Synthesis of 3-phenyl-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one derivatives and their antineoplastic activity

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Abstract

Reaction of 4-oxo-4*H*-chromen-3-carbaldehydes **1** with phenylacetic acids **2** under mild conditions or microwave irradiation led to 3-phenyl-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetates **3**. At stronger conditions by-products 5-(2-hydroxybenzoyl)-3-phenyl-2*H*-pyran-2-ones **4** and 5-hydroxy-2*H*,10a*H*-pyrano[2,3-*b*]chromen-2-ones **5** were also obtained. 5-Hydroxy- and 5-alkyloxy-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-ones **6** and **7**, respectively were easily prepared by reaction of **3** with water or alcohols. Twelve synthesized compounds were evaluated on their antineoplastic activities on 60 human tumour cell lines panels in NCI USA. According to the screening results 3-phenyl-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one was discovered as a new leading skeleton suitable for further development. Some SAR conclusions were made. Antitubuline mechanism for the most active compound **3g** has been proposed.

Keywords: Pyranochromenones, phenylacetic acids, condensation, rearrangement, antineoplastic activity, microwave irradiation, tubuline inhibitors

Introduction

Synthetic exploitation of 4-oxo-4*H*-chromen-3-carbaldehyde **1** and its derivatives results from their reactivity towards nucleophiles. The wide variety of heterocycles can be synthesized due to the presence of three electrophilic centres in the molecule of **1** and its ability to open or retain pyran-4-one ring.[1] Many of chromene derivatives exhibit significant biological activity, such as antitumour,[2,3] antimycobacterial[4] or antiviral.[5]

Phenylacetic acid is versatile synthon for synthesis of a diverse set of biologically active compounds, e.g. aldose reductase inhibitors[6] or hPPAR agonists.[7] According to our knowledge only condensation of 4-oxo-4*H*-chromen-3-carbaldehyde **1** with phenylacetic acid **2** in pyridine catalyzed by potassium *tert*-butoxide has been described as the method for synthesis of 3-styryl-4*H*-chromen-4-ones.[8] In connection with this, and as an extension of our studies on the synthesis,[9,10] photochemical[11] and theoretical[12] properties of chromene derivatives, we decided to investigate the reactions of 4-oxo-4*H*-chromene-3-carbaldehyde **1** with substituted phenylacetic acids **2** under different conditions, to determine the products and evaluate their antineoplastic activities.

Results and Discussion

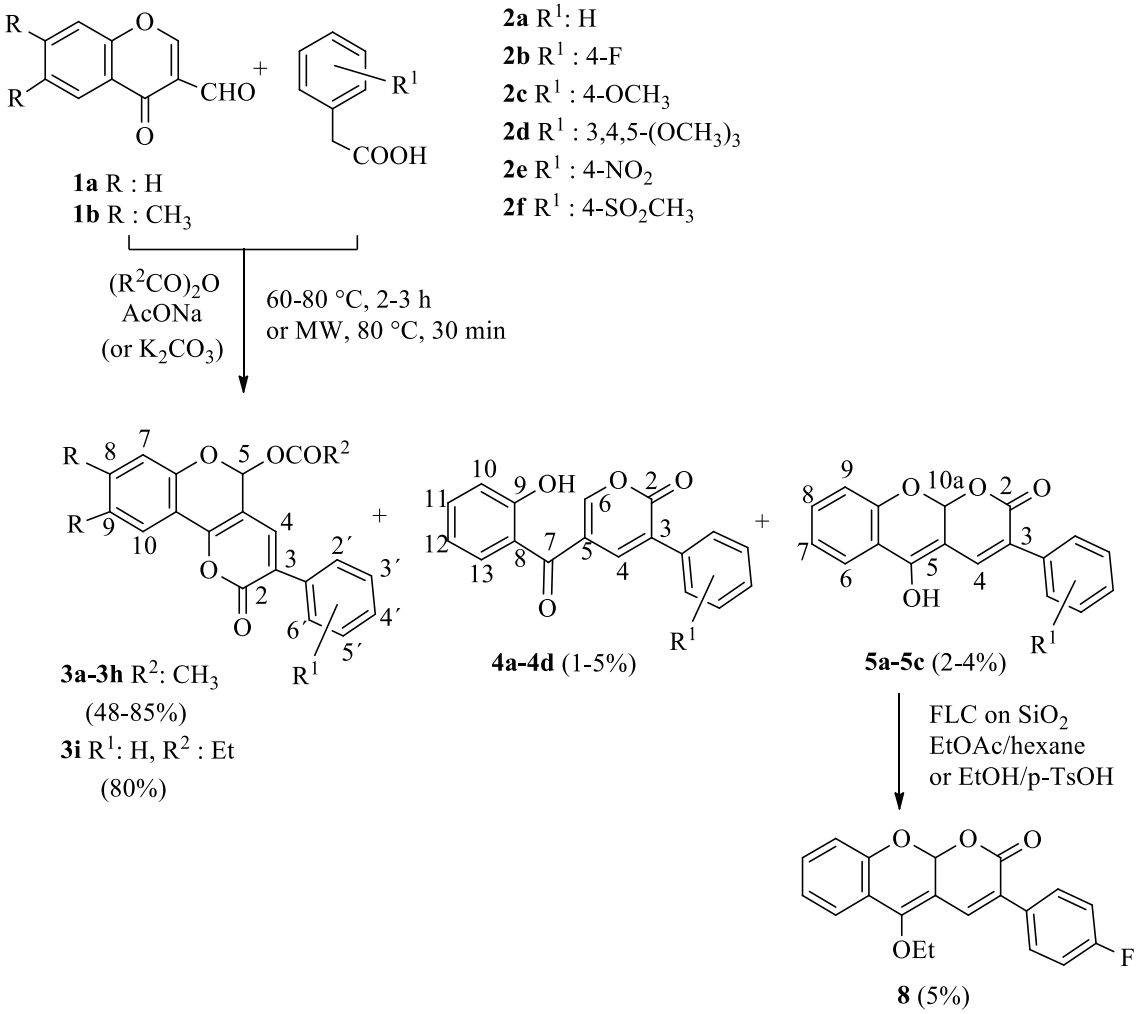
Chemistry

Several methods for the synthesis of pyrano[3,2-*c*]chromen-2-one derivatives were reported. *N*-(5-ethoxy-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-3-yl)benzamides were prepared by heating of azlactones in the presence of pyridine.[13] 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.[14]

Recently we developed convenient synthesis of 2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetates **3** by heating of equimolar quantities of chromene-3-carbaldehyde **1** and phenylacetic acids **2** in acetic anhydride in the presence of catalytic amount of sodium acetate or potassium carbonate.[15,16] When the mixture of **1** and **2** was heated at 60 – 80 °C for 2 - 3 h, the acetates **3a-3h** were isolated as only products in 48 - 81% yields. When the synthesis was accomplished in microwave oven at constant 80 °C, the yields of products **3** were slightly higher (68 - 85%), but the duration of the reaction was considerably shorter (30 min). The mixture of propionic anhydride with aldehyde **1a** and phenylacetic acid **2b** heated at 80 °C for 2h, or under microwave irradiation at 110 °C for 30 min both in the presence of potassium carbonate led to 3-(4-fluorophenyl)-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl propionate **3i** in 80% yield (Scheme 1).
In order to examine reaction profile and the thermal stability of acetates **3**, the mixture of **1** and **2** was treated at 100 °C for 12 – 22 h in Ac_2O. At these conditions, acetates **3** were isolated as the main products in 11 – 54%. The by-products 5-(2-hydroxybenzoyl)-3-phenyl-2*H*-pyran-2-ones **4** (1 – 5%) together with rearranged compounds 5-hydroxy-3-phenyl-2*H*,10a*H*-pyrano[2,3-*b*]chromen-2-ones **5** (2 – 4%) were obtained by flash chromatography of the crude reaction mixtures on SiO_2 (Scheme 1).

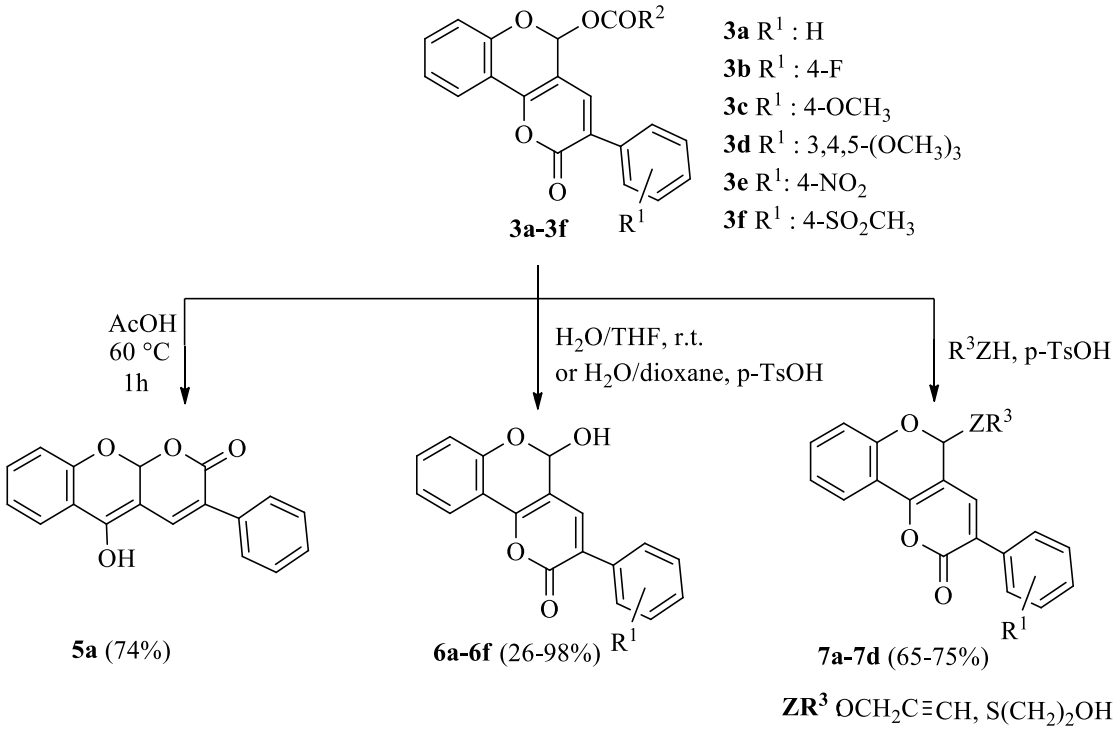
5-Hydroxy-3-phenyl-2*H*,10a*H*-pyrano[2,3-*b*]chromen-2-one **5a** was also prepared independently by rearrangement of **3a** in acetic acid with catalytic amount of p-TsOH at 60 °C within 1 h in 74% yield. The compound **5b** was partially converted to 5-ethoxy-3-(4-fluorophenyl)-2*H*,10a*H*-pyrano[2,3-*b*]chromen-2-one **8** during chromatography on SiO_2 by traces

of ethanol liberated from ethyl acetate eluent. Compound **8** can be also prepared from **5b** by its heating in EtOH in the presence of catalytic amount of p-TsOH (Scheme 2).



Scheme 1. The conditions for the synthesis of compounds **3a** – **8**.

We have observed that the acetyloxy group at C(5) in the compounds **3** easily undergoes nucleophilic substitution. The acetates **3** were partially converted into their alcohols **6** during flash chromatography purification on SiO_2. Therefore we have investigated the conditions for hydrolytic removing of AcO-group from **3** to prepare alcohols **6**. Acid catalyzed reaction of **3a** in the mixture of dioxane-water (3 : 1) at 50 °C within 2 h gave 74% of alcohol **6a**. Stirring of **3** with water in THF for 15 - 20 h at room temperature gave alcohols **6** in 26 – 98% yields (Scheme 2). Alkyloxy derivatives **7** were prepared by heating of **3** in alcohol (prop-2-yn-1-ol or 2-thioethanol) in the presence of catalytic amount of p-toluenesulfonic acid. The reaction heated at 60 °C for 3 h gave 65 – 75% of **7** compared to only 10 min microwave-assisted reaction that gives the yields 68 - 77% (Scheme 2).



Scheme 2. The conditions for the synthesis of compounds **5a** – **7d**.

Antineoplastic activity

Twelve from our prepared compounds were proposed to NCI USA for biological evaluation for anticancer properties (Table 1).[17]

Based on structures and obtained activities we can conclude that *para* substitution at Ph-C(3) in compounds **3** or **6** decreases antitumour properties with no relation to the electronic properties of substituents. Therefore in this case unsubstituted compounds are the most suitable (R^1: H or 4-F) **3g**, **3h**, **3b** and **6a**. Two methyl substituents in **3g**, **3h** lolcated in the skeleton on C(8) and C(9) seems to be important for the increase of antitumour activity.

Table 1. The One-dose pre-screening assay results performed on 60 human tumour cell lines incubated with experimental compounds at single concentration 10^{-5} M for 48 h

Compound	R^1	R	Mean Growth (%)	The Best (%)	Further evaluation
3a	H	H	[a]		N
3b	4-F	H	78.60	-35.40	60CC
3c	4-OMe	H	92.46	-25.88	N
3d	3,4,5-$(OCH_3)_3$	H	73.73	53.68	N
3e	4-NO_2	H	88.96	20.28	N

3g	H	Me	**10.92**	-42.23	60CC

Table 1. Continued

Compound	R^1	R	Mean Growth (%)	The Best (%)	Further evaluation
3h	4-F	Me	59.03	-7.18	60CC
6a	H	H	[b]		60CC
6b	4-F	H	75.59	3.53	N
6c	4- OCH_3	H	106.91	81.55	N
6e	4-NO_2	H	86.11	-13.61	N
6f	4-SO_2 CH_3	H	91.23	-3.93	N

[a,b]exceptionally two compounds have been pre-screened by an older method on three selected cell lines at single conc. 10^{-4} M. In this case to pass to further screenings at least one growth % should be below 32%: [a]NCI H460 – NSCLC lung cancer (growth 33%), MCF7 - breast cancer (96%), SF-268 – CNS cancer (107%); [b]NCI H460 (2%), MCF7 (17%), SF-268 (25%). Mean Growth (MID): represents average of growth % through all 60 human tumour cell lines at initial compound concentration 10^{-5} M compared to 100% for untreated tumour cell lines (controls). The Best inhibition activities are the border growth % observed in individual the most sensitive human tumour cell lines at One-dose compound concentration 10^{-5} M; **N**: no further screening indicated; **60CC**: means selection for screening on 60 human tumour cell line panel at five different concentrations from 10^{-4} M to 10^{-9} M. Negative values in the table represent cytotoxic influence of screened compound.

The most promising compounds **3b**, **3g**, **3h** and **6a**, derived from the results of One-dose pre-screening, were further evaluated at five different concentrations (10^{-5} to 10^{-9} M) on 60 human tumour cell line panels (Table 2).

Table 2. The results of five-Dose assay performed on 60 human tumour cell lines

Structure	GI_{50} [10^{-6} M] Mean growth inhibition 60CC and the best individual cell lines values	
3b	22.4	2.88
3g	**0.447**	**0.031**
3h	5.37	1.20
6a	7.41	1.62

Compound **3g** was shown to be the most active in slowing down tumour cell division compare to the control. Its structure contains unsubstituted phenyl group at C(3) and two methyl

substituents at C(8) and C(9) (Table 1). This compound possesses submicromolar mean growth inhibition activity over all 60 cancer cell lines panel (GI_{50}: 447 nM). Individual tumour cell lines (e.g. Leukemia, NSCLC, Melanoma) have shown even nanomolar sensitivities for this compound (GI_{50}: 31 - 63 nM, not stated in this paper).

The exact mechanism of antitumour activity of compound **3g** is not known. We believe that **3g** interacts with tubulines. The proposed mechanism is based on comparison of GI_{50} activity profile for individual tumour cell lines (kind of finger print) with profile of standard antitumour compounds having known antitumour mechanism. Compare analysis software is accessible *on-line* and it is free of charge.[18] The compare analysis has been done with **3g** inhibitor experimental GI_{50} values determined for all 60 human tumour cell lines (CC60 assays). These data were compared to sets of GI_{50} values of STANDARD_AGENTS taken from NCI database. A correlation between our most active compound **3g** (NSC:745523) with compounds possessing typical antitubuline interactions has been obtained: e.g. rhizoxin with NCI code NSC:S332598 (correlation coefficient, 0.597), maytansine NSC:S153858 (0.438), paclitaxel NSC:S125973 (0.400), S-trityl-L-cysteine NSC:S83265 (0.374), vinblastine sulfate NSC:S49842 (0.358). The list of compounds with known antitubuline mechanism is published in NCI databases.[19]

Conclusions

A new leading skeleton with 3-phenyl-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one structure has been identified thank to the NCI tumour cell lines screening possibilities. 3-Phenyl-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetates **3** were synthesized by the reaction of 4-oxo-4*H*-chromen-3-carbaldehydes **1** and substituted phenylacetic acids **2** in acetic anhydride. At stronger conditions, small amounts of two by-products 5-(2-hydroxybenzoyl)-3-phenyl-2*H*-pyran-2-ones **4** and 5-hydroxy-2*H*,10a*H*-pyrano[2,3-*b*]chromen-2-ones **5** were also obtained. Reaction of **3** with alcohols or water gave corresponding 5-hydroxy- and 5-alkyloxy 2*H*,5*H*-pyrano[3,2-c]chromen-2-ones **6** and **7**, respectively. Synthesis of some derivatives **3** and **7** were significantly accelerated under microwave irradiation and completed within 10–30 min. Based on the antitumour screening results and Beilstein database searches we found that 3-phenyl-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-ones represent a new type of antitumour compounds suitable for further development.[20] In our case the most active compounds were **3g**, **3h**, **3b** and **6a** (R^1: H or 4-F). *Para* substituents R^1 at Ph-C(3) in compounds **3** or **6** decreasing antitumour activity independently on their electronic properties. On the other site methyl groups at C(8) and C(9) in **3g**, **3h** seems to be important for observed activities. We proposed that the antitumour activity of **3g** performs *via* interaction with tubulines. This was based on comparisons of **3g** GI_{50} profile with similar GI_{50} CC60 profiles of the standard agents from NCI database.

Experimental Section

General. 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 in $CDCl_3$ or 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 or nujol. All microwave-assisted reactions were performed in an Initiator BIOTAGE microwave synthesizer. All solvents were distilled and dried appropriately prior to use. The course of reactions was monitored by TLC in ethyl acetate – hexane. Flash column chromatography was performed at SiO_2 (Merck Silica gel 60) with hexane/ethyl acetate as eluent. 4-Oxo-4*H*-chromene-3-carbaldehyde was synthesized according to the method, described by Nohara.[21] The other chemicals were purchased from the suppliers as the highest purity grade. Antitumour activities were determined *via* Developmental Therapeutics Program (DTP) NCI / NIH in USA.[22]

General procedures for (R^1-phenyl)-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetates (3a-3h)

Method A. A mixture of 4-oxo-4*H*-chromene-3-carbaldehyde **1a** or **1b** (20.0 mmol, 1.00 mol eq) and substituted phenylacetic acid **2** (20.0 mmol, 1.00 mol eq) in freshly distilled acetic anhydride (10 mL) and fused sodium acetate (45.0 mg, 1.14 mmol, 0.06 mol eq) was stirred for 2 h at 60 – 70 °C. After cooling the solid product **3** was filtered off, washed with cold diethyl ether and crystallized from dioxane abs.

Method B. A mixture of 4-oxo-4*H*-chromene-3-carbaldehyde **1a** (20.0 mmol, 1.00 mol eq) and substituted phenylacetic acid **2** (20.0 mmol, 1.00 mol eq) in freshly distilled acetic anhydride (10 mL) and potassium carbonate (10.0 mg, 0.72 mmol, 0.04 mol eq) was irradiated in microwave oven at 80 °C and 20W for 30 min. The product **3** was isolated and purified in the same manner as described in Method A.

Method C. A mixture of 4-oxo-4*H*-chromene-3-carbaldehyde **1a** (500.0 mg, 2.87 mmol, 1.00 mol eq) and substituted phenylacetic acid **2** (3.14 mmol, 1.09 mol eq) in freshly distilled acetic anhydride (1.5 mL) and fused sodium acetate (49.4 mg, 0.21 mmol, 0.07 mol eq) was stirred for 12-16 h at 100 °C under argon atmosphere. After cooling the yellow crystals were filtered off, washed with ethyl acetate (2 mL) and re-crystallized from ethyl acetate to give products **3**. After evaporation of solvents, the products **4** and **5**, respectively were separated by flash chromatography on SiO_2 with hexane/ethyl acetate (3:1).

3-Phenyl-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetate (3a). Method A, work-up as described above gave 81% of **3a**, (method B, 85%, method C, 11%) as yellow powder: mp 230–232 °C (ethyl acetate). Anal. calcd. for $C_{20}H_{14}O_5$ (334.3) requires: C, 71.85; H, 4.22. Found: C, 71.67; H, 4.24. IR *v*(KBr): 1717, 1651, 1638, 1565, 1550, 1490, 1460, 1376, 1222, 1198, 1116, 1043, 1001, 949, 893, 783, 760 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.00 (3H, s, CH_3),

7.13 (1H, d, *J* = 8.2, 0.8 Hz, H-7), 7.21 (1H, ddd, *J* = 7.7, 7.1, 0.8 Hz, H-9), 7.39 (1H, s, H-5), 7.41 -7.45 (3H, m, H-3',4',5'), 7.48 (1H, ddd, *J* = 7.7, 7.0, 1.4 Hz, H-8), 7.51 (1H, s, H-4), 7.68-7.71 (2H, m, H-2',6'), 7.92 (1H, dd, *J* = 7.7, 1.4 Hz, H-10); ^{13}C NMR (75 MHz, DMSO- d_6) δ 21.1, 91.3, 109.8, 114.5, 117.6, 122.1, 122.2, 124.7, 128.1, 128.1, 128.4, 128.4, 128.5, 132.5, 134.5, 139.9, 150.1, 152.7, 159.8, 172.1.

3-(4-Fluorophenyl)-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetate (3b). Method A, work-up as described above gave 60% of **3b** (method B, 70%, method C, 40%) as yellow powder: mp 244–246 °C (ethyl acetate). Anal. calcd. for $C_{20}H_{13}FO_5$ (352.3): C, 68.18; H, 3.72. Found: C, 68.41; H, 3.59. IR *v*(KBr): 1721, 1644, 1601, 1559, 1509, 1459, 1370, 1269, 1235, 1154, 1038, 937, 837, 760 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.02 (s, 3H, CH_3), 7.21 (dd, 1H, *J* = 8.3, 0.7 Hz, H-7), 7.25 -7.36 (m, 3H, H-9,3',5'), 7.45 (s, 1H, H-5), 7.54 (ddd, 1H, *J* = 8.3, 7.5, 1.6 Hz, H-8), 7.77-7.84 (m, 3H, H-10,2',6'), 8.00 (s, 1H, H-4). ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.7, 89.2, 106.0, 114.0, 115.2, 117.4, 117.4, 122.4, 123.4, 123.9, 130.2, 130.6, 133.0, 139.2, 150.9, 151.6, 151.6, 159.3, 162.1, 169.0.

3-(4-Methoxyphenyl)-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetate (3c). Method B, work-up as described above gave 68% of **3a** (method C, 54%) as yellow powder: mp 226–227 °C (ethyl acetate). Anal. calcd. for $C_{21}H_{16}O_6$ (364.4): C, 69.23; H, 4.43. Found: C, 69.61; H, 4.59. IR *v*(KBr):1713, 1648, 1609, 1559, 1513, 1459, 1250, 1177, 1030, 941, 826, 760 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 2.01 (s, 3H, CH_3), 3.80 (s, 3H, CH_3O), 6.99-7.06 (m, 2H, H-3',5'), 7.19 (dd, 1H, *J* = 8.2, 2.4 Hz, H-7), 7.27 (ddd, 1H, *J* = 8.0, 7.8, 2.4 Hz, H-9), 7.44 (s, 1H, H-5), 7.52 (ddd, 1H, *J* = 8.2, 7.8, 1.5 Hz, H-8), 7.68-7.75 (m, 2H, H-2',6'), 7.78 (dd, 1H, *J* = 8.0, 1.5 Hz, H-10), 7.92 (s, 1H, H-4). ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.8, 55.2, 89.3, 106.1, 113.8, 113.8, 114.2, 117.4, 122.3, 123.4, 124.7, 126.4, 129.4, 129.4, 132.7, 137.7, 150.1, 151.4, 159.5, 159.6, 169.1.

3-(3,4,5-Trimethoxyphenyl)-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetate (3d). Method A, work-up as described above gave 53% of **3d** (method C, 16%) as yellow powder: mp 175–180 °C (ethyl acetate). Anal. calcd. for $C_{23}H_{20}O_8$ (424.4): C, 65.09; H, 4.75. Found: C, 65.33; H, 4.44. IR *v* (KBr): 1733, 1648, 1555, 1462, 1374, 1331, 1239, 1192, 1127, 1003, 926, 764 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.02 (s, 3H, CH_3), 3.71 (s, 3H, CH_3O), 3.83 (s, 6H, 2 x CH_3O), 7.08 (s, 2H, H-2',6'), 7.21 (dd, 1H, *J* = 8.1, 1.0, H-7), 7.29 (ddd, 1H, *J* = 7.7, 7.3, 1.0 Hz, H-9), 7.44 (s, 1H, H-5), 7.54 (ddd, 1H, *J* = 8.1, 7.3, 1.3 Hz, H-8), 7.80 (dd, 1H, *J* = 7.7, 1.3 Hz, H-10), 8.02 (s, 1H, H-4). ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.7, 55.9, 60.0, 89.1, 105.7, 105.8, 114.0, 117.3, 122.3, 123.4, 124.7, 129.5, 132.9, 138.0, 138.8, 150.5, 151.4, 152.5, 159.1, 169.1.

3-(4-Nitrophenyl)-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetate (3e). Method A, work-up as described above gave 49% of **3e** (method B, 72%) as yellow powder: mp 249–251 °C (ethyl acetate). Anal. calcd. for $C_{20}H_{13}NO_7$ (379.3): C, 63.33; H, 3.45; N, 3.69. Found: C, 62.86; H, 3.90; N, 3.75. IR *v* (nujol): 1763, 1734, 1639, 1601, 1547, 1517, 1346, 1216, 1139, 1110, 1042, 1000, 938, 852, 767, 738, 691, 621. ^{1}H NMR (300 MHz, DMSO-d_6,) δ 2.02 (s, 3H, CH_3O), 7.22 (dd, 1H, *J* = 8.3, 1.0 Hz, H-7), 7.30 (ddd, 1H, *J* = 7.9, 7.5, 1.0 Hz, H-9), 7.46 (s, 1H, H-5), 7.57 (ddd, 1H, *J*= 8.3, 7.5, 1.6 Hz, H-8), 7.83 (dd, 1H, *J* = 7.9, 1.6 Hz, H-10), 8.02-8.08 (m, 2H, H-

2',6'), 8.23 (s, 1H, H-4), 8.30-8.36 (m, 2H, H-3',5'). ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.8, 89.1, 106.0, 113.8, 117.5, 122.5, 122.8, 123.5, 123.4, 129.1, 133.6, 140.8, 141.5, 146.9, 151.9, 152.3, 158.9, 169.1.

3-[(4-Methylsulfonyl)phenyl]-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetate (3f). Method A, work-up as described above gave 70% of **3f** as yellow powder: mp 240-242 °C (ethyl acetate). Anal. calcd. for $C_{21}H_{18}O_7S$ (412.4): C, 61.16; H, 3.91; S, 7.77. Found: C, 61.05; H, 4.36; S, 7.54. IR *v* (nujol): 1755, 1733, 1646, 1556, 1292, 1219, 1194, 1148, 1044, 999, 925, 846, 765 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.02 (s, 3H, CH_3O), 7.22 (dd, 1H, *J* = 8.2, 0.8 Hz, H-7), 7.30 (ddd, 1H, *J* = 7.9, 0.8 Hz, H-9), 7.48 (s, 1H, H-5), 7.57 (ddd, 1H, *J* = 8.2, 7.3, 1.4 Hz, H-8), 7.83 (dd, 1H, *J* = 7.9, 1.4 Hz, H-10), 8.00-8.04 (m, 4H, H-2',3',5',6'), 8.02 (s, 1H, H-4). ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.5, 43.9, 91.6, 110.2, 114.7, 118.1, 122.6, 122.8, 123.3, 127.4, 129.3, 133.3, 139.9, 140.6, 141.9, 151.6, 153.4, 159.9, 172.5.

8,9-Dimethyl-2-oxo-3-phenyl-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetate (3g). Method A, work-up as described above gave 74% of **3g** as yellow powder: mp 205–207 °C (ethyl acetate). Anal. calcd. for $C_{22}H_{18}O_5$ (362.4): C, 72.92; H, 5.01. Found: C, 73.09; H, 4.97. IR *v* (KBr): 3541, 3034, 2917, 1725, 1714, 1646, 1551, 1502, 1357, 1227, 1187, 1136, 1024, 1001, 935, 912, 887, 783, 699, 521 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): 1.97 (3H, s, CH_3), 2.27 (6H, s, 2 x CH_3), 7.01 (1H, s, H-7), 7.39 (1H, s, H-5), 7.40–7.48 (3H, m, H-3', 4', 5'), 7.54 (1H, s, H-4), 7.70–7.74 (2H, m, H-2',6'), 7.92 (1H, s, H-10); ^{13}C NMR (75 MHz, DMSO-d_6): 18.6, 19.8, 20.7, 89.5, 105.3, 109.0, 111.5, 118.1, 122.5, 123.9, 127.9, 128.0, 128.3, 128.5, 131.8, 134.4, 139.6, 142.8, 149.9, 151.5, 159.4, 169.1.

3-(4-Fluorophenyl)-8,9-dimethyl-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl acetate (3h). Method A, work-up as described above gave 73% of **3h** as yellow powder: mp 246–248 °C (ethyl acetate). Anal. calcd. for $C_{22}H_{17}FO_5$ (380.4): C, 69.47; H, 4.50. Found: C, 69.29; H, 4.47. IR *v* (KBr): 3443, 2926, 2857, 1728, 1686, 1638, 1547, 1510, 1459, 1377, 1357, 1303, 1233, 1163, 1105, 1051, 1030, 965, 893, 837, 774, 663, 527 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d6): 2.00 (3H, s, CH_3), 2.25 (3H, s, CH_3), 2.48 (3H, s, CH_3), 7.01 (1H, s, H-5), 7.29 (2H, ddd, *J* = 8.9, 7.4, 2.1 Hz, H-2', 6'), 7.37 (1H, s, H-7), 7.54 (1H, s, H-10), 7.77 (2H, ddd, *J* = 8.9, 7.7, 2.0 Hz, H-3', 5'), 7.95 (1H, s, H-4); ^{13}C NMR (75 MHz, DMSO-d6): 18.6, 19.8, 20.8, 91.2, 109.0, 111.9, 115.1, 115.3, 118.3, 122.3, 127.4, 130.1, 130.2, 131.9, 133.6, 134.9, 140.0, 142.0, 150.7, 150.9, 159.9, 161.6.

General procedures for 3-(4-fluorophenyl)-2-oxo-2*H*,5*H*-pyrano[3,2-*c*]chromen-5-yl propionate (3i)

Method D. A mixture of 4-oxo-4*H*-chromene-3-carbaldehyde **1** (3.00 mmol, 1.00 mol eq) and 4-fluorophenylacetic acid **2b** (3.00 mmol, 1.00 mol eq) in propionic anhydride (5 mL) and potassium carbonate (50.0 mg, 0.36 mmol, 0.12 mol eq) was stirred for 2 h at 80 °C. After cooling, diethyl ether (20 mL) was added and the mixture was cooled at -10 °C for 1 h. The solid product was filtered off, washed with cold diethyl ether and crystallized from dioxane to give 80% of **3i** as yellow powder.

Method E. A mixture of 4-oxo-4*H*-chromene-3-carbaldehyde **1** (1.00 mmol, 1.00 mol eq) and 4-fluorophenylacetic acid **2b** (1.00 mmol, 1.00 mol eq) in propionic anhydride (2 mL) and potassium carbonate (10.0 mg, 0.07 mmol, 0.002 mol eq) was irradiated in microwave oven at 110 °C and 20 W for 30 min. The product **3i** was isolated and purified in the same manner as described in Method A to give 82% of **3i** as yellow powder.

Method D, work-up as described above gave 80% of **3i** (method E, 82%) as yellow powder, m.p. 255-257 °C (ethyl acetate). Anal. calcd. for $C_{21}H_{15}FO_5$ (366.3): C, 69.85; H, 4.13. Found: C, 70.08; H, 4.16. IR *v* (nujol): 1748, 1730, 1645, 1557, 1292, 1220, 1195, 1152, 1045, 999, 930, 845, 770 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 0.99 (t, 3H, *J* = 7.4 Hz, CH_3), 2.30 (q, 2H, *J* = 7.4 Hz, CH_2), 7.20 (d, 1H, *J* = 8.2 Hz, H-7), 7.26 -7.35 (m, 3H, H-9,3',5'), 7.47 (s, 1H, H-5), 7.54 (ddd, 1H, *J* = 8.2, 7.1, 1.6 Hz, H-8), 7.77-7.83 (m, 3H, H-10,2',6'), 8.01 (s, 1H, H-4).

General procedure for 5-(2-hydroxybenzoyl)-3-(R^1-phenyl)-2*H*-pyran-2-ones (4a-4d)

Compounds **4** were isolated by flash chromatography from the mother liqueur obtained from the reaction after removing product **3** by filtration. (see Method C).

5-(2-Hydroxybenzoyl)-3-phenyl-2*H*-pyran-2-one (4a). Method C, work-up as described above gave 5% of **4a**, as yellow powder: mp 270-273 °C. R_f = 0.24 in EtOAc/hexane 1:1. Anal. calcd. for $C_{18}H_{12}O_4$ (292.3): C, 73.97; H, 4.14. Found: 73.74; H, 4.22. IR *v*(KBr): 1650, 1575, 1470, 1412, 1356, 1290, 1228, 1125, 1007, 909, 756 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 7.06-7.33 (m, 7H, H-4,6,Ph), 7.47 (ddd, 1H, *J* = 7.9, 7.0, 1.1 Hz, H-12), 7.51 (d, 1H, *J* = 8.6 Hz, H-10), 7.75 (ddd, 1H, *J* = 8.6, 7.0, 1.7 Hz, H-11), 8.08 (dd, 1H, *J* = 7.9, 1.7 Hz, H-13), OH not seen.

3-(4-Fluorophenyl)-5-(2-hydroxybenzoyl)-2*H*-pyran-2-one (4b). Method C, work-up as described above gave 2% of **4b**, as yellow powder: mp 290-292 °C. R_f = 0.22 in EtOAc/hexane 1:3. Anal. calcd. for $C_{18}H_{11}FO_4$ (310.1): C, 69.68; H, 3.57. Found: C, 69.18; H, 3.47. IR *v* (KBr): 1721, 1509, 1462, 1262, 1223, 1119, 1073, 1019, 799, 744, 694 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 7.17-7.25 (m, 2H, H-3',5'), 7.25-7.32 (m, 2H, H-2',6'), 7.51 (ddd, 1H, *J* = 8.0, 7.1, 1.0 Hz, H-12), 7.57 (d, 1H, *J*= 8.5, 1.0, H-10), 7.60 (d, 1H, *J* = 0.9, H-6), 7.77 (d, 1H, *J* = 0.9, H-4), 7.81 (ddd, 1H, *J* = 8.5, 7.1, 1.7 Hz, H-11), 8.09 (dd, 1H, *J* = 8.0, 1.7, H-13), 12.89 (br s, 1H, OH). ^{13}C NMR (300 MHz, DMSO-d_6) δ 115.5, 118.5, 119.0, 123.0, 125.3, 126.0, 130.1, 131.6, 131.8, 134.0, 134.6, 155.2, 156.4, 161.6, 167.5, 175.1.

5-(2-Hydroxybenzoyl)-3-(4-methoxyphenyl)-2*H*-pyran-2-one (4c). Method C, work-up as described above gave 1% of **4c**, as yellow powder: mp 235-238 °C. R_f = 0.19 in EtOAc/hexane 1:1. Anal. calcd. for $C_{19}H_{14}O_5$ (322.3): C, 70.80; H, 4.38. Found: C, 70.53; H, 4.64. IR *v* (KBr): 1725, 1515, 1468, 1274, 1230, 1121, 1081, 1025, 799, 748 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 3.77 (s, 1H, CH_3O), 6.92-6.98 (m, 2H, H-3',5'), 7.12-7.17 (m, 2H, H-2',6'), 7.51 (ddd, 1H, *J* = 8.0, 7.1, 1.0 Hz, H-12), 7.55 (d, 1H, *J* = 0.9, H-6), 7.57 (d, 1H, *J* = 8.5 Hz, H-10), 7.72 (d, 1H, *J* = 0.9 Hz, H-4), 7.81 (ddd, 1H, *J* = 8.5, 7.1, 1.7 Hz, H-11), 8.09 (dd, 1H, *J* = 8.0, 1.7 Hz, H-13), 12.73 (br s, 1H, OH). ^{13}C NMR (300 MHz, DMSO-d_6) δ 54.9, 113.9, 118.4, 119.1, 122.9, 125.2, 125.9, 127.4, 129.0, 130.2, 130.5, 134.5, 155.0, 156.2, 158.6, 167.8, 175.1.

5-(2-Hydroxybenzoyl)-3-(3,4,5-trimethoxyphenyl)-2*H*-pyran-2-one (4d). Method C, work-up as described above gave 5% of **4d**, as yellow powder: mp 230-233 °C. R_f = 0.26 in EtOAc/hexane 1:2. Anal. calcd. for $C_{21}H_{18}O_7$ (322.1): C, 70.80; H, 4.38. Found: C, 70.35; H, 4.51. IR *v* (KBr): 1655, 1578, 1466, 1412, 1354, 1285, 1227, 1123, 1003, 903, 756 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 3.80 (s, 6H, 2 x CH_3O). 3.89 (s, 3H, CH_3O), 6.48 (s, 2H, H-2',6'), 7.38 (dd, 1H, *J* = 8.6, 0.9 Hz, H-10), 7.43 (ddd, 1H, *J* = 8.0, 0.9 Hz, H-12), 7.46 (d, 1H, *J* = 0.7 Hz, H-6), 7.67 (ddd, 1H, *J* = 8.6, 7.0, 1.6 Hz, H-11), 8.18 (d, 1H, *J* = 0.7 Hz, H-4), 8.27 (dd, 1H, *J* = 8.0, 1.6 Hz, H-13), OH not seen.

General procedures 5-hydroxy-3-(R^1-phenyl)-2*H*,10a*H*-pyrano[2,3-*b*]chromen-2-ones (5a–5c)

Method F. Compound **3a** (10.0 mmol) was dissolved in acetic acid (10 mL) and the solution was stirred at 60 °C for 1 h. After cooling the yellow precipitate was filtered off, washed with diethyl ether and crystallized from toluene or acetic acid.

Method G. Compounds **5** as the side products were isolated by flash chromatography from the filtrate obtained after products **3** isolation (see Method C).

5-Hydroxy-3-phenyl-2*H*,10a*H*-pyrano[2,3-*b*]chromen-2-one (5a). Method F, work-up as described above gave 77% of **5a** (method G, 2 %) as yellow powder: mp 256-258 °C. R_f = 0.62 in EtOAc/hexane 1:1. Anal. calcd. for $C_{18}H_{12}O_4$ (292.3): C, 73.97; H, 4.14. Found: C, 74.18; H, 4.16. IR *v* (KBr): 3450, 3060, 1731, 1715, 1645, 1610, 1557, 1489, 1460, 1371, 1355, 1316, 1296, 1271, 1233, 1204, 1180, 1113, 1037, 1019, 951, 912, 785, 759, 737 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 6.73 (1H, s, H-10a), 7.15 (1H, s, H-4), 7.17 (1H, dd, *J*= 8.2, 1.0 Hz, H-9), 7.21 (1H, ddd, *J* = 7.6, 7.4, 1.0 Hz, H-7), 7.35–7.41 (3H, m, H-3', 4', 5'), 7.42 (1H, ddd, *J*= 8.2, 7.1, 1.5 Hz, H-8), 7.47–7.51 (2H, m, H-2', 6'), 7.89 (1H, dd, *J* = 7.7, 1.5 Hz, H-6), 8.12 (1H, s, OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 30.7, 96.9, 107.5, 114.3, 117.7, 122.1, 122.6, 122.8, 125.0, 128.0, 128.3, 128.6, 132.5, 134.4, 139.4, 150.3, 151.8, 159.4.

3-(4-Fluorophenyl)-5-hydroxy-2*H*,10a*H*-pyrano[2,3-*b*]chromen-2-one (5b). Method G, work-up as described above gave 4% of **5b**, as yellow powder: mp 260-262 °C. R_f = 0.52 in EtOAc/hexane 1:1. Anal. calcd. for $C_{18}H_{11}FO_4$ (310.3): C, 69.68; H, 3.57. Found: C, 69.44; H, 3.79. IR *v* (KBr): 3450, 1730, 1715, 1645, 1615, 1557, 1485, 1375, 1355, 1298, 1271, 1230, 1204, 1180, 1040, 1025, 951, 918, 785 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 6.90 (s, 1H, H-10a), 7.23 (ddd, 1H, *J* = 7.7, 7.4, 1.1 Hz, H-7), 7.28 (dd, 1H, *J* = 8.3, 1.1 Hz, H-9), 7.24-7.33 (m, 2H, H-3',5'), 7.53 (ddd, 1H, *J* = 8.3, 7.4, 1.5 Hz, H-8), 7.56 (s, 1H, H-4), 7.58-7.65 (m, 2H, H-2', 6'), 7.72 (dd, 1H, *J* = 7.7, 1.5 Hz, H-6), OH not seen.

5-Hydroxy-3-(4-methoxyphenyl)-2*H*,10a*H*-pyrano[2,3-*b*]chromen-2-one (5c). Method G, work-up as described above gave 2% of **5b**, as yellow powder: mp 260-262 °C. R_f = 0.34 in EtOAc/hexane 1:1. Anal. calcd. for $C_{19}H_{14}O_5$ (322.3): C, 70.80; H, 4.38. Found: 70.63; H, 4.52. IR *v* (KBr): 3440, 1728, 1720, 1651, 1615, 1559, 1480, 1375, 1358, 1292, 1277, 1206, 1188, 1025, 955, 919, 780 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 3.81 (s, 3H, CH_3O), 6.90 (s, 1H, H-10a), 7.01-7.03 (m, 2H, H-3',5'), 7.23 (ddm, 1H, *J* = 7.6, 4.4 Hz, H-7), 7.30-7.32 (m, 1H, H-9),

7.50 (s, 1H, H-4), 7.45-7.60 (m, 3H, H-8, 2',6'), 7.70-7.74 (m, 1H, H-6), OH not seen. ^{13}C NMR (300 MHz, DMSO-d_6) δ 55.2, 93.1, 107.2, 113.8, 114.6, 117.6, 122.3, 123.1, 124.8, 126.3, 129.2, 132.5, 137.0, 149.8, 151.3, 159.5, 159.6.

General procedures for 5-hydroxy-3-(R^1-phenyl)-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-ones (6a–6f)

Method H. A mixture of acetate **3** (0.41 mmol, 1.00 mol eq) and p-toluenesulfonic acid (15 mg, 0.09 mmol, 0.22 mol eq) was heated in 50 mL of mixture dioxane–water (3:1) at 50 °C for 2 h. After cooling the solid product was filtered off, washed with cold diethyl ether and crystallized from dioxane.

Method I. A mixture of acetate **3** (0.41 mmol, 1.00 mol eq), and water (0.5 mL, 27.8 mmol, 67.80 mol eq) in 0.5 mL of THF was stirred at room temperature for 15 h. Then the mixture was diluted with ethyl acetate (20 mL) and extracted with water (3 x 20 mL). The organic layer was dried with Na_2SO_4. After filtration and evaporation of ethyl acetate, the solid product was crystallized from dioxane.

5-Hydroxy-3-phenyl-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one (6a). Method H, work-up as described above gave 74% of **6a** as yellow powder: mp 150–151 °C (dioxane), R_f = 0.62 in EtOAc/hexane 1:1. Anal. calcd. for $C_{18}H_{12}O_4$ (292.3): C, 73.97; H, 4.14. Found: C, 74.19; H, 4.13. IR *v* (KBr): 3381, 3030, 1760, 1705, 1639, 1610, 1546, 1464, 1371, 1352, 1220, 1191, 1175, 1042, 1018, 973, 933, 899, 758, 696 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.51 (1H, d, *J* = 6.4 Hz, OH), 6.43 (1H, d, *J* = 6.6 Hz, H-5), 7.07 (1H, dd, *J* = 8.1, 1.0 Hz, H-7), 7.13 (1H, ddd, *J* = 8.1, 1.0 Hz, H-8), 7.39 -7.44 (4H, m, H-9,3',4',5'), 7.49 (1H, s, H-4), 7.66–7.71 (2H, m, H-2',6'), 7.87 (1H, dd, *J* = 7.8, 1.7 Hz, H-10). ^{13}C NMR (75 MHz, DMSO-d_6) δ 91.1, 109.6, 114.4, 117.5, 121.9, 122.0, 124.5, 124.8, 125.2, 127.9, 128.2, 132.3, 134.4, 134.8, 139.7, 152.6, 159.7, 168.3.

3-(4-Fluorophenyl)-5-hydroxy-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one (6b). Method I, work-up as described above gave 78% of **6b** as yellow powder: mp 290 °C dec., R_f = 0.52 in EtOAc/hexane 1:1. Anal. calcd. for $C_{18}H_{11}FO_4$ (310.3): C, 69.68; H, 3.57. Found: C, 69.41; H, 3.36. IR *v* (nujol): 3388, 1702, 1636, 1548, 1301, 1266, 1156, 1038, 983, 840, 761 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.43 (d, 1H, *J* = 6.6 Hz, H-5), 7.12 (dd, 1H, *J* = 8.3, 1.1 Hz, H-7), 7.17 (ddd, 1H, *J* = 7.8, 7.3, 1.1 Hz, H-9), 7.23-7.36 (m, 2H, H-3',5'), 7.47 (ddd, 1H, *J* = 8.3, 7.3, 1.7 Hz, H-8), 7.73 (dd, 1H, *J* = 7.8, 1.7 Hz, H-10), 7.74 (d, 1H, *J* = 6.6 Hz, H-7), 7.77-7.83 (m, 2H, H-2',6'), 7.88 (s, 1H, H-4). ^{13}C NMR (300 MHz, DMSO-d_6) δ 91.2, 109.7, 114.4, 115.2, 117.6, 122.1, 122.2, 123.7, 130.2, 130.9, 132.4, 139.7, 150.1, 152.7, 159.8, 162.1.

5-Hydroxy-3-(4-methoxyphenyl)-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one (6c). Method I, work-up as described above gave 98% of **6c** as yellow powder: mp 235-238 °C, R_f = 0.34 in EtOAc/hexane 1:1. Anal. calcd. for $C_{19}H_{14}O_5$ (322.3): C, 70.80; H, 4.38. Found: C, 70.59; H, 4.09. IR *v* (nujol): 3346, 1725, 1645, 1602, 1552, 1513, 1288, 1245, 1184, 1113, 1044, 1021, 979, 902, 879, 842, 767, 735, 687 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.81 (s, 3H, CH_3O), 6.42 (d, 1H, *J* = 6.6 Hz, H-5), 6.99-7.05 (m, 2H, H-3',5'), 7.12 (dd, 1H, *J* = 8.3, 1.1 Hz, H-7),

7.17 (ddd, 1H, *J* = 7.8, 7.4, 1.1 Hz, H-9), 7.46 (ddd, 1H, *J* = 8.3, 7.4, 1.7 Hz, H-8), 7.72 (dd, 1H, *J* = 7.8, 1.1 Hz, H-10), 7.72 (d, 1H, *J* = 6.6 Hz, H-7), 7.69-7.76 (m, 2H, H-2',6'), 7.80 (s, 1H, H-4). ^{13}C NMR (300 MHz, DMSO-*d*$_6$) δ 55.1, 91.2, 109.7, 113.7, 113.7, 114.5, 117.4, 121.9, 121.9, 124.2, 126.6, 129.2, 129.2, 132.1, 138.1, 149.2, 152.4, 159.4, 159.8.

5-Hydroxy-3-(3,4,5-trimethoxyphenyl)-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one (6d). Method I, work-up as described above gave 80% of **6d** as yellow powder: mp 219-221 °C, R_f = 0.21 in EtOAc/hexane 1:1. Anal. calcd. for $C_{21}H_{18}O_7$ (382.4): C, 65.96; H, 4.74. Found: C, 65.67; H, 4.44. IR *v* (nujol): 3432, 1725, 1645, 1587, 1561, 1332, 1294, 1233, 1121, 1041, 999, 877, 821, 764 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 3.71 (s, 3H, CH_3O), 3.83 (s, 6H, CH_3O), 6.42 (d, 1H, *J* = 6.6 Hz, H-5), 7.08 (s, 2H, H-2',6'), 7.13 (dd, 1H, *J* = 8.3, 1.0 Hz, H-7), 7.18 (ddd, 1H, *J* = 7.9, 7.4, 1.0 Hz, H-9), 7.48 (ddd, 1H, *J* = 8.3, 7.4, 1.0 Hz, H-8), 7.72 (dd, 1H, *J* = 7.9, 1.6 Hz, H-10), 7.75 (d, 1H, *J* = 6.6 Hz, H-7), 7.93 (s, 1H, H-4). ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ 56.0, 56.0, 60.1, 91.3, 105.7, 109.7, 114.5, 117.6, 122.1, 124.4, 129.9, 132.4, 137.9, 137.9, 139.5, 149.8, 152.5, 152.6, 159.7.

5-Hydroxy-3-(4-nitrophenyl)-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one (6e). Method I, work-up as described above gave 70% of **6e** as yellow powder: mp 192-195 °C, R_f = 0.24 in EtOAc/hexane 1:1. Anal. calcd. for $C_{18}H_{11}NO_6$ (337.3): C, 64.10; H, 3.29; N, 4.15. Found: C, 63.63; H, 3.57; N, 4.28. IR *v* (nujol): 3538, 1737, 1698, 1636, 1546, 1518, 1346, 1325, 1221, 1156, 1039, 974, 855, 776 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 6.46 (d, 1H, *J* = 6.6 Hz, H-5), 7.14 (dd, 1H, *J* = 8.3, 0.9 Hz, H-7), 7.19 (ddd, 1H, *J*= 7.9, 7.4, 0.9 Hz, H-9), 7.51 (ddd, 1H, *J* = 8.3, 7.4, 1.5 Hz, H-8), 7.76 (dd, 1H, *J* = 7.9, 1.5 Hz, H-10), 7.82 (d, 1H, *J* = 6.6 Hz, OH), 8.02 -8.08 (m, 2H, H-2',6'), 8.12 (s, 1H, H-4), 8.28-8.34 (m, 2H, H-3',5'). ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ 91.2, 109.7, 114.3, 117.7, 123.5, 122.2, 122.5, 129.2, 130.2, 133.0, 141.1, 142.0, 146.9, 151.5, 153.0, 159.3.

5-Hydroxy-3-[(4-methylsulfonyl)phenyl]-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one (6f). Method I, work-up as described above gave 26% of **6f** as yellow powder: mp 181-183 °C, R_f = 0.31 in EtOAc/hexane 1:1. Anal. calcd. for $C_{19}H_{14}O_6S$ (370.1): C, 61.61; H, 3.81; S, 8.66. Found: C, 61.49; H, 3.51; S, 8.29. IR *v*(nujol): 3424, 1729, 1644, 1604, 1593, 1553, 1486, 1409, 1296, 1270, 1203, 1142, 1090, 1041, 988, 958, 845, 766 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 3.26 (s, 3H, SO_2CH_3), 7.14 (d, 1H, *J* = 7.0 Hz, H-5), 7.19 (ddd, 1H, *J* = 7.7, 7.3, 1.0 Hz, H-9), 7.50 (ddd, 1H, *J* = 8.3, 7.4, 1.6 Hz, H-8), 7.76 (dd, 1H, *J* = 7.7, 1.6 Hz, H-10), 7.80 (d, 1H, *J* = 7.0 Hz, H-7), 7.96-8.02 (m, 4H, H-2',3',5',6'), 8.05 (s, 1H, H-4). ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ 43.5, 91.2, 109.7, 114.3, 117.7, 122.2, 122.4, 122.8, 126.9, 128.9, 132.9, 139.4, 140.1, 141.5, 151.2, 152.9, 159.5.

General procedures for 3-(R^1-phenyl)-5-(R^3-oxy)-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-ones (7a-7d)

Method J: A solution of acetate **3** (3.00 mmol) and p-toluenesulfonic acid (8.0 mg, 0.04 mmol) in prop-2-yn-1-ol (10 mL) was heated at 60 °C for 3 h. After cooling, 30 mL of diethyl ether was added, the formed precipitate filtered off, washed with diethyl ether and crystallized from dioxane.

Method K: A solution of acetate **3** (2.00 mmol) and p-toluenesulfonic acid (5.0 mg, 0.03 mmol) in prop-2-yn-1-ol (4 mL) was irradiated in microwave oven at 60 °C and 10W for 10 min. The products **7** were isolated and purified in the same manner as described in Method A.

3-Phenyl-5-(prop-2-yn-1-yloxy)-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one (7a). Method J, work-up as described above gave 70% of **7a** (method K, 75%) as yellow powder: mp 185-188 °C. Anal. calcd. for $C_{21}H_{14}O_4$ (330.4): C, 76.35; H, 4.27. Found: C, 76.59; H, 4.46. IR *v* (nujol): 1718, 1710, 1652, 1565, 1488, 1465, 1297, 1190, 1042, 1017, 981, 897, 785, 680 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 3.56 (t, 1H, *J* = 2.4 Hz, CH), 4.47 (d, 2H, *J* = 2.4 Hz, CH_2), 6.41 (s, 1H, H-5), 7.24 (ddd, 1H, *J* = 7.7, 7.1, 0.9 Hz, H-9), 7.53 (ddd, 1H, *J* = 8.3, 7.2, 1.6 Hz, H-8), 7.72 (d, 1H, *J* = 7.5 Hz, H-7), 7.73 (ddd, 2H, *J* = 7.9, 7.1, 1.2 Hz, H-2',6'), 7.76 (dd, 1H, *J* = 8.2, 1.8 Hz, H-10), 7.73-7.84 (m, 3H, H-3',4',5'), 7.87 (s, 1H, H-4).

3-(4-Methoxyphenyl)-5-(prop-2-yn-1-yloxy)-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one (7b). Method J, work-up as described above gave 74% of **7b** (method K, 77%) as yellow powder: mp 202-205 °C. Anal. calcd. for $C_{22}H_{16}O_5$ (360.4): C, 73.33; H, 4.48. Found: C, 73.54; H, 4.57. IR *v*(nujol): 1718, 1707, 1652, 1565, 1480, 1464, 1298, 1190, 1050, 1018, 976, 898, 788, 675 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 3.55 (t, 1H, *J* = 2.4 Hz, CH), 4.46 (d, 1H, *J* = 2.4 Hz, CH_2), 6.39 (s, 1H, H-5), 7.03 (ddd, 2H, H-3',5'), 7.22 (d, 1H, *J* = 8.0 Hz, H-7), 7.24 (ddd, 1H, *J* = 7.7, 1.1 Hz, H-9), 7.51 (ddd, 1H, *J* = 8.0, 1.6 Hz, H-8), 7.71 (ddd, 2H, *J* = 6.9, 2.1 Hz, H-2',6'), 7.75 (dd, 1H, *J* = 7.7, 1.6 Hz, H-10), 7.79 (s, 1H, H-4).

3-(4-Nitrophenyl)-5-(prop-2-yn-1-yloxy)-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one (7c). Method J, work-up as described above gave 65% of **7c** (method K, 68%) as yellow powder: mp 232-235 °C. Anal. calcd. for $C_{21}H_{13}NO_6$ (375.3): C, 67.20; H, 3.49; N, 3.73. Found: C, 67.51; H, 3.58; N, 3.99. IR *v*(nujol): 1720, 1710, 1645, 1560, 1485, 1460, 1295, 1197, 1045, 1015, 975, 900, 788, 672 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 3.57 (t, 1H, *J* = 2.4 Hz, CH), 4.48 (d, 2H, *J* = 2.4 Hz, CH_2), 6.42 (s, 1H, H-5), 7.26 (d, 1H, *J* = 7.7 Hz, H-7), 7.28 (ddd, 1H, *J* = 7.8, 0.9 Hz, H-9), 7.56 (ddd, 1H, *J* = 7.8, 1.2 Hz, H-8), 7.80 (dd, 1H, *J* = 7.8, 1.2 Hz, H-10), 8.06 (ddd, 2H, *J* = 9.0, 2.0 Hz, H-2',6'), 8.10 (s, 1H, H-4), 8.32 (ddd, 2H, *J* = 9.0, 2.0 Hz, H-3',5').

5-[(2-Hydroxyethyl)thio]-3-phenyl-2*H*,5*H*-pyrano[3,2-*c*]chromen-2-one (7d). A solution of acetate **3a** (1.10 g, 3.00 mmol,), 2-thioethanol (0.25 mL, 1.10 mol eq) and p-toluenesulfonic acid (8.0 mg, 0.04 mmol) in CH_3NO_2 (3 mL) was stirred and heated at 60 °C for 3 h. After cooling, 30 mL of diethyl ether was added, the formed solid was filtered off, washed with diethyl ether and crystallized from dioxane to give 75% of **7d** as yellow powder: mp 184-186 °C. Anal. calcd. for $C_{20}H_{16}O_4S$ (352.4): C, 68.16; H, 4.58; S, 9.10. Found: C, 68.41; H, 4.43; S, 9.22. IR *v* (nujol): 1716, 1648, 1615, 1555, 1480, 1295, 1260, 1190, 1145, 1070, 1042, 978, 912, 895, 771, 689 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 2.73-2.93 (m, 2H, CH_2O), 3.40 (br, 1H, OH), 3.49-3.74 (m, 2H, CH_2S), 6.97 (s, 1H, H-5), 7.11 (d, 1H, *J* = 8.1 Hz, H-7), 7.12 (ddd, 1H, *J* = 7.7, 0.5 Hz, H-9), 7.21(ddd, 1H, *J* = 8.1, 1.5 Hz, H-8), 7.41-7.51 (m, 3H, H-10, 2',6'), 7.69-7.71 (m, 3H, H-3',4',5'), 7.78 (s, 1H, H-4).

5-Ethoxy-3-(4-fluorophenyl)-2*H*,10a*H*-pyrano[2,3-*b*]chromen-2-one (8). Compound **8** was isolated in 5% (from the crude reaction mixture obtained by synthesis of **5b**) by flash

chromatography in ethyl acetate/hexane (1:2) as yellow powder: mp 160-164 °C, R_f = 0.66 in EtOAc/hexane 1:2. Anal. calcd. for $C_{20}H_{15}FO_4$ (338.3): C, 71.00; H, 4.47. Found: C, 71.18; H, 4.33. IR *v* (KBr): 1717, 1644, 1605, 1555, 1509, 1462, 1370, 1262, 1231, 1157, 1069, 1022, 968, 907, 841, 760 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 1.13 (dd, 3H, *J* = 7.0 Hz, CH_3), 3.78 (dq, 1H, *J* = 9.8, 6.9 Hz, CH_2), 3.89 (dq, 1H, *J* = 9.8, 7.0 Hz,CH_2), 6.29 (s, 1H, H-10a), 7.19 (dd, 1H, *J* = 8.3, 0.9 Hz, H-9), 7.22 (ddd, 1H, *J* = 7.7, 7.5, 0.9 Hz, H-7), 7.35 – 7.27 (m, 2H, H-3',5'), 7.51 (ddd, 1H, *J* = 8.3, 7.5, 1.5 Hz, H-8), 7.75 (dd, 1H, *J* = 7.7, 1.5 Hz, H-6), 7.84 – 7.55 (m, 2H, H-2',6'), 7.92 (s, 1H, H-4).

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References

1. Ghosh, C. K. *Heterocycles* **2004**, 63, 2875.
2. Ishar, M. P. S.; Singh, G.; Singh, S.; Sreenivasan, K. K.; Singh, G. *Bioorg. Med. Chem. Lett.* **2006**, 16, 1366.
3. Nawrot-Modranka, J.; Nawrot, E.; Graczyk, J. *Eur. J. Med. Chem.* **2006**, 41, 1301.
4. El-Shaaer, H. M.; Foltínová, P.; Lácová, M.; Chovancová, J.; Stankovičová, H. *Farmaco* **1998**, 53, 224.
5. Kirkiacharian, S.; Thuy, D. T.; Sicsic, S.; Bakhchinian, R.; Kurkjian, R.; Tonnaire, T. *Farmaco* **2002**, 57, 703.
6. Rakowitz, D.; Gmeiner, A.; Schröder, N.; Matuszczak, B. *Eur. J. Parm. Sci*. **2006**, 27, 188.
7. Santini, C.; Berger, G. D.; Han, W.; Mosley, R.; MacNaul, K.; Berger, J.; Doebber, T.; Wu, M.; Moller, D. E.; Tolman, R. L.; Sahoo, S. P. *Bioorg. Med. Chem. Lett*. **2003**, 13, 1277.
8. Silva, V. L. M.; Silva, A. M. S.; Pinto, D. C. G. A.; Cavaleiro, J. A. S.; Patonay, T. *Synlett* **2004**, 2717.
9. Gašparová, R.; M. Lácová, M.; Krutošíková, A. *Collect. Czech. Chem. Commun*. **2005**, 70 2101.
10. Stankovičová, H.; Gáplovský, A.; Lácová, M.; Chovancová, J.; Puchala, A. *J. Heterocycl. Chem.* **2006**, 43, 843.
11. Gáplovský, A.; Donovalová, J.; Lácová, M.; Mračnová, R.; El-Shaaer, H. M. *J. Photochem. Photobiol., A* **2000**, 136, 61.
12. Stankovičová, H.; Fabian, W. M. F.; Lácová, M. *Molecules* **1997**, 1, 223.

13. Ghosh, C. K.; Bandyopadhyay, C. *Indian J. Chem., Sect. B* **1984**, 23, 1048.
14. Ghosh, C. K.; Bandyopadhyay, C.; Biswas, S.; Chandravarty, A. K. *Indian J. Chem., Sect. B* **1990**, 29, 814.
15. Lácová, M.; Stankovičová, H.; Boháč, A.; Kotzianová, B. *Tetrahedron* **2008**, 64, 9646.
16. Lácová, M.; Gašparová, R.; Koiš, P.; Boháč, A.; El-Shaaer, H. M. *Tetrahedron* **2010**, 66, 1410.
17. http://dtp.nci.nih.gov/screening.html
18. http://dtp.nci.nih.gov/compare/
19. http://dtp.nci.nih.gov/docs/compare/examples/antitub.html
20. Lácová, M.; Boháč, A. Patent Application No. PP5006 – 2006.
21. Nohara A.; Umetani T.; Sanno Y. *Tetrahedron* **1974**, 30, 3553.
22. https://dtps7.ncifcrf.gov/