6, 131.9, 135.4, 139.1, 153.0, 154.8, 160.0, 161.3, 163.1. Anal. Calcd for C₂₁H₁₅ClN₂O₄: C, 63.89; H, 3.83; N, 7.10. Found: C, 63.61; H, 3.67; N, 7.03.

5-(6-Chloro-2-p-tolyl-4H-chromen-4-ylidene)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione (6f):

Red crystals (yield: 69%), m.p. 203-204 °C; 
¹H NMR (300 MHz, CDCl₃): δ 2.46 (3H, s, CH₃), 3.42 (6H, s, 2 × >NCH₃), 7.36 (2H, d, J=9 Hz, H-3’, 5’), 7.58 (1H, d, J=9 Hz, H-8), 7.68 (1H, dd, J=9.0 & 2.3 Hz, H-7), 7.96 (2H, d, J=8.2 Hz, H-2’, 6’), 8.01 (1H, d, J=2.3 Hz, H-5), 9.10 (1H, s, H-3 of 2-chromene moiety). 
¹³C NMR (75 MHz, CDCl₃): δ 21.7, 28.4, 99.7, 111.4, 119.4, 122.4, 127.3, 127.8, 130.2, 130.4, 130.9, 134.8, 144.2, 151.9, 153.0, 161.0, 162.0, 162.7. ESMS: m/z calculated for C₂₂H₁₈ClN₂O₄ [M + H]⁺: 409.09, found 409.39

5-(6-Chloro-2-(4-methoxyphenyl)-4H-chromen-4-ylidene)-1,3-dimethylpyrimidine-2,4,6 (1H,3H,5H)-trione (6g):

Red crystals (yield: 80%), m.p. 209-210 °C; 
¹H NMR (300 MHz, CDCl₃): δ 3.42 (6H, s, 2 × >NCH₃), 3.92 (3H, s, OCH₃), 7.03 (2H, d, J=9.0 Hz, H-3’, 5’), 7.58 (1H, d, J=9 Hz, H-8), 7.67 (1H, dd, J=9 & 2.4 Hz, H-7), 8.00 (1H, br.s, H-5), 8.05 (2H, d, J=9 Hz, H-2’, 6’), 9.03 (1H, s, H-3 of 2-chromene moiety). 
¹³C NMR (75 MHz, CDCl₃): δ 28.4, 55.7, 99.6, 111.2, 115.0, 119.3, 122.4, 122.8, 129.4, 130.4, 130.9, 134.7, 152.0, 152.9, 161.0, 162.2, 163.9. Anal. Calcd for C₂₂H₁₇ClN₂O₅: C, 62.20; H, 4.03; N, 6.59. Found: C, 62.07; H, 4.18; N, 6.68.

5-(2-(4-Methoxyphenyl)-6-methyl-4H-chromen-4-ylidene)-1,3-dimethylpyrimidine-2,4,6 (1H,3H,5H)-trione (6h):

Red crystals (yield: 83%), m.p. 217-218 °C, IR (KBr pellet): 1699, 1628, 1551, 1515, 1430, 1466, 1363, 1262, 1224, 1120, 1025, 904, 838, 862, 804, 776, 754, 719, 643 cm⁻¹; 
¹H NMR (300 MHz, CDCl₃): δ 2.47 (3H, s, CH₃), 3.42 (6H, s, 2 × >NCH₃), 3.92 (3H, s, OCH₃), 7.04 (2H,d, J=9.0 Hz, H-3’, 5’), 7.56 (2H, br.s, H-7 & H-8), 7.82 (1H, br.s, H-5), 8.07(2H,d, J=8.9 Hz, H-2’, 6’), 8.93 (1H, s, H-3 of 2-chromene moiety). 
¹³C NMR (75 MHz, CDCl₃): δ 21.4, 28.3, 55.7, 97.7, 111.9, 114.9, 117.6, 121.5, 123.1, 129.4, 131.0, 134.9, 136.5, 152.3, 153.1, 162.2, 162.8, 136.0, 163.7 Anal. Calcd for C₂₃H₂₀N₂O₅: C, 68.31; H, 4.98; N, 6.93. Found: C, 68.14; H, 4.88; N, 7.11.
1,3-Dimethyl-5-(6-methyl-2-p-tolyl-4H-chromen-4-ylidene)pyrimidine-2,4,6(1H,3H,5H)-trione (6i):

Red crystals (yield: 78%), m.p. 181-182 °C, IR: 1701, 1630, 1546, 1546, 1511, 1358, 1275, 1192, 1131, 1054, 816, 778, 764, 719, 643; \(^1\)H NMR (300 MHz, CDCl\(_3\)): δ 2.46 (3H, s, CH\(_3\)), 2.48 (3H, s, CH\(_3\)), 3.42 (6H, s, 2 × >NCH\(_3\)), 7.36 (2H, d, J=7.8 Hz, H-3', 5'), 7.58 (2H, s, H-7,8), 7.82 (1H, s, H-5), 7.98 (2H, d, J=7.8 Hz, H-2', 6'), 9.00 (1H, s, H-3 of 2-chromene moiety), \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): δ 21.4, 21.7, 28.3, 98.3, 112.1, 117.7, 121.5. 127.3, 128.1, 130.1, 131.0, 134.9, 136.7, 143.9, 152.2, 153.2, 162.1, 162.8, 163.1 ESMS: m/z calculated for C\(_{23}\)H\(_{20}\)N\(_2\)O\(_4\) [M + H]\(^+\): 389.1501, found 389.1541.

5-(8-Methoxy-2-phenyl-4H-chromen-4-ylidene)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione (6j):

Red crystals (yield: 81%), m.p. 219-220 °C, IR: 2923, 2852, 1692, 1550, 1501, 1402, 1450682, 701, 723, 755, 775, 802, 863, 1068, 1140, 122874, 1353; \(^1\)H NMR (300 MHz, CDCl\(_3\)): δ 3.42 (6H, s, 2 × >NCH\(_3\)), 4.06 (3H, s, OCH\(_3\)), 7.22 (1H, d, J=7.9 Hz, H-7), 7.32 (1H, t, J=8.4 Hz, H-6), 7.53-7.59 (4H, m, Ar-H), 8.14(2H, dd, J= 7.8 & 2.0 Hz, H-2', 6'), 9.14 (1H, s, H-3 of 2-chromene moiety). ESMS: m/z calculated for C\(_{22}\)H\(_{18}\)N\(_2\)O\(_5\) [M + H]\(^+\): 391.12, found 391.20.

5-(8-Methoxy-2-p-tolyl-4H-chromen-4-ylidene)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione (6k):

Red crystals (yield: 85%), m.p. 231-232 °C, \(^1\)H NMR (300 MHz, CDCl\(_3\)): δ 2.46 (3H, s, CH\(_3\)), 3.41 (6H, s, 2 × >NCH\(_3\)), 4.05 (3H, s, OCH\(_3\)), 7.20-7.37 (4H, m, Ar-H), 7.54 (1H, d, J=8.0 Hz, H-5), 8.04 (2H, d, J= 8.1Hz, H-2', 6'), 9.08 (1H, s, H-3 of 2-chromene moiety), ESMS: m/z calculated for C\(_{23}\)H\(_{20}\)N\(_2\)O\(_4\) [M + H]\(^+\): 405.13, found 405.11.

1,3-Dimethyl-5-(2-(thiophen-2-yl)-4H-chromen-4-ylidene)pyrimidine-2,4,6(1H,3H,5H)-trione (6l):

Red crystals (yield: 25%), m.p. 243-244 °C, \(^1\)H NMR (300 MHz, CDCl\(_3\)): δ 3.42 (6H, s, 2 × >NCH\(_3\)), 7.26-7.28 (1H, m, ), 7.41 (1H, t, J=7.5 Hz, Ar-H), 7.61 (1H, d, J = 8.1 Hz), 7.72-7.76 (2H, m, Ar-H), 7.96 (1H, d, J = 3.6 Hz, H-5 of thiophene moiety), 8.01 (1H, d, J = 8.3 Hz, Ar-H), 8.93 (1H, s, H-3 of 2-chromene moiety) \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): δ28.5, 99.1, 111.3, 118.0, 121.6, 124.9, 129.4, 131.0, 132.0, 133.1, 135.1, 152.5, 154.3, 158.2, 162.1, 162.9. HRMS: m/z calculated for C\(_{19}\)H\(_{15}\)N\(_2\)O\(_4\)S [M + H]\(^+\): 366.0674, found 367.0747.
2,4,6-Trimethyl-4,12-dihydro-1H-6,12-methanobenzo[7,8][1,3]dioxocino[4,5-d]pyrimidine-1,3(2H)-dione (12):

Colourless crystals (yield: 42%), m.p. 98-99 °C. $^1$H NMR (300 MHz, CDCl$_3$): δ 1.91 (3H, s, CH$_3$), 2.14 and 2.23 (each 1H, br. d, J = 13.5 Hz, CH$_2$), 3.28 (3H, s, >NCH$_3$), 3.34 (3H, s, >NCH$_3$), 4.21 (1H, br. s, aliph. CH), 6.85 (1H, d, J = 7.4 Hz, Ar-H), 6.91 (1H, t, J = 7.4 Hz, Ar-H), 7.12 (1H, t, J = 7.4 Hz, Ar-H), 7.40 (1H, d, J = 7.5 Hz, Ar-H).

$^{13}$C NMR (75 MHz, CDCl$_3$): δ 25.5, 26.7, 28.0, 28.7, 31.2, 91.8, 102.3, 116.0, 122.0, 126.3, 127.8, 128.0, 150.7, 155.1, 161.6. HRMS: m/z calculated for C$_{16}$H$_{17}$N$_2$O$_4$ [M + H]$^+$: 301.1110, found 301.1295.
$^{1}$H and $^{13}$C Spectra:

**Compound 6a**
Compound 6b

Table 2. 6b
300 MHz, CDCl3

Table 2. 6b
75 MHz, CDCl3
Compound 6c

Table 2, 6c
500 MHz, CDCl₃

Table 2, 6c
75 MHz, CDCl₃
Compound 6d

Table-2, 6d
300 MHz, CDCl₃

Table-2, 6d
75 MHz, CDCl₃
**Compound 6e**

Table-2, 6e
75 MHz, CDCl₃
**Compound 6f**

Table-2, 6f

300 MHz, CDCl₃

Table-2, 6f

75 MHz, CDCl₃
Compound 6g

Table 2, 6g
300 MHz, CDCl₃

Table 2, 6g
75 MHz, CDCl₃
Compound 6h

Table-2, 6h
300 MHz, CDCl₃

Table-2, 6h
75 MHz, CDCl₃
Compound 6i

Table-2, 6i
300 MHz, CDCl₃

Table-2, 6i
75 MHz, CDCl₃
**Compound 6j**

![Compound 6j](image)

**Compound 6k**

![Compound 6k](image)
Compound 6l
Compound 12
Interaction study of 6a, 6b and 6i with β-lg

Fluorescence titration of 10μM of β-lg with 1-9 μM of 6a and Plot of F₀/F vs [compound 6a] as per the Stern-Volmer equation

Fluorescence titration of 10μM of β-lg with 1-9 μM of 6b and Plot of F₀/F vs [compound 6b] as per the Stern-Volmer equation

Fluorescence titration of 10μM of β-lg with 1-10 μM of 6i and Plot of F₀/F vs [compound 6i] as per the Stern-Volmer equation
UV-Vis spectrum of 6i and its change with addition of protein solution