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Synthesis, reactions and antineoplastic activity of 3-(2-oxo-2*H*-chromen-3-yl)-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromene derivatives

Research Article

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Abstract: The key 3-(2-oxo-2*H*-chromen-3-yl)-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetates **3** were synthesized in high yields by cyclocondensation of 4-oxo-4*H*-chromen-3-carbaldehydes **1** with coumarin-3-acetic acids **2** under mild conditions. The reaction pathway involves aldol condensation and subsequent intramolecular lactonization to afford 2-oxo-2*H*,5*H*-pyrano[3,2-c]chromene skeleton **3**. Further treatment of acetates **3** with alcohols, water or nitrogen containing compounds led to 5-alkoxy-, 5-hydroxy- or 5-acylamino-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-ones **4**–**6** *via* nucleophilic substitution of acetyloxy group at C-5. Acetates and hydroxyl derivatives **3** and **5** undergo facile rearrangement in an acid medium yielding 5-hydroxypyrano[2,3-*b*]chromen-2(10a*H*)-ones **7**.

Twelve prepared compounds were evaluated on their antineoplastic activities on 60 human tumour cell line panels in NCI USA. The obtained biological results confirmed that 3-(2-oxo-2*H*-chromen-3-yl)-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one represents a new leading skeleton suitable for further antitumour activity study.

Keywords: *Pyranochromenes • Coumarin • Condensation • Nucleophilic substitution • Rearrangement • Microwave*

1. Introduction

From a synthetic point of view, 4-oxo-4*H*-chromene and coumarin derivatives are versatile synthons for synthesis of a diverse set of rearranged products and new heterocyclic systems [1,2]. Many chromene and coumarin derivatives exhibit a broad spectrum of biological properties, such as antitumour [3], antibacterial [4] or antiviral [5]. In addition, several chromene derivatives have been identified in angiogenesis [6] and also as selective estrogen receptor modulators [7]. Moreover, coumarins are also known for their important photochromic characteristics [8]. Synthetic exploitation of 4-oxo-4*H*-chromen-3-carbaldehyde **1** and its derivatives results from their reactivity towards nucleophiles. The presence of three electrophilic centers in the molecule **1** and the ability to open or retain the pyrone ring [9,10] can give access to a wide variety of heterocycles [11,12]. Because 4-oxo-4*H*-chromenes and coumarins have found many applications in medicine [13,14], the synthesis of new types of chromene derivatives is therefore of high interest.

The present study is a follow-up paper to the previous articles dealing with the synthesis, kinetics, theoretical and photochemical properties and biological activity of several series of chromene derivatives [15-17].

The aim of this study was to determine the reaction conditions for coupling two chromene (4-oxo- and 2-oxo-) moieties **1**, **2** in one skeleton under conditions different to Doebner reaction in order to synthesize new potential

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anticancer compounds. Our research was based on recently discovered antitumour properties of 2-oxo-2*H*,5*H*-pyrano[3,2-c]chromene skeleton [18].

2. Experimental procedure

Melting points were determined on a Kofler hot plate apparatus and are uncorrected. ^{1}H NMR / ^{13}C NMR spectra were obtained on a 300 MHz / 75 MHz VARIAN GEMINI 2000 spectrometer DMSO-d_6 with tetramethylsilane as an internal standard. Elemental analyses were performed on CARLO ERBA STRUMENTAZIONE 1106 apparatus. IR spectra were recorded on FTIR-ATR REACT IR 1000 spectrometer in KBr. All solvents were distilled and dried appropriately prior to use. The course of the reactions were monitored by TLC in hexane / ethyl acetate (3:1). Flash column chromatography was performed on SiO_2 (Merck Silica gel 60 F_{254}) with hexane / ethyl acetate (3:1) as eluent. All microwave assisted reactions were performed in an Initiator BIOTAGE microwave synthesizer. 4-Oxo-4*H*-chromene-3-carbaldehydes were synthesized according to the method described by Nohara [19]. 7-R^3-Coumarin-3-acetic acids **2** were synthesized according to the method described in [20]. The other chemicals were purchased from the suppliers as the highest purity grade.

2.1. 3-(2-Oxo-2*H*-chromen-3-yl)-2-oxo-2*H*,5*H*-pyrano[3,2-c]chromen-5-yl acetates (3a-3m)

Method A. Substituted chromone-3-carboxaldehyde **1** (2.26 mmol), 7-R^3-coumarin-3-acetic acid **2** (2.26 mmol) and anhydrous sodium acetate (8 mg, 0.2 mmol) were dissolved under stirring in freshly distilled acetic anhydride (15 mL). The mixture was heated at 90 -100 ºC for 2 h. After cooling the formed solid product was filtered off, washed with diethyl ether, and re-crystallized from toluene or nitromethane.

Method B. Substituted chromone-3-carboxaldehyde **1** (2.26 mmol), 7-R^3-coumarin-3-acetic acid **2** (2.26 mmol) and anhydrous sodium acetate (8 mg, 0.2 mmol) in freshly distilled acetic anhydride (4 mL) were irradiated in a microwave oven for 10 min at 400W. The product **3** was isolated and purified in the same manner as described in Method A.

3-(2-Oxo-2*H*-chromen-3-yl)-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetate (3a). Method A, work-up as described above gave 71% (method B, 75%) of **3a**, as yellow powder: m.p. 230–232°C (toluene). Anal. calcd. for $C_{23}H_{14}O_7$ (402.4): C, 68.66; H, 3.51. Found: C, 68.48; H, 3.49. IR (KBr): 3422, 3066, 2965, 2572, 1722, 1645, 1607, 1552, 1514, 1491, 1460, 1375, 1320, 1290, 1251, 1189, 1159, 1120, 1043, 996, 919, 842, 757 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): 8.55 (s, 1 H, H-4‘); 8.23 (s, 1 H, H-4); 7.80 (d, 1 H, *J* = 7.6 Hz, H-5’); 7.66 (d, 1 H, *J* = 8.5 Hz, H-10); 7.59 (ddd, 1 H, *J* = 0.8, 7.2, 7.4 Hz, H-7’); 7.36 (d, 1 H, *J* = 7.1 Hz, H-8); 7.35 (d, 1 H, *J* = 7.6 Hz, H-8’), 7.34 (ddd, 1 H, *J* = 0.8, 7.2, 7.4 Hz, H-6’); 7.12 (ddd, 1 H, *J* = 0.9, 7.5, 8.0 Hz, H-9); 7.07 (d, 1 H, *J* = 7.9 Hz, H-7); 6.40 (s, 1 H, H-5); 1.98 (s, 3 H, AcO). ^{13}C NMR (75 MHz, DMSO-d_6): 172.7, 159.7, 159.4, 153.3, 153.0, 151.4, 142.5, 142.3, 132.7, 132.2, 128.7, 124.7, 122.7, 121.9, 120.5, 118.9, 118.3, 117.5, 116.0, 114.4, 109.0, 91.7, 21.0.

9-Methyl-3-(2-oxo-2*H*-chromen-3-yl)-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetate (3b). Method A, work-up as described above gave 69% (method B, 72%) of **3b**, as yellow powder: m.p. 268-271°C (toluene). Anal. calcd. for $C_{24}H_{16}O_7$ (416.4): C, 69.23; H, 3.87. Found: C, 69.01; H, 3.89. IR (KBr): 3400, 3008, 2545, 1718, 1702, 1620, 1605, 1564, 1495, 1368, 1320, 1285, 1245, 1190, 1118, 1050, 995, 915, 850, 763 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): 8.66 (s, 1 H, H-4‘); 8.34 (s, 1 H, H-4); 7.72 (s, 1 H, H-10); 7.61 (d, 1 H, *J* = 7.6 Hz, H-5’); 7.55 (ddd, 1 H, *J* = 0.8, 7.4, 7.8 Hz, H-7’); 7.36 (d, 1 H, *J* = 7.2 Hz, H-8); 7.35 (d, 1 H, *J* = 7.7 Hz, H-8’); 7.35 (ddd, 1 H, *J* = 0.8, 7.4, 7.7 Hz, H-6’); 7.27 (d, 1 H, *J* = 7.5 Hz, H-7); 7.02 (s, 1 H, H-5); 2.34 (s, 3 H, AcO); 2.04 (s, 3 H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): 197.4, 169.6, 159.9, 159.8, 153.7, 153.2, 150.6, 142.9, 141.7, 134.3, 133.1, 132.4, 128.9, 124.8, 119.9, 119.1, 118.2, 116.4, 113.6, 105.5, 89.9, 21.0, 20.7.

8,9-Dimethyl-3-(2-oxo-2*H*-chromen-3-yl)-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetate (3c). Method A, work-up as described above gave 82% of **3c**, as yellow powder: m.p. 276-278°C (toluene). Anal. calcd. for $C_{25}H_{18}O_7$ (430.4): C, 69.76; H, 4.22. Found: C, 69,58; H, 4.19. IR (KBr): 3380, 3065, 2969, 1728, 1650, 1609, 1552, 1491, 1460, 1378, 1324, 1290, 1249, 1189, 1159, 1120, 1045, 999, 919, 842, 760 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): 8.45 (s, 1 H, H-4‘); 8.15 (s, 1 H, H-4); 7.82 (dd, 1 H, *J* = 1.5, 7.4 Hz, H-5’); 7.69 (ddd, 1 H, *J* = 1.4, 7.4, 8.3 Hz, H-7’); 7.58 (s, 1 H, H-10); 7.47 (d, 1 H, *J* = 8.2 Hz, H-8’); 7.43 (s, 1 H, H-7); 7.41 (ddd, 1 H, *J* = 1.03, 1.17, 7.47 Hz, H-6’); 7.05 (s, 1 H, H-5); 2.36 (s, 3 H, AcO); 2.29 (s, 3 H, CH_3); 2.29 (s, 3 H, CH_3). The ^{13}C NMR spectrum was non-measurable because of the low solubility of **3c**.

9-Fluoro-3-(2-oxo-2*H*-chromen-3-yl)-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetate (3d). Method A, work-up as described above gave 78% of **3d**, as yellow powder: m.p. 295-297 °C decomp. (toluene). Anal. calcd. for $C_{23}H_{13}FO_7$ (420.3): C, 65.72; H, 3.12; F, 4.52. Found: C, 65.93; H, 3.18; F, 4.76. IR (KBr):

3353, 3057, 1730, 1655, 1608, 1550, 1497, 1377, 1285, 1190, 1162, 1120, 1045, 990, 921, 850 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): 8.47 (s, 1 H, H-4'); 8.20 (s, 1 H, H-4); 7.83 (s, 1 H, H-10); 7.69 (ddd, 1 H, *J* = 0.9, 7.5, 7.9 Hz, H-7'); 7.60 (d, 1 H, *J* = 7.5 Hz, H-8); 7.47 (d, 1 H, *J* = 7.7 Hz, H-7); 7.45 - 7.39 (m, 2 H, H-5',6'); 7.35 (d, 1 H, *J* = 7.6 Hz, H-8'); 6.45 (s, 1 H, H-5); 2.03 (s, 3 H, AcO). The ^{13}C NMR spectrum was non-measurable because of the low solubility of **3d**.

9-Chloro-3-(2-oxo-2*H*-chromen-3-yl)-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetate (3e). Method A, work-up as described above gave 80% of **3e**, as yellow powder: m.p. 297-298°C (toluene). Anal. calcd. for $C_{23}H_{13}ClO_7$ (436.8): C, 63.24; H, 3.00; Cl, 8.12. Found C, 63.08; H, 3.03; Cl, 7.97. IR (KBr): 3350, 3070, 2970, 1732, 1654, 1605, 1552, 1493, 1460, 1370, 1285, 1205, 1185, 1159, 1120, 1045, 1005, 921, 850 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): 8.68 (s, 1 H, H-4'); 8.38 (s, 1 H, H-4); 7.88 (s, 1 H, H-10); 7.62 (d, 1 H, *J* = 7.6 Hz, H-5'); 7.58 (ddd, 1 H, *J* = 0.9, 7.5, 7.9 Hz, H-7'); 7.38 (d, 1 H, *J* = 7.3 Hz, H-8); 7.35 (d, 1 H, *J* = 7.7 Hz, H-8'); 7.34 (ddd, 1 H, *J* = 0.9, 7.5, 7.8 Hz, H-6'); 7.12 (d, 1 H, *J* = 7.5 Hz, H-7); 7.07 (s, 1 H, H-5); 2.06 (s, 3 H, AcO). ^{13}C NMR (75 MHz, DMSO-d_6): 169.3, 159.9, 159.2, 153.3, 151.5, 150.4, 143.4, 141.1, 133.0, 132.6, 129.0, 128.8, 124.9, 122.8, 119.5, 119.3, 119.1, 119.0, 116.3, 115.4, 106.2, 89.7, 20.9.

9-Nitro-3-(2-oxo-2*H*-chromen-3-yl)-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetate (3f). Method A, work-up as described above gave 85% (method B, 87%) of **3f**, as yellow powder: m.p. 284-287°C (nitromethane). Anal. calcd. for $C_{23}H_{13}NO_9$ (447.4): C, 61.75; H, 2.93; N, 3.13. Found: C, 61.91; H, 2.94; N, 2.99. IR (KBr): 3395, 3065, 2976, 1760, 1705, 1695, 1655, 1602, 1581, 1550, 1501, 1490, 1437, 1305, 1245, 1207, 1198, 1120, 1090, 1006, 970, 920, 795 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): 8.89 (s, 1 H, H-10); 8.74 (s, 1 H, H-4'); 8.48 (s, 1 H, H-4); 8.34 (d, 1 H, *J* = 8.9 Hz, H-8); 7.64 (d, 1 H, *J* = 8.1 Hz, H-5'); 7.61 (ddd, 1 H, *J* = 0.9, 7.5, 8.0 Hz, H-7'); 7.37 (d, 1 H, *J* = 7.9 Hz, H-8'); 7.36 (ddd, 1 H, *J* = 0.9, 8.0, 7.8 Hz, H-6'); 7.27 (d, 1 H, *J* = 7.9 Hz, H-7); 7.20 (s, 1 H, H-5); 2.09 (s, 3 H, AcO). ^{13}C NMR (75 MHz, DMSO-d_6): 168.9, 159.8, 159.7, 156.2, 153.3, 150.5, 143.8, 143.4, 140.4, 132.8, 129.1, 127.9, 125.0, 120.4, 119.4, 119.1, 118.9, 118.5, 116.5, 114.6, 106.6, 89.7, 20.9.

8-Acetyloxy-3-(2-oxo-2*H*-chromen-3-yl)-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetate (3g). Method A, work-up as described above gave 71% (method B, 74%) of **3g**, as yellow powder: m.p. 281-283°C (toluene). Anal. calcd. for $C_{25}H_{16}O_9$ (460.4): C, 65.22; H, 3.50. Found: 65.41; H, 3.53. IR (KBr): 2920, 2865, 1754, 1720, 1620, 1555, 1451, 1485, 1375, 1324, 1221, 1190, 1120, 1085, 1040, 1014, 961, 922, 768 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): 8.70 (s, 1 H, H-4'); 8.41 (s, 1 H, H-4); 7.88 (d, 1 H, *J* = 8.1 Hz, H-5'); 7.62 (d, 1 H, *J* = 8.1 Hz, H-10); 7.55 (d, 1 H, *J* = 8.0 Hz, H-8'); 7.37 – 7.26 (m, 3H, H-9, 6', 7'); 6.90 (s, 1 H, H-7); 6.49 (s, 1 H, H-5); 2.32 (s, 3 H, AcO); 2.03 (s, 3 H, AcO). ^{13}C NMR (75 MHz, DMSO-d_6):168.8, 167.6, 159.4, 154.1, 150.4, 150.0, 145.8, 145.6, 143.0, 141.9, 132.3, 128.9, 124.8, 124.2, 119.2, 118.6, 118.3, 116.4, 116.0, 114.6, 111.4, 107.5, 92.3, 21.2, 20.9.

9-Acetyloxy-3-(2-oxo-2*H*-chromen-3-yl)-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetate (3h). Method A, work-up as described above gave 74% (method B, 77%) of **3h**, as yellow powder: m.p. 299-301°C (toluene). Anal. calcd. for $C_{25}H_{16}O_9$ (460.4): C, 65.22; H, 3.50. Found: C, 65.38; H, 3.51. IR (KBr): 2915, 2860, 1757, 1723, 1621, 1556, 1453 (m), 1488 (w), 1378, 1325, 1214, 1198, 1118, 1088, 1040, 1007, 955, 914, 764 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): 8.46 (s, 1 H, H-4'); 8.08 (s, 1 H, H-4); 7.81 (d, 1 H, *J* = 7.9 Hz, H-5'); 7.66 (ddd, 1 H, *J* = 0.9, 7.6, 8.1 Hz, H-7'); 7.46 (d, 1 H, *J* = 7.9 Hz, H-8'); 7.41 (s, 1 H, H-10); 7.40 (ddd, 1 H, *J* = 0.9, 7.6, 7.9 Hz, H-6'); 7.23 (d, 1 H, *J* = 7.9 Hz, H-8); 7.05 (d, 1 H, *J* = 7.8 Hz, H-7); 6.44 (s, 1 H, H-5); 2.28 (s, 3 H, AcO); 1.92 (s, 3 H, AcO). ^{13}C NMR (300 MHz, DMSO-d_6): 169.4, 160.0, 159.4, 153.2, 150.4, 145.7, 145.7, 143.2, 141.3, 132.4, 128.9, 124.8, 124.7, 120.5, 119.7, 119.1, 118.6, 118.5, 116.3, 116.1, 114.8, 106.7, 96.8, 20.9, 20.8.

7,8-Diacetyloxy-3-(2-oxo-2*H*-chromen-3-yl)-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetate (3i). Method A, work-up as described above gave 69% of **3i**, as yellow powder: m.p. 230-232°C (toluene). Anal. calcd. for $C_{27}H_{18}O_{11}$ (518.4): C, 62.55; H, 3.50. Found C, 62.37; H, 3.52. IR (KBr): 3105, 1760, 1649, 1600, 1580, 1505, 1489, 1401, 1350, 1302, 1295, 1244, 1187, 1150, 1102, 999, 841, 795 cm^{-1}. ^1H NMR(300 MHz, DMSO-d_6): 8.46 (s, 1 H, H-4'); 8.20 (s, 1 H, H-4); 7.81 (d, 1 H, *J* = 7.7 Hz, H-5'); 7.75 (d, 1 H, *J* = 8.0 Hz, H-10); 7.69 (ddd, 1 H, *J* = 7.4, 7.1, 0.8 Hz, H-7'); 7.54 (d, 1 H, *J* = 7.1 Hz, H-8'); 7.41 (ddd, 1 H, *J* = 1.0, 7.1, 7.4 Hz, H-6'); 7.25 (d, 1 H, *J* = 8.5 Hz, H-9); 6.45 (s, 1 H, H-5); 2.33 (s, 3 H, AcO); 2.29 (s, 3 H, AcO); 1.99 (s, 3 H, AcO). ^{13}C NMR (300 MHz, DMSO-d_6): 172.0, 168.9, 168.2, 167.5, 158.9, 158.6, 152.9, 151.1, 146.1, 145.0, 143.1, 141.5, 132.7, 131.9, 128.9, 125.0, 121.1, 120.2, 118.6, 118.2, 116.1, 113.3, 105.2, 89.1, 20.9, 20.6, 19.9.

3-Oxo-2-(2-oxo-2*H*-chromen-3-yl)-3*H*,12*H*-benzo[*h*]pyrano[3,2-*c*]chromen-12-yl acetate (3j). Method A, work-up as described above gave 67% of **3j** as yellow powder: m.p. 286-289°C (toluene). Anal. calcd. for $C_{27}H_{16}O_7$ (452.4): C, 71.68; H, 3.56. Found: 71.79; H, 3.54. IR (KBr):3386, 3065, 2950, 1701, 1699,

1640, 1617, 1603, 1520, 1487, 1410, 1318, 1250, 1205, 1197, 1163, 1110, 1042, 1012, 945, 815, 763 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): 8.53 (s, 1 H, H-4'); 8.33 (d, 1 H, *J* = 8.5 Hz, H-5'); 8.23 (s, 1 H, H-4); 7.88 -7.81 (m, 2 H, H-8,9); 7.64 – 7.59 (m, 4 H, Ar-H); 7.38-7.35 (m, 2H, H-6',7'); 7.25 (d, 1H, *J* = 7.5 Hz, H-8'); 6.67 (s, 1 H, H-5); 1.96 (s, 3 H, AcO). ^{13}C NMR (300 MHz, DMSO-d_6): 172.3, 156.6, 159.3, 152.9, 152.3, 150.3, 142.4, 142.3, 135.4, 132.1, 128.7, 128.4, 127.9, 126.4, 124.7, 124.5, 122.6, 121.5, 120.8, 118.9, 118.4, 117.5, 115.9, 108.7, 107.9, 92.2, 20.7.

3-(7-Methoxy-2-oxo-2*H*-chromen-3-yl)-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetate (3k). Method A, work-up as described above gave 81% of **3k** as yellow powder: m.p. 294-296°C (toluene). Anal. calcd. for $C_{24}H_{16}O_8$ (432.4): C, 66.67; H, 3.73. Found: C, 66.82; H, 3.51. IR (KBr): 2958, 2927, 2858, 1722, 1645, 1614, 1552, 1506, 1460, 1375, 1259, 1197, 1159, 1074, 1020, 981, 935, 895, 796, 757 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): 8.41 (s, 1 H, H-4'); 8.16 (s, 1 H, H-4); 7.82 (dd, 1 H, *J* = 1.4, 7.7 Hz, H-10); 7.74 (d, 1 H, *J* = 8.8 Hz, H-5'); 7.56 (ddd, 1 H, *J* = 1.4, 7.8, 8.3 Hz, H-8); 7.48 (s, 1 H, H-5); 7.29 (ddd, 1 H, *J* = 1.0, 7.7, 7.8 Hz, H-9); 7.22 (dd, 1 H, *J* = 1.0, 8.3 Hz, H-7); 7.08 (d, 1 H, *J* = 2.4 Hz, H-8'); 7.01 (dd, 1 H, *J* = 2.4, 8.8 Hz, H-6'); 3.89 (s, 3 H, MeO); 2.03 (s, 3 H, AcO). ^{13}C-NMR (75 MHz, DMSO-d_6): 169.0, 163.1, 158.9, 158.6, 155.0, 151.7, 151.4, 143.2, 141.2, 133.4, 130.1, 123.5, 122.6, 120.1, 117.5, 117.3, 113.9, 113.0, 112.2, 105.4, 100.4, 89.2, 56.1, 20.7.

3-(7-Acetyloxy-2-oxo-2*H*-chromen-3-yl)-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetate (3l). Method A, work-up as described above gave 65% of **3l** as yellow powder: m.p. 239 – 243°C. (nitromethane). Anal. calcd. for $C_{25}H_{16}O_9$ (460.4): C, 65.22; H, 3.50. Found: C, 65.43; H, 3.47. IR (KBr): 2929, 2864, 1760, 1725, 1617, 1551, 1455, 1486, 1378, 1320, 1196, 1127, 1046, 1007, 914, 764 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): 8.48 (s, 1 H, H-4'); 8.09 (s, 1 H, H-4); 7.78 (d, 1 H, *J* = 8.6 Hz, H-5'); 7.75 (dd, 1 H, *J* = 1.5, 7.8 Hz, H-10); 7.50 (ddd, 1 H, *J* = 1.5, 7.5, 8.3 Hz, H-8); 7.36 (d, 1 H, *J* = 2.2 Hz, H-8'); 7.22 (dd, 1 H, *J* = 2.2, 8.6 Hz, H-6'); 7.19 (ddd, 1 H, ,*J* = 1.0, 7.5, 7.8 Hz, H-9); 7.14 (dd, 1 H, *J* = 1.0, 8.3 Hz, H-7); 6.45 (s, 1 H, H-5); 2.33 (s, 3 H, AcO); 1.91 (s, 3 H, AcO). ^{13}C-NMR (75 MHz, DMSO-d_6): 168.9, 168.6, 158.4, 158.3, 153.4, 153.2, 151.74, 151.72, 142.4, 141.7, 133.4, 129.8, 123.4, 122.6, 120.3, 119.6, 119.0, 117.4, 116.4, 113.8, 109.8, 105.3, 89.1, 20.8, 20.6.

3-[7-(*N,N*-Dimethylamino)-2-oxo-2*H*-chromen-3-yl]-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetate (3m). Method A, work-up as described above gave 56% of **3m** as orange powder: m.p. 284 – 286°C (nitromethane). Anal. calcd. for $C_{25}H_{19}NO_7$ (445.4): C, 67.41; H, 4.30; N, 3.14. Found: C, 67.71; H, 4.11; N, 2.98. IR (KBr): 1729, 1621, 1521, 1471, 1374, 1305, 1201, 1139, 1004, 938, 818, 768 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): 8.33 (s, 1 H, H-4'); 8.14 (s, 1 H, H-4); 7.80 (dd, 1 H, *J* = 1.5, 7.9 Hz, H-10); 7.56 (d, 1 H, *J* = 8.9 Hz, H-5'); 7.54 (ddd, 1 H, *J* = 1.5, 7.7, 8.2 Hz, H-8); 7.46 (s, 1 H, H-5); 7.28 (ddd, 1 H, *J* = 1.0, 7.7, 7.9 Hz, H-9); 7.02 (dd, 1 H, *J*) = 1.0, 8.2 Hz, H-7); 6.78 (dd, 1 H, *J* = 2.3, 8.9 Hz, H-6'); 6.61 (d, 1 H, *J* = 2.3 Hz, H-8'); 3.06 (s, 6 H, Me_2N); 2.02 (s, 3 H, AcO). ^{13}C-NMR (75 MHz, DMSO-d_6): 171.7, 168.9, 159.6, 159.2, 155.3, 153.2, 152.6, 151.5, 149.7, 143.0, 140.6, 132.3, 129.6, 121.9 and 121.9, 119.8, 117.4, 114.3, 113.1, 109.6, 109.1, 107.9, 96.7, 91.3, 20.9.

2.2. 5-Alkoxy-3-(2-oxo-2*H*-chromen-3-yl)-2*H*,5*H*-pyrano[3,2-c]chromen-2-ones (4a-4d)

Method A: A solution of acetate **3** (10 mmol) and 4-methylbenzenesulfonic acid (8 mg, 0.04 mmol) in ethanol (10 mL) was stirred and heated at 60°C for 3 h. After cooling, the formed solid compound was filtered off, washed with diethyl ether and re-crystallized from ethanol.

Method B: A solution of acetate **3** (10 mmol), appropriate alcohol (hexan-1-ol or prop-2-yn-1-ol) (10.5 mmol) and 4-methylbenzenesulfonic acid (8 mg, 0.04 mmol) in toluene (10 mL) was stirred and heated at 100°C for 2 h. After cooling, the solid was filtered off, washed with diethyl ether and re-crystallized from toluene.

5-Ethoxy-9-methyl-3-(2-oxo-2*H*-chromen-3-yl)-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one (4a). Method A, work-up as described above gave 72% of **4a** as yellow powder: m.p. 189-191°C (ethanol). Anal. calcd. for $C_{24}H_{18}O_6$ (402.4): C, 71.64; H, 4.51. Found: C, 71.83; H, 4.55. IR (KBr): 3089, 2940, 1705, 1698, 1641, 1545, 1495, 1460, 1402, 1327, 1264, 1212, 1151, 1129, 1090, 1017, 995, 908, 761 cm^{-1}.^{1}H-NMR (300 MHz, DMSO-d_6): 8.48 (s, 1 H, H-4'); 8.12 (s, 1 H, H-4); 7.82 (dd, 1 H, *J* = 1.4, 7.8 Hz, H-5'); 7.67 (ddd, 1 H, *J* = 1.5, 7.8, 8.3 Hz, H-7'); 7.57 (d, 1 H, *J* = 1.5 Hz, H-10); 7.47 (dd, 1 H, *J* = 8.3 Hz, H-8); 7.41 (ddd, 1 H, *J* = 1.1, 7.6, 7.9 Hz, H-6'); 7.33 (dd, 1 H, *J* = 1.1, 8.3 Hz, H-8'); 7.09 (d, 1 H, *J* = 8.3 Hz, H-7); 6.31 (s, 1 H, H-5); 3.82 (2H, q, *J* = 7.0 Hz, CH_2); 2.35 (s, 3 H, CH_3); 1.12 (t, 3 H, *J* = 7.0 Hz, CH_3). The ^{13}C NMR spectrum was non-measurable because of the low solubility of **4a**.

9-Chloro-5-ethoxy-3-(2-oxo-2*H*-chromen-3-yl)-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one (4b). Method A, work-up as described above gave 74% of **4b** as yellow powder: m.p. 208-212°C (ethanol). Anal. calcd. for

$C_{23}H_{15}ClO_6$ (422.8): C, 65.34; H, 3.58; Cl, 8.38. Found: C, 65.49; H, 3.55; Cl, 7.99. IR (KBr): 3090, 2935, 1708, 1701, 1644, 1555, 1501, 1468, 1410, 1325, 1271, 1220, 1155, 1137, 1094, 1020, 999, 902, 756 cm^{-1}.^{1}H-NMR (300 MHz, DMSO-d_6): 8.73 (s, 1 H, H-4'); 8.39 (s, 1 H, H-4); 7.85 (s, 1 H, H-10); 7.62 (d, 1 H, *J* = 7.8 Hz, H-5'); 7.57 (ddd, 1 H, *J* = 1.0, 7.9, 8.1 Hz, H-7'); 7.46 (d, 1 H, *J* = 7.7 Hz, H-8); 7.39 (d, 1 H, *J* = 7.7 Hz, H-8'); 7.35 (ddd, 1 H, *J* = 1.1, 7.9, 8.1 Hz, H-6'); 7.05 (d, 1 H, *J* = 8.1 Hz, H-7); 6.11 (s, 1 H, H-5); 3.79 (q, 2 H, *J* = 7.2 Hz, CH_2); 1.22 (t, 3 H, *J* = 7.2 Hz, CH_3). ^{13}C-NMR (75 MHz, DMSO-d_6): 160.0, 159.7, 153.2, 151.5, 151.1, 143.1, 141.8, 132.6, 132.4, 129.0, 127.9, 124.8, 122.8, 119.6, 119.2, 119.0, 118.8, 116.3, 115.9, 108.2, 97.3, 64.8, 15.8.

5-Hexyloxy-3-(2-oxo-2*H*-chromen-3-yl)-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one (4c). Method B, work-up as described above gave 67% of **4c** as yellow powder: m.p. 253-256°C (toluene). Anal. calcd. for $C_{27}H_{24}O_6$ (444.5): C, 72.96; H, 5.44. Found: C, 73.21; H, 5.47. IR (KBr): 3084, 2915, 1711, 1698, 1640, 1555, 1497, 1459, 1410, 1325, 1270, 1225, 1158, 1139, 1101, 1025, 989, 889, 756 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): 8.72 (s, 1 H, H-4'); 8.37 (s, 1 H, H-4); 7.97 (d, 1 H, *J*=8.7 Hz, H-5'); 7.61-7.59 (m, 2 H, H-7', 10); 7.37 (ddd, 1 H, *J* = 1.5, 7.8, 8.0 Hz, H-8); 7.35 -7.28 (m, 2 H, H-6',8'); 7.19 (d, 1 H, *J* = 7.9 Hz, H-9); 7.08 (d, 1 H, *J* = 7.9 Hz, H-7); 6.10 (s, 1 H, H-5); 3.95 (q, 2H *J* = 6.5 Hz, OCH_2); 1.64 -1.66 (m, 2 H, CH_2); 1.24 -1.25 (m, 6 H, 3 x CH_2); 0.84 (t, 3 H, *J* = 6.2 Hz, CH_3). ^{13}C-NMR (75 MHz, DMSO-d_6): 159.0, 152.9, 151.1, 143.0, 142.5, 133.6, 132.4, 131.2, 128.9, 124.8, 122.1, 121.2, 118.7, 117.4, 116.0, 114.0, 109.1, 91.1, 20.1.

3-(7-Methoxy-2-oxo-2*H*-chromen-3-yl)-9-methyl-5-(prop-2-yn-1-yloxy)-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one (4d). Method B, work-up as described above gave 68% of **4d** as yellow powder: m.p. 317-319 °C decomp. (toluene). Anal. calcd. for $C_{26}H_{18}O_7$ (442.4): C, 70.58; H, 4.10. Found: C, 70.79; H, 4.14. IR (KBr): 3095, 2899, 1710, 1700, 1645, 1565, 1499, 1455, 1412, 1325, 1272, 1225, 1163, 1144, 1089, 1032, 984, 865, 748 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): 8.45 (s, 1 H, H-4'); 8.08 (s, 1 H, H-4); 7.74 (d, 1 H, *J* = 8.7 Hz, H-5'); 7.58 (s, 1 H, H-10); 7.36 (d, 1 H, *J* = 8.2 Hz, H-8); 7.14 (d, 1 H, *J* = 8.3 Hz, H-7); 7.09 (d, 1H, *J* = 2.4 Hz, H-8'); 7.02 (dd, 1 H, *J* = 1.2, 8.7 Hz, H-6'); 6.38 (s, 1 H, H-5); 4.45 (d, 1H, *J* = 1.8 Hz, CH_2); 3.89 (s, 3 H, OCH_3); 3.56 (s, 1 H, CH); 2.36 (s, 3 H, CH_3). ^{13}C-NMR (75 MHz, DMSO-d_6): 162.4, 159.1, 158.9, 154.9, 152.8, 151.3, 151.1, 150.0, 142.9, 137.9, 132.1, 131.6, 130.1, 127.6, 122.1, 119.4, 117.6, 117.2, 114.2, 112.9, 112.3, 107.1, 100.4, 79.2, 78.0, 20.2.

2.3. 5-Hydroxy-3-(2-oxo-2*H*-chromen-3-yl)-2*H*,5*H*-pyrano[3,2-c]chromen-2-ones (5a-5f)

Method A: Compound **3** or **4** (0.33 mmol) and catalytic amount of 4-methylbenzenesulfonic acid (5.6 mg, 0.033 mmol) was heated in 20 mL of dioxane-water (3:1) at 70°C for 1h. The solid product was cooled to room temperature, filtered off and re-crystallised from dioxane.

Method B: Compound **3l** (0.14 mmol) was added into the solution of 96% H_2SO_4 (3.25 mL), H_2O (1.3 mL) and EtOH (1.95 mL) (5:3:2). The mixture was stirred at 20°C for 10 min. Then H_2O (25 mL) was added and the yellow solution was stirred at 20°C for 5 h. The formed yellow precipitate was filtered off, washed with H_2O and re-crystallized from dioxane.

5-Hydroxy-3-(2-oxo-2*H*-chromen-3-yl)-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one (5a). Method A, work-up as described above gave 71% of **5a** as yellow powder: m.p. 283 - 285°C (dioxane). Anal. calcd. for $C_{21}H_{12}O_6$ (360.3): C, 70,00; H, 3,36. Found: C, 70,24; H, 3,39. IR (KBr): 3498, 3080, 1705, 1695, 1630, 1543, 1495, 1461, 1400, 1320, 1264, 1212, 1150, 1127, 1085, 1014, 995, 908, 768 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): 8.47 (s, 1 H, H-4'); 8.09 (s, 1 H, H-4); 7.87 (d, 1H, *J* = 5.5 Hz, OH); 7.81 (s, 1 H, H-10); 7.74 (d, 1 H, *J* = 8.3 Hz, H-5'); 7.67 (ddd, 1 H, *J* = 1.2, 8.0, 8.2 Hz, H-8); 7.47 (d, 1H, *J* = 7.5 Hz, H-8'); 7.41 (ddd, 1 H, *J* = 1.2, 7.9, 8.3 Hz, H-7'); 7.18 (ddd, 1 H, *J* = 1.2, 7.6, 8.72 Hz, H-6'); 7.13 (d, 1 H, *J* = 8.2 Hz, H-7); 6.47 (d, 1 H, *J* = 5.4 Hz, H-5). ^{13}C-NMR (75 MHz, DMSO-d_6): 158.9, 158.7, 152.8, 150.8, 142.6, 142.4, 132.7, 132.4, 132.2, 128.7, 124.8, 122.2, 122.0, 121.1, 119.0, 118.7, 118.6, 117.5, 115.2, 114.1, 108.9, 91.1.

5-Hydroxy-9-methyl-3-(2-oxo-2*H*-chromen-3-yl)-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one (5b). Method A, work-up as described above gave 71% of **5b** as yellow powder: m.p. 266 - 267°C (dioxane). Anal. calcd. for $C_{22}H_{14}O_6$ (374.3): C, 70,59; H, 3,77. Found: C, 70,38; H, 3,74. IR (KBr): 3461, 3092, 2907, 1709, 1695, 1627, 1540, 1498, 1459, 1395, 1307, 1257, 1210, 1150, 1115, 1059, 1020, 999, 912, 771 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): 8.47 (s, 1 H, H-4'); 8.08 (s, 1 H, H-4); 7.74 (ddd, 1 H, *J* = 1.0, 7.4, 8.1 Hz, H-7'); 7.67 (dd, 1 H, *J* = 1.9, 8.2 Hz, H-8); 7.82 (d, 1 H, *J* = 7.8 Hz, H-5'); 7.54 (d, 1 H, *J* = 8.3 Hz, H-10); 7.52 (brs, 1 H, OH); 7.41 (dd, 1 H, *J* = 1.8, 8.1 Hz, H-8'); 7.30 (ddd, 1 H, *J* = 1.0, 7.5, 8.0 Hz, H-6'); 7.02 (d, 1 H, *J* = 8.2 Hz, H-7); 6.40 (d, 1 H, *J* = 6.6 Hz, H-5); 2.34 (s, 3 H, CH_3). The ^{13}C NMR spectrum was non-measurable because of the low solubility of **5b**.

8,9-Dimethyl-5-hydroxy-3-(2-oxo-2*H*-chromen-3-yl)-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one (5c). Method

A, work-up as described above gave 69% of **5c** as yellow powder: m.p. 198-200°C (dioxane). Anal. calcd. for $C_{23}H_{16}O_6$ (388.4): C, 71.13; H, 4.15. Found: C, 71.31; H, 4.18. IR (KBr): 2941, 1719, 1615, 1559, 1509, 1499, 1462, 1398, 1318, 1264, 1215, 1157, 1058, 1025, 997, 894 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): 8.46 (s, 1 H, H-4'); 8.07 (s, 1 H, H-4); 7.83 (dd, 1 H, *J* = 1.4, 7.7 Hz, H-5'); 7.69 (d, 1 H, *J* = 6.57 Hz, OH); 7.74 (ddd, 1 H, *J* = 1.3, 8.2, 8.5 Hz, H-7'); 7.51 (s, 1 H, H-10); 7.45 (d, 1 H, *J* = 8.2 Hz, H-8'); 7.30 (ddd, 1 H, *J* = 1.1, 7.7, 8.5 Hz, H-6'); 6.94 (s, 1 H, H-7); 6.39 (d, 1 H, *J* = 6.5 Hz, H-5); 2.27 (s, 6 H, 2 CH_3). The ^{13}C NMR spectrum was non-measurable because of the low solubility of **5c**.

5-Hydroxy-3-(7-methoxy-2-oxo-2*H*-chromen-3-yl)-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one (5d). Method A, work-up as described above gave 72% of **5d** as yellow powder: m.p. 311-315°C (dioxane). Anal. calcd. for $C_{22}H_{14}O_7$ (390.3): C, 67.69; H, 3.62. Found: C, 67.91; H, 3.48. IR (KBr): 2934, 2868, 1722, 1617, 1552, 1502, 1463, 1378, 1274, 1251, 1208, 1127, 1069, 980, 757 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): 8.43 (s, 1 H, H-4'); 8.07 (s, 1 H, H-4); 7.79 (d, 1 H, *J* = 6.9 Hz, OH); 7.74 (dd, 1 H, *J* = 1.6, 7.6 Hz, H-10); 7.74 (d, 1 H, *J* = 8.5 Hz, H-5'); 7.49 (ddd, 1 H, *J* = 1.6, 7.2, 8.3 Hz, H-8); 7.18 (ddd, 1 H, *J* = 1.0, 7.2, 7.6 Hz, H-9); 7.13 (dd, 1 H, *J* = 1.0, 8.3 Hz, H-7); 7.07 (d, 1 H, *J* = 2.5 Hz, H-8'); 7.01 (dd, 1 H, *J* = 2.5, 8.5 Hz, H-6'); 6.44 (d, 1 H, *J* = 6.9 Hz, H-5); 3.89 (s, 3 H, MeO). ^{13}C-NMR (75 MHz, DMSO-d_6): 62.9, 159.1, 154.8, 152.8, 150.5, 142.9, 141.9, 132.7, 130.1, 122.3, 122.1, 119.4, 117.6, 117.4, 114.3, 113.0, 112.3, 109.1, 100.4, 91.2, 56.1.

5-Hydroxy-3-(7-acetyloxy-2-oxo-2*H*-chromen-3-yl)-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one (5e). Method A, work-up as described above gave 64% of **5e** as yellow powder: m.p. 284-286°C (dioxane). Anal. calcd. for $C_{23}H_{14}O_8$ (418.4): C, 66.03; H, 3.37. Found: C, 65.83; H, 3.09. IR (KBr): 3076, 2930, 2864, 1764, 1725, 1644, 1617, 1548, 1494, 1435, 1374, 1320, 1193, 1127, 1004, 934, 911, 760 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): 8.48 (s, 1 H, H-4'); 8.09 (s, 1 H, H-4); 7.87 (d, 1 H, *J* = 8.6 Hz, H-5'); 7.83 (d, 1 H, *J* = 6.6 Hz, OH); 7.75 (dd, 1 H, *J* = 1.5, 7.8 Hz, H-10); 7.50 (ddd, 1 H, *J* = 1.5, 7.9, 8.2 Hz, H-8); 7.36 (d, 1 H, *J* = 2.2 Hz, H-8'); 7.22 (dd, 1 H, *J* = 2.2, 8.6 Hz, H-6'); 7.19 (ddd, 1 H, *J* = 0.8, 7.8, 7.9 Hz, H-9); 7.14 (dd, 1 H, *J* = 0.8, 8.2 Hz, H-7); 6.45 (d, 1 H, *J* = 6.6 Hz, H-5); 2.33 (s, 3 H, AcO). The ^{13}C NMR spectrum was non-measurable because of the low solubility of **5e**.

5-Hydroxy-3-[(7-(*N,N*-dimethylamino)-2-oxo-2*H*-chromen-3-yl]-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one (5f). Method A, work-up as described above gave 62% of **5f** as orange powder: m.p. 293-296°C (dioxane). Anal. calcd. for $C_{23}H_{17}NO_6$ (403.4): C, 68.48; H, 4.25; N, 3.47. Found: C, 68.22; H, 3.99; N, 3.21. IR (KBr): 1787, 1718, 1617, 1594, 1521, 1463, 1386, 1301, 1251, 1208, 1146, 1062, 1019, 977, 907, 826, 760 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): 8.35 (s, 1 H, H-4'); 8.06 (s, 1 H, H-4); 7.77 (d, 1 H, *J* = 6.5 Hz, OH); 7.73 (dd, 1 H, *J* = 1.6, 7.7 Hz, H-10); 7.56 (d, 1 H, *J* = 8.8 Hz, H-5'); 7.48 (ddd, 1 H, *J* = 1.6. 7.4, 8.3 Hz, H-8); 7.18 (ddd, 1 H, *J* = 0.8, 7.4, 7.7 Hz, H-9); 7.12 (dd, 1 H, *J* = 0.8, 8.3 Hz, H-7); 6.78 (dd, 1 H, *J* = 2.2, 8.8 Hz, H-6'); 6.61 (d, 1 H, *J* = 2.2 Hz, H-8'); 6.42 (d, 1 H, *J* = 6.5 Hz, H-5); 3.07 (s, 6 H, Me_2N). ^{13}C NMR (75 MHz, DMSO-d_6): 159.7, 159.4, 155.4, 153.3, 152.7, 149.7, 143.2, 140.79, 140.78, 132.46, 132.45, 129.8, 122.1, 122.0, 119.8, 117.6, 114.4, 113.1, 109.7, 109.2, 108.0, 96.7, 91.3.

5-hydroxy-3-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one (5g). Method B, work-up as described above gave 62% of **5g** as yellow powder: m.p. 260-262 °C (dioxane). Anal. calcd. for $C_{21}H_{12}O_7$ (376.3): C, 67.02; H, 3.21. Found: C, 66.89; H, 3.33. IR (KBr) 3630, 3283, 2974, 2935, 1707, 1614, 1552, 1498, 1460, 1375, 1321, 1243, 1204, 1158, 1128, 1043, 981, 903, 850, 811, 765 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): 10.77 (br s, 1 H, OH); 8.38 (s, 1 H, H-4'), 8.05 (s, 1 H, H-4); 7.79 (d, 1 H, *J* = 6.7 Hz, OH), 7.74 (dd, 1 H, *J* = 1.6, 7.8 Hz, H-10), 7.64 (d, 1 H, *J* = 8.6 Hz, H-5'), 7.49 (ddd, 1 H, *J* = 1.6, 7.5 8.3 Hz, H-8), 7.18 (ddd, 1 H, *J* = 1.0, 7.5, 7.8 Hz, H-9), 7.13 (dd, 1 H, *J* = 1.0, 8.3 Hz, H-7), 6.84 (dd, 1 H, *J* = 2.3, 8.6 Hz, H-6'), 6.79 (d, 1 H, *J* = 2.3 Hz, H-8'), 6.43 (d, 1 H, *J* = 6.7 Hz, H-5). ^{13}C NMR (75 MHz, DMSO-d_6): 161.9, 159.2, 159.2, 155.0, 152.8, 150.3, 143.2, 141.8, 132.7, 130.4, 122.2, 122.1, 119.6, 117.6, 116.3, 114.3, 113.7, 111.2, 109.1, 101.9, 91.2.

2.4. 5-R^4-Amino-3-(2-oxo-2*H*-chromen-3-yl)-2*H*,5*H*-pyrano[3,2-c]chromen-2-ones (6a-6f)

The solution of nitrogen containing compound (formamide, acetylamide, 4-methylphenylsulfonamide, ethyl carbamate or 1,3-oxazolidin-2-one) (10 mmol) in nitromethane (7 mL) was added to the solution of acetate **3** (10 mmol) in nitromethane (7 mL) in the presence of 4-methylbenzenesulfonic acid (15 mg, 0.075 mmol). The mixture was refluxed for 3 h. After cooling, the formed solid product was filtered off, washed with cold diethyl ether and re-crystallized from nitromethane.

9-Chloro-5-(*N*-formylamino)-3-(2-oxo-2*H*-chromen-3-yl)-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one (6a). Work-up as described above gave 69% of **6a** as orange powder: m.p. 290-292 °C decomp. (nitromethane). Anal. calcd. for $C_{22}H_{12}ClNO_6$ (421.8): C, 62.65; H, 2.87; Cl, 8.41; N, 3.32. Found: C, 62.81; H, 2.91; Cl, 8.04; N, 3.49. IR (KBr): 3060, 2995, 2970,

1700, 1650, 1600, 1545, 1490, 1370, 1300, 1295, 1198, 1100, 1022, 990, 908, 824, 756 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): 9.57 (d, 2/3H, *J* = 9.6 Hz, CH=O); 9.30 (t, 1/3H, J = 9.4 Hz, NH); 8.47 (d, 1/3H, *J* = 9.7 Hz, CH=O); 8.46 (s, 1 H, H-4'); 8.09 (s, 1 H, H-10); 8.04 (s, 1 H, H-4); 7.83 (dd, 1 H, *J* = 1.0, 7.7 Hz, H-5'); 7.69 – 7.65 (m, 1 H, H-8'); 7.55 – 7.51 (m, 2 H, H-7, H-7'); 7.48 (d, 1 H, *J* = 8.0 Hz, H-8); 7.42 (ddd, 1 H, *J* = 0.7, 7.5, 7.7 Hz, H-6'); 7.15 (t, 2/3H, *J* = 9.4 Hz, NH); 6.97 (d, 2/3 H, *J* = 6.5 Hz, H-5); 6.85 (d, 1/3 H, *J* = 6.1 Hz, H-5). ^{13}C NMR (75 MHz, DMSO-d_6): 164.6, 161.1, 158.6, 158.5, 153.0, 151.5, 150.0, 132.6, 132.5, 129.0, 126.4, 124.9, 121.7, 121.1, 121.0, 120.5, 119.6, 118.6, 116.2, 116.1, 107.3, 73.2.

5-(*N*-Formylamino)-3-(7-methoxy-2-oxo-2*H*-chromen-3-yl)-9-methyl-2*H*,5*H*-pyrano[3,2-*c*] chromen-2-one (6b). Work-up as described above gave 71% of **6b** as orange powder: m.p. 240-242°C decomp. (nitromethane). Anal. calcd. for $C_{24}H_{17}NO_7$ (431.4): C, 66.82; H, 3.97; N, 3.25. Found: C, 67.08; H, 3.90; N, 3.54. IR (KBr): 3105, 2995, 2965, 1705, 1650, 1598, 1545, 1495, 1366, 1295, 1195, 1103, 1031, 995, 889, 825 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): 9.54 (d, 2/3 H, *J* = 9.0 Hz, CH=O); 9.25 (t, 1/3 H, *J* = 10.5 Hz, NH); 8.47 (d, 1/3H, *J* = 9.2 Hz, CH=O); 8.41 (s, 1 H, H-4'); 8.08 (t, 1/3 H, *J* = 10.1 Hz, NH); 8.00 (s, 1 H, H-4); 7.76 (d, 1 H, *J* = 8.7 Hz, H-5'); 7.57 (d, 1 H, *J* = 1.1 Hz, H-10); 7.46 (d, 1 H, *J* = 8.1 Hz, H-8); 7.26 (d, 1 H, *J* = 8.2 Hz, H-6'); 6.99 (d, 1 H, *J* = 8.3 Hz, H-7); 7.27 (dd, 1 H, *J* = 2.2, 8.3 Hz, H-6'); 7.08 (d, 1 H, *J* = 2.2 Hz, H-8'); 6.92 (d, 2/3 H, *J* = 6.0 Hz, H-5); 6.76 (d, 1/3 H, *J* = 6.1 Hz, H-5); 3.88 (s, 3 H, MeO); 2.28 (s, 3 H, CH_3). The ^{13}C NMR spectrum was non-measurable because of the low solubility of **6b**.

5-(*N*-acetylamino)-9-methyl-3-(2-oxo-2*H*-chromen-3-yl)-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one (6c). Work-up as described above gave 78% of **6c** as orange powder: m.p. 244-247°C (nitromethane). Anal. calcd. for $C_{24}H_{17}NO_6$ (415.4): C, 69.39; H, 4.12; N, 3.37. Found: C, 69.58; H, 4.17; N, 3.46. IR (KBr): 3031, 2990, 2944, 1712, 1654, 1610, 1558, 1497, 1370, 1295, 1198, 1105, 1025, 991, 846, 757 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): 9.31 (d, 1 H, *J* = 8.9 Hz, NH); 8.44 (s, 1 H, H-4'); 8.00 (s, 1 H, H-4); 7.83 (dd, 1 H, *J* = 1.4, 7.8 Hz, H-5'); 7.67 (ddd, 1 H, *J* = 1.5, 7.8, 8.0 Hz, H-7'); 7.55 (d, 1 H, *J* = 1.3 Hz, H-10); 7.47 (d, 1 H, *J* = 8.2 Hz, H-8); 7.41 (ddd, 1 H, *J* = 1.4, 7.8, 8.0 Hz, H-6'); 7.28 (dd, 1 H, *J* = 1.6, 7.8 Hz, H-8'); 6.96 (d, 1 H, *J* = 8.3 Hz, H-7); 6.82 (d, 1 H, *J* = 8.8 Hz, H-5); 2.35 (s, 3 H, CH_3); 1.83 (s, 3 H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): 169.5, 158.9, 152.9, 151.6, 151.0, 142.8, 142.7, 142.3, 133.8, 132.5, 131.6, 129.0, 125.5, 122.4, 121.4, 119.3, 118.7, 117.6, 116.1, 114.5, 106.9, 74.3, 22.7, 20.2.

9-Chloro-5-[*N*-(ethoxycarbonyl)amino]-3-(7-methoxy-2-oxo-2*H*-chromen-3-yl)-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one (6d). Work-up as described above gave 76% of **6d** as orange powder: m.p. 215-218 °C (nitromethane). Anal. calcd. for $C_{25}H_{18}ClNO_8$ (495.9): C, 60.55; H, 3.66; N, 2.82; Cl, 7.15. Found: C, 60.72; H, 3.69; N, 3.06; Cl, 7.31. IR (KBr): 3260, 3050, 2985, 1705, 1699, 1620, 1515, 1495, 1380, 1312, 1250, 1200, 1145, 1060, 1005, 960, 801, 752 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): 8.79 (d, 1 H, *J* = 9.3 Hz, NH); 8.41 (s, 1 H, H-4'); 8.03 (s, 1 H, H-4); 7.75 (d, 1 H, *J* = 8.7 Hz, H-5'); 7.67 (d, 1 H, *J* = 2.4 Hz, H-10); 7.49 (dd, 1 H, *J* = 2.7, 8.7 Hz, H-7); 7.13 (d, 1 H, *J* = 8.7 Hz, H-8); 7.08 (d, 1 H, *J* = 2.4 Hz, H-8'); 7.01 (dd, 1 H, *J* = 2.4, 8.7 Hz, H-6'); 6.74 (d, 1 H, *J* = 9.0 Hz, H-5); 4.08 (q, 2 H, *J* = 7.2, 7.2, 7.5 Hz, CH_2); 3.89 (s, 3 H, OMe); 1.17 (t, 3 H, *J* = 6.9 Hz. CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): 162.9, 158.9, 158.6, 154.8, 151.2, 149.6, 143.1, 141.2, 132.1, 130.1, 130.0, 126.0, 121.4, 120.4, 119.6, 117.1, 116.3, 112.9, 112.1, 107.2, 100.3, 77.4, 60.5, 55.9, 14.25.

5-[*N*-(4-methylphenyl)sulfonamino]-3-(2-oxo-2*H*-chromen-3-yl)-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one (6e). Work-up as described above gave 79% of **6e** as yellow powder: m.p. 273-275 °C (nitromethane). Anal. calcd. for $C_{28}H_{19}NO_7S$ (513.5): C, 65.49; H, 3.73; N, 2.73; S, 6.24. Found: C, 65.71; H, 3.78; N, 2.98; S, 6.61. IR (KBr): 3035, 1718, 1624, 1599, 1530, 1499, 1400, 1340, 1242, 1150, 1010, 896, 779 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): 9.51 (d, 1 H, *J* = 9.2 Hz, NH); 8.45 (s, 1 H, H-4'); 7.93 (s, 1 H, H-4); 7.85 (dd, 1 H, *J* = 1.4, 7.6 Hz, H-5'); 7.72 - 7.68 (m, 3 H, H-7',2'',6''); 7.49 (d, 1 H, *J* = 7.3 Hz, H-10); 7.46 -7.39 (m, 2 H, H-7',8'); 7.38 (d, 2H, *J* = 8.2 Hz, H-3'',5''); 7.32 (ddd, 1 H, *J* = 1.6, 7.3, 7.9 Hz, H-6'); 7.13 (ddd, 1 H, *J* = 1.5, 7.6, 7.9 Hz, H-9); 6.62 (d, 1 H, *J* = 8.4 Hz, H-7); 6.27 (d, 1 H, *J* = 8.0 Hz, H-5); 2.40 (s, 3 H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): 160.3, 158.6, 158.6, 152.9, 151.5, 151.4, 142.9, 141.7, 138.9, 136.6, 132.7, 132.4, 129.4, 128.9, 126.5, 125.5, 124.8, 123.0, 122.5, 122.3, 121.0, 119.3, 118.6, 117.0, 114.6, 105.9, 79.0, 20.9.

9-Methyl-5-(2-oxo-1,3-oxazolidin-3-yl)-3-(2-oxo-2*H*-chromen-3-yl)-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one (6f). Work-up as described above gave 72% of **6f** as yellow powder: m.p. 185-187 °C (nitromethane). Anal. calcd. for $C_{25}H_{17}NO_7$ (443.4): C, 67.72; H, 3.86; N, 3.16. Found: C, 67.96; H, 3.89; N, 3.39. IR (KBr): 3086, 2988, 1709, 1694, 1616, 1525, 1493, 1375, 1310, 1264, 1207, 1145, 1071, 1012, 968, 822, 767 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): 8.48 (s, 1 H, H-4'); 7.99 (s, 1 H, H-4); 7.84 (dd, 1 H, *J* = 1.5, 7.5 Hz, H-5'); 7.68 (ddd, 1 H, *J* = 1.0, 8.2, 8.4 Hz, H-7'); 7.54 (d, 1 H, *J* = 1.3 Hz, H-10); 7.09 (d, 1H, *J* = 8.2 Hz, H-8'); 7.42 (ddd, 1 H, *J* = 0.9, 8.1, 8.4 Hz, H-6'); 7.31 (dd, 1 H, *J* =

1.5, 8.4 Hz, H-8); 7.04 (d, 1 H, *J* = 8.2 Hz, H-7); 6.93 (s, 1 H, H-5); 4.31 (q, 1H, *J* = 3.9, 4.2, 4.8 Hz CH_2); 4.18 (q, 1H, *J* = 8.4, 8.7, 8.7 Hz, CH_2); 3.47 (q, 1H, *J* = 3.9, 3.9, 4.8 Hz, CH_2); 3.19 (q, 1H, *J* = 8.7, 8.7, 8.9 Hz, CH_2); 2.35 (s, 3 H, CH_3). The ^{13}C NMR spectrum was unmeasurable because of the low solubility of **6f**.

2.5. 5-Hydroxy-3-(2-oxo-2*H*-chromen-3-yl) pyrano[2,3-b]chromen-2(10aH)-ones (7a–7c)

Method A: Compound **3** (10 mmol) was dissolved in acetic acid (10 mL) and the solution was stirred at 60°C for 2-3 h. After cooling, the yellow precipitate was filtered off, washed with diethyl ether and re-crystallized from toluene.

Method B: Compound **3l** or **5e** (10 mmol) was dissolved in 30% sulphuric acid (40 mL) and the solution was stirred at 100°C for 10 h. Yellow-green precipitate was filtered off, washed with water, then washed with diethyl ether and re-crystallized from toluene.

7-Fluoro-5-hydroxy-3-(2-oxo-2*H*-chromen-3-yl) pyrano[2,3-*b*]chromen-2(10a*H*)-one (7a). Method A, work-up as described above gave 70% of **7a** as yellow powder: m.p. 295-298 °C (toluene). Anal. calcd. for $C_{21}H_{11}FO_6$ (378.3): C, 66.67; H, 2.93; F, 5.02. Found: C, 66.691; H, 3.04; F, 5.38. IR (KBr): 3024, 2905, 1705, 1675, 1599, 1550, 1500, 1480, 1332, 1304, 1255, 1187, 1165, 1052, 945, 899, 826, 801 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): 8.49 (s, 1 H, H-4'); 8.12 (s, 1 H, H-4); 7.88 (brs, 1H, OH); 7.82 (dd, 1 H, *J* = 1.5, 7.8 Hz, H-5'); 7.68 (ddd, 1 H, *J* = 1.5, 7.2, 8.6 Hz, H-7'); 7.51 (d, 1H, *J* = 3.2 Hz, H-6); 7.49 (d, 1 H, *J* = 8.8 Hz, H-8'); 7.45 (ddd, 1 H, *J* = 0.9, 7.2, 7.8 Hz, H-6'); 7.37 (dd, 1 H, *J* = 3.2, 8.8 Hz, H-8); 7.19 (dd, 1 H, *J* = 4.4, 8.9 Hz, H-9); 6.45 (s, 1 H, H-10a). ^{13}C NMR (75 MHz, DMSO-d_6): 153.1, 151.8, 150.3, 142.9, 142.1, 132.4, 132.2, 129.0, 128.8, 128.5, 126.9, 124.8, 122.2, 120.1, 119.1, 118.9, 116.2, 115.6, 109.6, 106.3, 91.9.

7-Chloro-5-hydroxy-3-(2-oxo-2*H*-chromen-3-yl) pyrano[2,3-*b*]chromen-2(10a*H*)-one (7b). Method A, work-up as described above gave 68% of **7a** as yellow powder: m.p. 275 - 277 °C (toluene). Anal. calcd. for $C_{21}H_{11}ClO_6$ (394.8): C, 63,89; H, 2,81; Cl, 8,98. Found: C, 64,13; H, 2,89; Cl, 9,31. IR (KBr): 3020, 2900, 1705, 1670, 1600, 1550, 1499, 1480, 1330, 1300, 1255, 1180, 1164, 1055, 945, 895, 821, 799 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): 8.47 (s, 1 H, H-4'); 8.11 (s, 1 H, H-4); 7.94 (brs, 1H, OH); 7.83 (dd, 1 H, *J* = 1.5, 7.9 Hz, H-5'); 7.69 (d, 1H, J = 2.6 Hz, H-6); 7.68 (ddd, 1 H, *J* = 1.5, 7.2, 8.8 Hz, H-7'); 7.53 (dd, 1 H, *J* = 2.6, 8.7 Hz, H-8); 7.47 (dd, 1 H, *J* = 0.9, 8.8 Hz, H-8'); 7.41 (ddd, 1 H, *J* = 0.9, 7.2, 7.9 Hz, H-6'); 7.18 (d, 1 H, *J* = 8.7 Hz, H-9); 6.48 (s, 1 H, H-10a). The ^{13}C NMR spectrum was non-measurable because of the low solubility of **7b**.

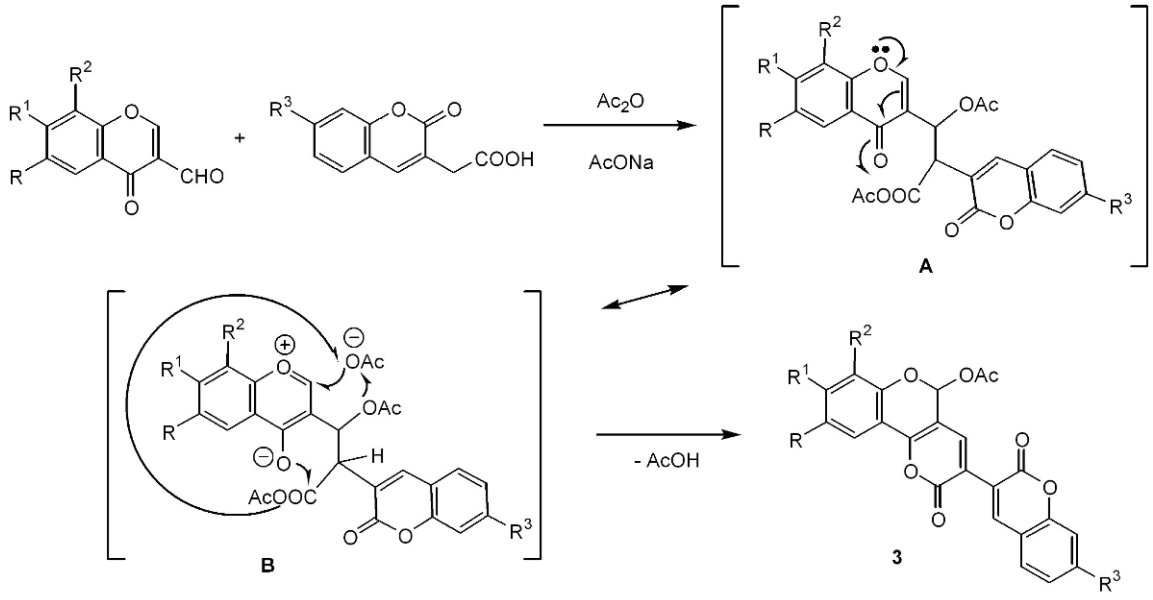
5-Hydroxy-3-(7-hydroxy-2-oxo-2*H*-chromen-3-yl) pyrano[2,3-*b*]chromen-2(10a*H*)-one (7c). Method B, work-up as described above gave 75% of **7c** as yellow-green powder: m.p. 237 - 239 °C (toluene). Anal. calcd. for $C_{21}H_{12}O_7$ (376.3): C, 67.02; H, 3.21. Found: C, 66.89; H, 3.02. IR (KBr): 3630,, 3599, 2927, 2858, 1715, 1692, 1614, 1468, 1359, 1328, 1228, 1197, 1159, 1128, 1027, 942, 896, 850, 787, 764 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): 12.75 (brs, 1 H, OH), 10.70 (brs, 1 H, OH), 8.55 (s, 1 H, Hz, H-4'), 8.11 (dd, 1 H, *J* = 1.6, 8.2 Hz, H-6), 8.10 (s, 1 H, H-4), 7.85 (ddd, 1 H, *J* = 1.6, 7.1, 8.7 Hz, H-8), 7.70 (dd, 1 H, *J* = 1.0, 8.7 Hz, H-9), 7.63 (d, 1 H, *J* = 8.5 Hz, H-5'), 7.54 (ddd, 1 H, *J* = 1.0, 7.1, 8.2 Hz, H-7),

6.99 (d, 1 H, *J* = 1.1 Hz, H-10a), 6.84 (dd, 1 H, *J* = 2.3, 8.5 Hz, H-8'), 6.77 (d, 1 H, *J* = 2.3 Hz, H-6'). ^{13}C NMR (75 MHz, DMSO-d_6): 174.6,167.3, 161.6, 159.6, 155.7, 155.4, 155.1, 141.7, 134.4, 133.9, 130.2, 125.8, 125.7, 125.4, 123.2, 122.0, 120.3, 118.5, 113.6, 111.5, 101.9.

3. Results and discussion

Synthesis of pyrano[3,2-*c*]chromen-2-one skeleton from azlactones or acrylates was already reported by Ghosh [21,22]. *N*-(5-Ethoxy-2-oxo-2*H*,5*H*-pyrano[3,2-*c*] chromen-3-yl)benzamides were prepared by heating of corresponding azlactones in the presence of pyridine [21]. Ethyl 5-ethoxy-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromene-3-carboxylate was obtained by reaction of ethyl 2-cyano-3-(4-oxo-4*H*-chromen-3-yl)acrylate in DMF-ethanol [22]. Among the condensations of coumarin-3-acetic acids only the reaction of **2** with furan-2-carbaldehydes was described [23] to obtain Perkin condensation products. Recently, we developed convenient synthesis of 2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetates **3** by heating equimolar quantities of chromene-3-carbaldehyde **1** and substituted acetic acids **2** in acetic anhydride in the presence of catalytic amount of sodium acetate or potassium carbonate [24,25].

3-(2-Oxo-2*H*-chromen-3-yl)-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetates **3** (Scheme 1) were isolated as the only products after 2 h of heating at 90–100ºC in high yields (56–85%). The isolation of acetates **3** from reaction mixtures was simple because they are formed as the first precipitates. Under these reaction conditions no traces of other products were found in the reaction mixtures according to the TLC and 1H-NMR analyse. Prolonged reaction time or increase in reaction temperature leads to a mixture of various by-products [24].



3a R = H, R^1= H, R^2 = H, R^3 = H
3b R = Me, R^1= H, R^2 = H, R^3 = H
3c R = Me, R^1= Me, R^2 = H, R^3 = H
3d R = F, R^1= H, R^2 = H, R^3 = H
3e R = Cl, R^1= H, R^2 = H, R^3 = H
3f R = NO_2, R^1= H, R^2 = H, R^3 = H

3g R = OAc, R^1= H, R^2 = H, R^3 = H
3h R = H, R^1= OAc, R^2 = H, R^3 = H
3i R = H, R^1= OAc, R^2 = OAc, R^3 = H
3j R,R^1= CH=CH-CH=CH, R^2 = H, R^3 = H
3k R = H, R^1= H, R^2 = H, R^3 = OMe
3l R = H, R^1= H, R^2 = H, R^3 = OAc
3m R = H, R^1= H, R^2 = H, R^3 = NMe_2

Scheme 1. Synthesis of 3-(2-oxo-2*H*-chromen-3-yl)-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetates **3**.

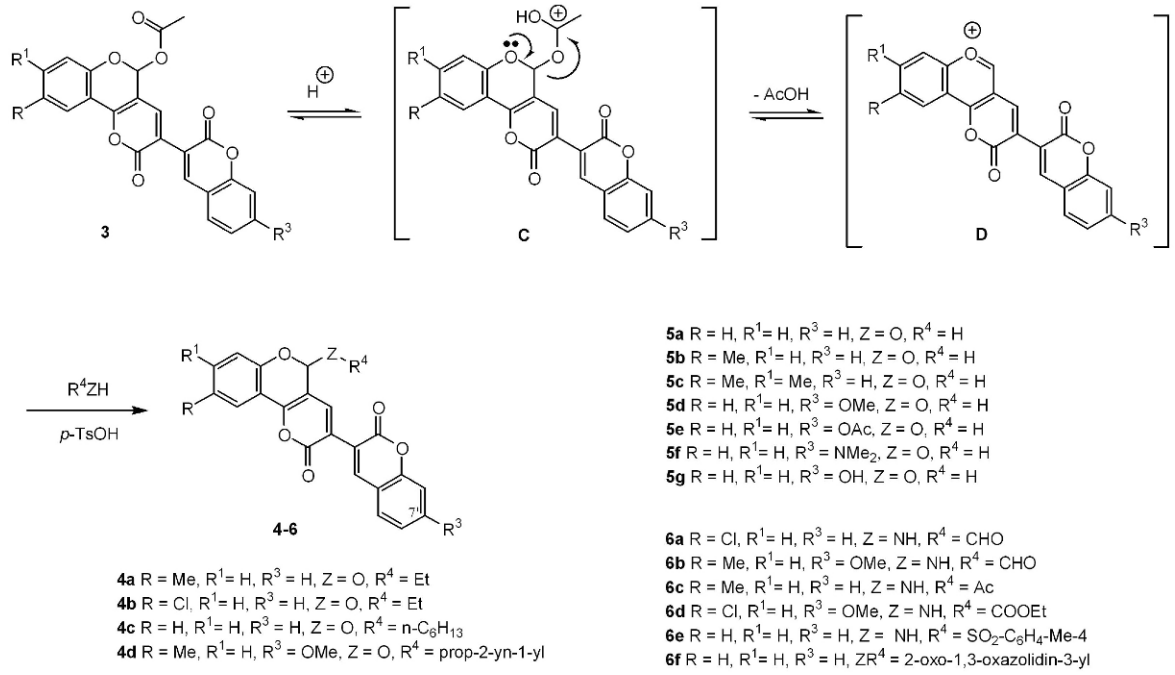
The mechanism, assumed for the formation of **3** involves the reaction of aldehydes **1** with the mixed *in situ* formed anhydride of acetic and coumarin-3-acetic acids **2** yielding acetylated aldol reaction intermediate **A**, which can exist in the form of pyrylium salt **B**. We proposed that the next step involves intramolecular nucleophilic attack of enolate anion from C-4 to the carboxylate C=O bond of **B** together with concomitant attack of acetate anion at C-2 position of pyrylium ring. Induced lactonisation as described above led to acetate **3**. Compounds **3** were also isolated when the same reaction was carried out in a microwave oven. In this case the yields of **3** were comparable to those, obtained under conventional heating (72–87%), but the duration under microwave irradiation was considerably shorter (10 min) and obtained product **3** was pure enough (^{1}H NMR spectra) to be used for the next reaction without any further purification.

The acetyloxy group at C-5 of acetates **3** easily undergoes nucleophilic substitution under acidic catalysis. Heating and stirring of **3** in ethanol in the presence of catalytic amount of 4-methylbenzenesulfonic acid for 3 h at 60°C yielded ethoxy derivatives **4a** and **4b** in 72 and 74% yields, respectively. In cases were the used alcohol is a solid or is expensive, it can be dissolved in toluene or nitrobenzene together with acetate **3** and 4-methylbenzenesulfonic acid. Heating of the reaction mixture at 100°C for 2 h yielded derivatives **4c** and **4d** in 67 and 68% yields, respectively.

Compounds **3** or **4** can be converted to 5-hydroxy-3-(2-oxo-2*H*-chromen-3-yl)-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-ones **5** in good yields (62-72%) by their heating at 70°C for 1h in dioxane-water media in the presence of catalytic amount of 4-methylbenzenesulfonic acid (Scheme 2).

Depending on the reaction media and temperature, 3-(7-acetyloxy-2-oxo-2*H*-chromen-3-yl)-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetate **3l** can give access to three different derivatives. When **3l** was treated with 4-methylbenzenesulfonic acid in dioxane-water (3:1) at 70°C for 1h, only C-5 acetyl group is removed to give 5-hydroxy-3-(7-acetyloxy-2-oxo-2*H*-chromen-3-yl)-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one **5e.** Both C-5 and C-7' acetyl groups were removed under stirring of **3l** in the solution of 96% H_2SO_4, H_2O and EtOH (5:3:2) for 10 min. The reaction temperature has to be kept under 20°C, because heating **3l** in 30% H_2SO_4 at elevated temperature (100°C) led to rearranged 5-hydroxy-3-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)pyrano[2,3-*b*]chromen-2(10a*H*)-one **7c**.

Treatment of acetates **3** with nitrogen containing compound (formamide, acetylamide,



Scheme 2. Exploitation of **3** for the synthesis of 5-substituted 3-(2-oxo-2*H*-chromen-3-yl)-2*H*,5*H*-pyrano[3,2-*c*]chromene derivatives **4-6**.

Table 1. The biological activity of 3a, 3m, 5b, 5d, 5f and 5g obtained from their antineoplastic screening on 60 cancer cell lines at NCI USA. The results represent concentration of compounds that are necessary to slow down the human tumor cells proliferation to half (GI_{50}) in average for all 60 cell lines (the 2nd column) or in the best cases for particular cancer cell lines (the 3rd column).

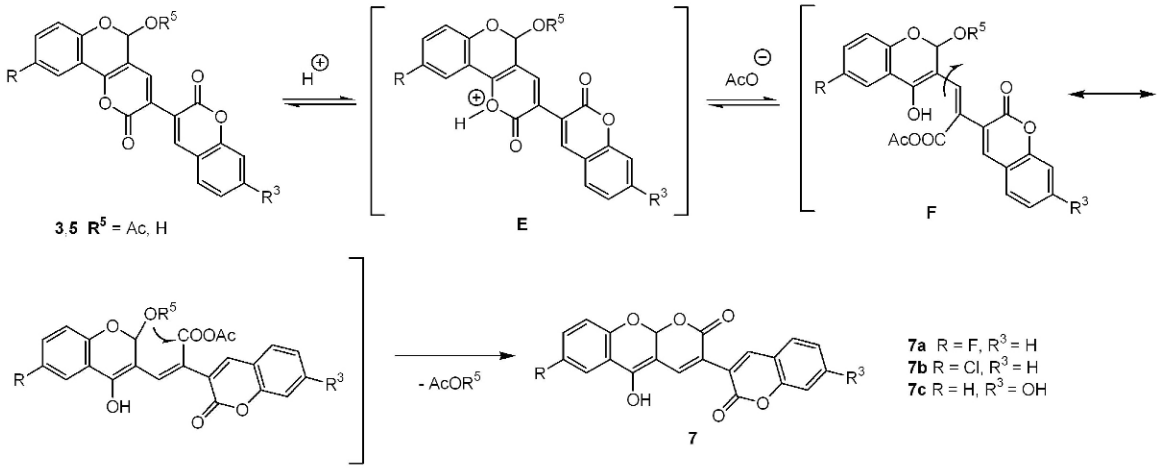
Compound	GI_{50} [×10^{-6} M] [a]	Panel / Cell line / the best GI_{50} value [10^{-6} M]
3a	22.3	Ovarian Cancer / SK-OV-3 / 13.7; Melanoma / MALME-3M / 13.9
3m	79.4	Leukemia / RPMI-8226 / 0.39; Ovarian Cancer / OVCAR-4 / 1.29; Colon Cancer / HCT-116 / 1.42; NSCLC[b] / EKVX / 1.78
5b	20.4	Melanoma / MALME-3M / 1.02; Renal Cancer / ACHN / 1.02
5d	15.9	CNS Cancer / SF-295 and SNB-75/ < 0.01 and 5.73, resp.
5f	3.9	Leukemia / SR and MOLT-4/ 0.44 and 0.92, resp.; Ovarian Cancer / OVCAR-4 / 0.55; Melanoma / SK-MEL-5 / 0.75; Breast Cancer / T-47D / 1.01; NSCLC[b] / NCI-H23 / 1.41; Colon Cancer / HCT-116 / 1.44
5g	17.8	Leukemia / SR and MOLT-4 / 2.65 and 4.33, resp.; Renal Cancer / RXF 393 / 4.50; NSCLC[b] / NCI-H23 / 5.53

[a] *an average value of GI_{50} (Growth Inhibition 50 %) value over the all 60 human cancer cell lines used in the assay panel at NCI USA*
[b] *NSCLC: Non-Small Cell Lung Cancer*

4-methylphenylsulfonamide, ethyl carbamate or 1,3-oxazolidin-2-one) in the presence of 4-methylbenzenesulfonic acid after 3h led to 5-R^4-aminosubstituted 3-(2-oxo-2*H*-chromen-3-yl)-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-ones **6** in 69-79% yields (Scheme 2). The mechanism, assumed for the formation of **4-6** involves the protonation of acetyloxy group of **3** (structure **C**) and cleavage of acetic acid led to the 2-oxo-2*H*-pyrano[3,2-*c*]chromen-6-ium **D**, which is further converted to product **4-6** by the addition of nucleophile (water, alcohol or nitrogen group) [25].

Rearrangement of 5-substituted 3-(2-oxo-2*H*-chromen-3-yl)-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromenes **3** or **5** to 5-hydroxy-3-(2-oxo-2*H*-chromen-3-yl) pyrano[2,3-*b*]chromen-2(10a*H*)-ones **7** readily occurs in acidic media (*e.g.* by acetic or sulphuric acid) and elevated temperature. Formation of **7** can be explained by the protonation of **3** or **5** to give intermediate **E**. Addition of acetyloxy anion (or water) to the protonated **E** and the ring opening lead to structure **F**. The intramolecular rotation around single bond in the molecule **F** and a final 1,6-ring closure results derivatives **7** in 68-75 % yields (Scheme 3).

Compounds **3a**, **3c**, **3d**, **3e**, **3k**, **3m**, **4a**, **4c, 5b**, **5d**, **5f** and **5g** were screened on their antineoplastic activity in NCI USA. Substances **3c**, **3d**, **3e**, **3k**, **4a** and **4c** were



Scheme 3. Rearrangement of **3** or **5** leads to the synthesis of 5-hydroxy-3-(2-oxo-2*H*-chromen-3-yl)pyrano[2,3-*b*]chromen-2(10a*H*)-ones **7**.

not active enough to be selected for the next screening. Compounds **3a**, **3m**, **5b**, **5d**, **5f** and **5g** were tested on 60 human cancer cell lines panel at 5 concentrations (from 10^{-4} M to 10^{-8} M). The most interesting results are depicted in Table 1.

4. Conclusions

3-(2-Oxo-2*H*-chromen-3-yl)-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetates **3** were synthesized by reaction of 4-oxo-4*H*-chromen-3-carbaldehydes **1** with coumarin-3-acetic acids **2** in acetic anhydride in the presence of sodium acetate. Reaction of **3** with alcohols gave corresponding 5-alkoxy-3-(2-oxo-2*H*-chromen-3-yl)-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-ones **4** by two ways. Either acetate **3** reacted with excess of ethanol in the presence of 4-methylbenzenesulfonic acid at mild conditions, or the reaction of **3** with alcohol (hexan-1-ol or prop-2-yn-1-ol) was carried out in toluene under acidic catalysis. Synthesis of some derivatives **4** was accelerated under microwave irradiation and completed within 10 min. Similarly, acid catalyzed reaction of acetates **3** with water yielded 5-hydroxy-3-(2-oxo-2*H*-chromen-3-yl)-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-ones **5**. Reaction of **3** with nitrogen containing compound (formamide, acetylamide, 4-methylphenylsulfonamide, ethyl carbamate or 1,3-oxazolidin-2-one) gave 5-R^4-aminosubstituted 2*H*,5*H*-pyrano[3,2-*c*]chromen-2-ones **6**. Derivatives **3** or **5** readily rearrange to 5-hydroxypyrano[2,3-*b*]chromen-2(10a*H*)-ones **7** under acid catalysis at elevated temperature.

Twelve prepared compounds were under antineoplastic activity evaluation. Six of them **3a**, **3m**, **5b**, **5d**, **5f** and **5g** passed pre-screening selection procedure and were further tested at different concentrations (from 10^{-4} M to 10^{-8} M) on 60 human tumor cell line panel at NCI USA. According the results from Table 1, we can conclude that all six screened compounds were able to slow down human cancer cells proliferation at micromolar concentration. For compound **5d** in one particular case (CNS Cancer / SF-295) GI_{50} activity was stated to be better as 10 nM. In this case a validation of this activity would be necessary to exclude possible false positive result. Even though the compound **3m has the lowest average GI_{50} value, in one cell line** (Leukemia / RPMI-8226) GI_{50} reaches the interesting concentration of 390 nM. Among all screened substances the compound **5f** has the most effective antineoplastic properties for both the average GI_{50} value (obtained through all 60 screened human cancer cell lines) and also for several particular cell lines where GI_{50} activities of **5f** were obtained at submicromolar concentrations. Based on the biological results substances possessing 3-(2-oxo-2*H*-chromen-3-yl)-2-oxo-2*H*,5*H*-pyrano[3,2-c]chromene skeleton are promising compounds with potential antineoplastic properties. Further structure activity relationship research is required in order to improve their antitumor activity.

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References

[1] C. K. Ghosh, Heterocycles 63, 2875 (2004)
[2] R.Gašparová, M. Lácová, Molecules 10, 937 (2005)
[3] D.H. Nam, K.Y. Lee, C.S. Moon, Y.S. Lee, Eur. J. Med. Chem. 45, 4288 (2010)
[4] T. Raj, R.K. Bhatia, R.K. Sharma, V. Gupta, D. Sharma, M.P.S. Ishar, Eur. J. Med. Chem. 44, 3209 (2009)
[5] J. Ungwitayatorn, C. Wiwat, W. Samee, P. Nunthanavanit, N. Phosrithong, J. Mol. Struct. 1001, 152 (2011)
[6] S. Lee, K. Sivakumar, W.-S. Shin, F. Xie, Q. Wang, Bioorg. Med. Chem. Lett. 16, 4596 (2006)
[7] N. Chen, N. Jain, J. Xu, M. Reuman, X. Li, R.K. Russell, Z. Sui, Tetrahedron Lett. 47, 5909 (2006)
[8] C.N. Huang, P.Y. Kuo, C.H. Lin, D.Y. Yang, Tetrahedron, 63, 10025 (2007)
[9] J. Quiroga, A. Rengifo, B. Insuasty, R. Abonia, M. Nogueras, A. Sanchez, Tetrahedron Lett. 43, 9061 (2002)
[10] O. Fatunsin, V.O. Iaroshenko, S. Dudkin, S. Mkrtchyan, A. Villinger, P. Langer, Tetrahedron Lett. 51, 4693 (2010)
[11] M.A. Ibrahim, Tetrahedron, 65, 7687 (2009)
[12] M. Terzidis, C.A. Tsoleridis, J. Stephanidou-Stephanatou, Tetrahedron 63, 7828 (2007)
[13] J. Nawrot-Modranka, E. Nawrot, J. Graczyk, J. Eur. J. Med. Chem. 41, 1301 (2006)
[14] L.J. Legoabe, A. Petzer, J.P. Petzer Eur. J. Med. Chem. 49, 343 (2012)
[15] R. Gašparová, M. Lácová, A. Krutošíková, Collect. Czech. Chem. Commun. 70, 2101 (2005)
[16] H. Stankovičová, A. Gáplovský, M. Lácová, J. Chovancová, A. Puchala, J. Heterocycl. Chem. 43, 843 (2006)
[17] M. Lácová, R. Gašparová, P. Koiš, A. Boháč, H.M. El-Shaaer, Tetrahedron 66, 1410 (2010)
[18] M. Lácová, A. Boháč, Patent SK 287542 B6 (2011)
[19] A. Nohara, T. Umetani, Y. Sanno, Tetrahedron 30, 3553 (1974)
[20] S. Kováčová, L. Kováčiková, M. Lácová, A. Boháč, M. Sališová, Chem. Papers 64, 806 (2010)
[21] C.K.Ghosh, C. Bandyopadhyay, Indian J. Chem., Sect. B 23, 1048 (1984)
[22] C.K.Ghosh, C. Bandyopadhyay, S. Biswas, A.K. Chandravarty, Indian J. Chem., Sect. B 29, 814 (1990)
[23] A.Y. Bochkov, V.N. Yarovenko, M.M. Krayushkin, T.A. Chibisova, T.M. Valova, V.A. Barachevskii, V.F. Traven, I.P. Beletskaya, Russian J. Org. Chem. 44, 595 (2008)
[24] L. Kováčiková, R. Gašparová, A. Boháč, M. Ďurana, M. Lácová, ARKIVOC 11, 188 (2010)
[25] M. Lácová, R. Gašparová, P. Koiš, A. Boháč, H.M. El-Shaaer, Tetrahedron 66,1410 (2010)