Synthesis and Antitumour Activity of New Derivatives of Flavone-8-acetic Acid (FAA). Part 1: 6-Methyl Derivatives

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Summary

A range of 17 derivatives of flavone-8-acetic acid (FAA) with a 6-methyl substituent have been prepared and their anti-tumour activity evaluated *in vitro* against a panel of human and murine tumour cell lines and *in vivo* against MAC 15A. While many of the compounds show activity comparable to FAA *in vitro*, this essentially disappears *in vivo*, possibly due to degradation before the compounds can reach the tumour site.

Introduction

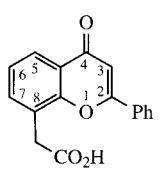
Flavone-8-acetic acid **1** (FAA, NSC347512, LM975) is a synthetic flavonoid[1] with near universal activity against solid murine tumour models[2,3] but little or no activity in the clinic[4]. Whilst the mechanism of action of FAA against murine tumours is still not yet fully understood[5], the response of these tumours to FAA is characterised by rapid vascular collapse, hemorrhagic necrosis, and the involvement of an immunological component[6,7]. The aim of this study is to investigate the mechanism of action of FAA by conducting structure activity studies on a series of novel analogues. A comparison between the properties of active and inactive analogues may provide useful information on the mechanism of action of this novel class of compound. The range of analogues for which activity data is available is in fact somewhat limited. Since the original report[1] where a good number analogues were examined, only one paper has reported activity data for simple analogues[8]. A further large number of compounds have been described in patents[9], but only for a few of these has the activity been reported. Useful *in vivo* activity has been reported for analogues of **1** with a 1-cyclopentenyl or 1-cyclohexenyl substituent in place of phenyl at the 2-position[10], for a wide range of substituted xanthenone-4-acetic acids **2**[11], and most recently for the flavone-8-carboxylic acid derivatives **3**[12].

We report here the preparation and *in vitro* and *in vivo* activity of some new simple derivatives of **1** with a methyl group at the 6-position[13]. The structure activity studies were essentially divided into two stages; an initial screen against a panel of human and murine cell lines *in vitro* with the aim of establishing the cytotoxic potency of a novel compound thereby providing an estimate of a suitable starting dose *in vivo* and secondly, all compounds were tested *in vivo* against the murine colon tumour line MAC 15A grown subcutaneously which is responsive to FAA[14].

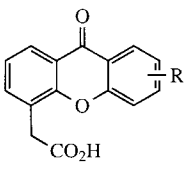
Results and Discussion

Synthesis

The compounds were prepared following a patent procedure[15] starting from 2-hydroxy-3-methoxymethyl-5-methylacetophenone **7**. This was obtained in a four-step sequence from *p*-cresol by acetylation to give **4** and Fries rearrangement to **5**[16], chloromethylation using HCl and formaldehyde to give **6**, and reaction with methanol in the presence of hydrochloric acid and iron powder[15]. Generation of the dianion of **7** using NaH and condensation with the required aromatic ester then gave the 1,3-diones **8**. It is well known that 1,3-diketones can exist in different tautomeric forms and in the case of **8** the three possible forms **8A–C** present different opportunities for hydrogen bonding as shown. The ^1H and ^{13}C NMR spectra of all the compounds **8** showed the presence of a single tautomeric form in solution and the ^{13}C signals for the 1,3-dione function, exemplified by the values for **8c** of δ_C 195.6, 177.1 and 92.8, clearly exclude the diketone form **8A**. An enol structure was also confirmed by the ^1H signals at δ_H 6–7 (1 H), 12–13 (1 H), and 15–16 (1 H) for all the compounds, with the latter two values suggesting structure **8B** where two favourable hydrogen bonding interactions across a six-membered ring are possible rather than **8C** with only one.



1



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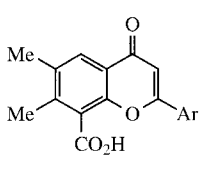

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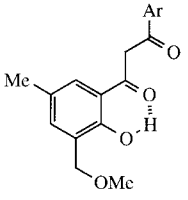
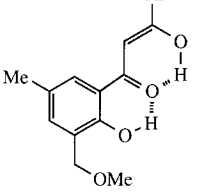
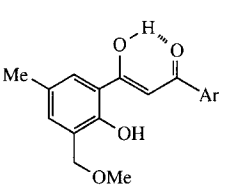


8A



8B



8C

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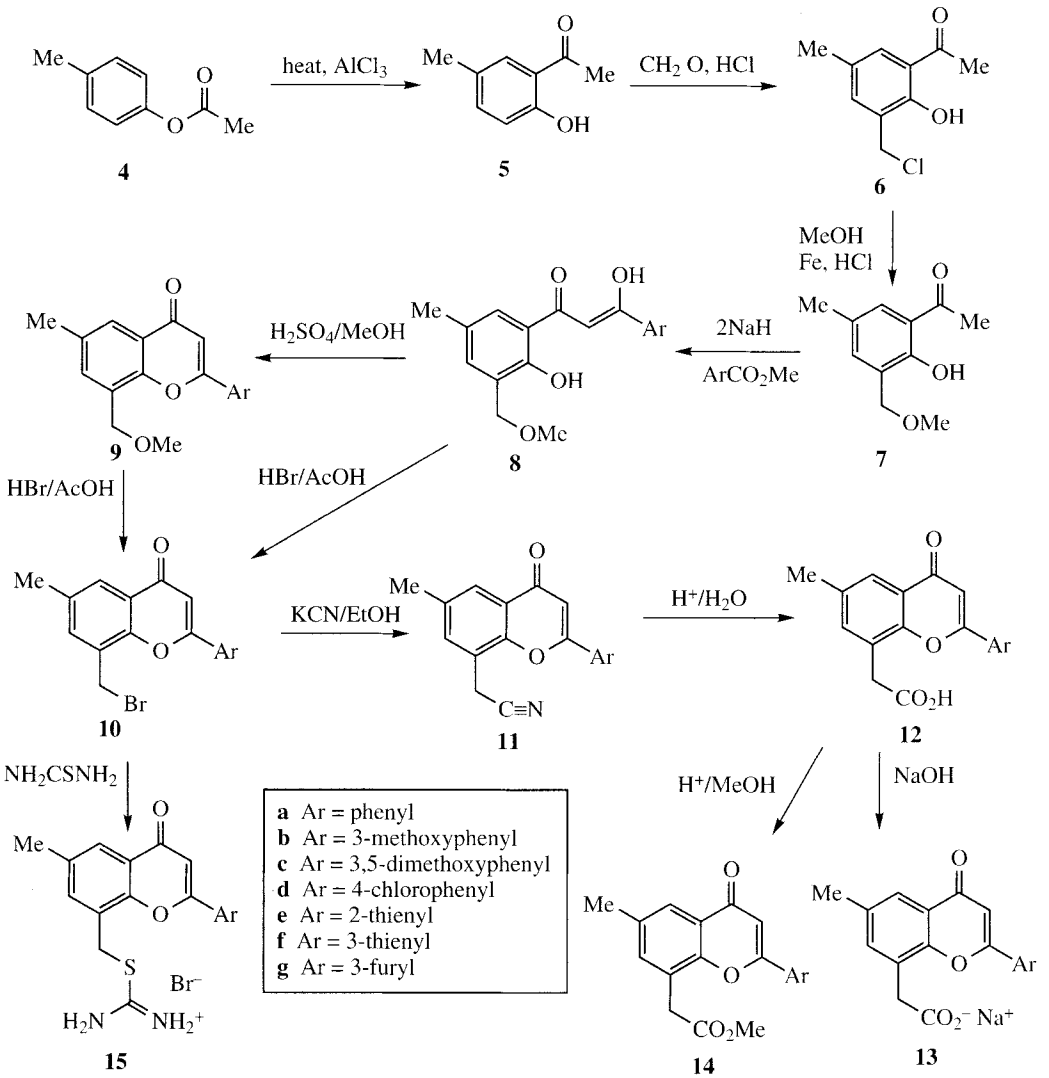

The 1,3-diones could be cyclised directly to flavones **10** with concomitant formation of the 8-bromomethyl group, or alternatively cyclised to **9** by treatment with H_2SO_4 in methanol, and then converted to **10**. Direct reaction worked well for benzenoid substituents **a-d** while the two-step method was found to be preferable for heterocyclic substituents **e-g**. The bromides **10** were then converted to nitriles **11** by reaction with KCN, and acid hydrolysis afforded the desired carboxylic acids **12**. Some of the acids **12** had low solubility in suitable media for screening and so they were converted to the more soluble sodium salts **13** by simple treatment with NaOH. Other derivatives readily prepared were the methyl esters **14** and thiouronium salts **15** and these were of interest since analogues of this type lacking the 6-methyl group were reported to have some activity[1]. Using these methods sixteen examples of compounds **12-15** were obtained in reasonable overall yield and gave the expected spectroscopic and microanalytical data.

Anti-Tumour Activity in Vitro

A panel of human and murine tumour cell lines was employed. These included the following cell lines; MAC 15A[3](derived from an ascitic murine adenocarcinoma of the colon), MAC 16[7] (slow growing, solid and cachectic murine adenocarcinoma of the colon), MAC 26[7] (well differentiated solid murine adenocarcinoma of the colon) WEHI-3B[17] (murine myelo-monocytic leukaemia), K562[18] (human chronic myelogenous leukaemia with erythroid characteristics), HT-29[19], HCLO, DLD-1[20], and HCT-18 (human adenocarcinomas of the colon), HRT-18[21] (human rectal adenocarcinoma), and BM (murine bone marrow).

Chemosensitivity was assessed using an MTT assay[22] following the continuous (96 hours) exposure of cell lines to each compound. All results were expressed in terms of % survival, taking the control absorbance values to represent 100% survival. From the dose response curves constructed, IC_{50} values were estimated. FAA was reconstituted in saline. The final concentration of solutions used was less than 0.01% and solvent controls were used throughout. Solubility problems were encountered with compounds **12f,g**, **14a,b,e**, and **15a,e** in that all proved insoluble in physiologically acceptable solvents and so chemosensitivity data could not be obtained. The *in vivo* activity of these could however be measured since they could be dissolved in suitable vehicles for intraperitoneal injection (see below).

Table 1: Chemosensitivity of tumour cell lines *in vitro*

Compound	*in vitro* IC_{50} value (μM)										
	MAC 15A	MAC 16	MAC 26	WEHI	K562	HCLO	HT-29	DLD1	HCT 18	HRT 18	BM
1	540	640	740	—	1110	>1790	1390	>1790	875	340	—
12a	560	1020	320	710	480	920	610	850	650	270	610
12b	310	130	520	260	—	340	680	520	1170	520	860
12c	340	—	370	93	135	540	—	—	—	310	175
12e	530	700	320	330	370	870	1670	850	570	320	780
13a	660	790	1270	—	940	>1580	1170	>1580	1300	1420	—
13b	200	490	>1450	—	380	>1450	1130	>1450	>1450	>1450	—
13e	310	680	900	—	570	>1550	930	1240	1090	1520	—
13f	1090	1040	>1550	—	930	>1550	1550	>1550	>1550	>1550	—
13g	200	880	1630	—	1080	>1630	1400	>1630	>1630	>1630	—

The responses of human and murine tumour cell lines to FAA **1** and its analogues are presented in Table 1. The results presented clearly demonstrate that FAA and its analogues are cytotoxic *in vitro*. Cytotoxic potency however is low with the majority of compounds inducing cell kills at IC_{50} values of greater that 300 μM. In the case of MAC 15A cells the most active compounds were **13b** and **13g**, with **13a** and **13f** being the least active. The remaining compounds **12a,b,c,e** and **13e** were of comparable cytotoxicity to **1**.

Anti-Tumour Activity in Vivo

The tumour line used in this study was MAC 15A grown subcutaneously as a poorly differentiated, solid tumour in NMRI mice which has previously been shown to respond to FAA[14]. Chemotherapy began when tumours had reached a size that could be accurately measured and had an established vasculature. Anti-tumour activity was assessed by tumour weights. All drugs were administered intraperitoneally at comparable doses to **1** which was used as a positive control.

The responses of MAC 15A tumours grown subcutaneously to FAA and its analogues are presented in Table 2. As positive controls FAA was administered in two solvents, 20% cremophor/saline and arachis oil. In the case of FAA **1** administered in 20% cremophor/saline good anti-tumour activity was observed (83% tumour inhibition) with significant differences ($T_{0.01}$) between treated and control tumour weights. FAA administered in arachis oil however proved to be less active (53% tumour inhibition) and more toxic (3/5 deaths) suggesting that this vehicle may alter the pharmacokinetic profile of FAA *in vivo*. No significant differences between treated and control tumour weights were observed in mice treated with **12a,b,c,g**, **13a,b,e,g**, **14a,b,e**, and **15a,d,e**. In the case of **12e** treated tumour weights at 300 mg kg^{-1} were significantly greater ($T_{0.05}$) than control tumour weights. Significant ($T_{0.05}$) activity was observed with **12f** at 200 and 300 mg kg^{-1} (59 and 45% tumour inhibition respectively) and **13f** at 200 mg kg^{-1} (56% tumour inhibition). At 300 mg kg-1 **13f** was toxic (4/5 deaths). In a repeat experiment **13f** at 250 mg kg^{-1} was not active and no hemorrhagic necrosis was observed with any of the compounds tested.

Table 2: Response of MAC 15A tumours to FAA analogues *in vivo*

Compound	Vehicle	% Tumour growth inhibition at dose (mg/kg)		
		100	200	300
1 (FAA)	A	—	83	—
1 (FAA)	B	—	53[a]	—
12a	A	0	0	0
12b	C	3	0	20
12c	C	0	18	8
12e	A	11	0	0
12f	A	39	59	45
12g	A	46	43	8
13a	D	32	4	16
13b	D	39	27	25
13e	D	14	36	0
13f	D	36	56	94[b]
13g	D	21	21	36
14a,b,e	B	0	0	—
15a,d	B	0	0	—
15e	B	6	15	—

A 20% crem/saline, B arachis oil, C NaOH/saline, D saline

[a] 3/5 deaths

[b] 4/5 deaths; retest at 250 mg/kg gave 0% TGI

The data for most of the compounds in Table 2 appear not to show a conventional dose response relationship. However it is known that FAA **1** and other compounds such as the xanthenone acetic acids **2** show a very steep dose response relationship and anti-tumour activity is seen only very close

to the maximum tolerated dose. The most important feature of the data in Table 2 is that even at doses close to the toxicity level most of the compounds **12–15** do not show significant activity.

The results of this study clearly demonstrate that a very narrow structure activity relationship exists. Whilst the cytotoxic potency of all these compounds are comparable *in vitro* (Table 1), the presence of a single methyl group on the molecule **12a** effectively abolishes the activity against MAC 15A tumours *in vivo*. Substitution by an OH group at the 6-position of **1** was previously reported to completely remove its activity against C38 *in vivo*[1] and these results are also consistant with those of Atwell *et al.*[8] where modifications to the nucleus of the molecule led to inactive compounds. It seems possible that the main effect of the 6-methyl group here is to provide a handle for degradation of the compounds *in vivo* before they can reach the tumour site. If this is the case, then the good *in vitro* activity observed for electron rich aryl groups such as methoxyphenyl, furyl, and thienyl may translate to improved *in vivo* activity for the corresponding 6-unsubstituted FAA analogues. We are currently determining the activity of a wide range of these prepared by a new improved route and the results will be reported shortly.

Acknowledgement

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Experimental

Melting points were determined on a Reichert hot-stage microscope and are uncorrected. NMR spectra were recorded at 300 MHz for ^1H and at 75 MHz for ^{13}C on a Bruker AM300 instrument using solutions in $CDCl_3$ or CD_3SOCD_3 and are reported in ppm relative to Me_4Si as internal standard with coupling constants J in Hz. Infrared spectra were obtained using a Perkin-Elmer SP-1200 spectrophotometer on thin films for liquids and on Nujol mulls for solids. Mass spectra were obtained on a Finnigan-Incos 50 mass spectrometer using electron impact at 70 eV. Dry THF was freshly distilled from potassium benzophenone ketyl under N_2. Solutions of products were dried over anhydrous $MgSO_4$ and evaporated under reduced pressure.

3-Chloromethyl-2-hydroxy-5-methylacetophenone (**6**)

To a stirred solution of paraformaldehyde (12 g, 0.4 mol) in concentrated HCl (200 ml) at 20 °C was added 2-hydroxy-5-methylacetophenone[16] **5** (50 g, 0.33 mol). The mixture was heated at 70–80 °C for 7 h and left for 12 h at 20 °C. The lower organic layer was separated and dissolved in dichloromethane (250 ml). This solution was washed well with water and 10% aqueous $NaHCO_3$, dried, and evaporated. The residual semi-solid was extracted with hexane (3 × 50 ml) and the residue upon evaporation Kugelrohr distilled to give **3** as a yellow oil (62 g, 78%) which crystallised on standing; bp (oven temp.) 240 °C/1 Torr (ref.[15] 110–116 °C/0.4 Torr), mp 38–40 °C.– IR 3410–3450 cm^{-1} (br, OH), 1650 (C=O), 1620 (C=C).– ^1H NMR ($CDCl_3$) δ = 2.76 (s, 3 H, 5-CH_3), 2.48 (s, 3 H, $COCH_3$), 4.48 (s, 2 H, CH_2Cl), 8.08 (d, J = 2, 1 H, 4-H), 8.16 (d, J = 2, 1 H, 6-H), 13.12 (s, 1 H, exchangeable with D_2O, 2-OH).

2-Hydroxy-3-methoxymethyl-5-methylacetophenone (**7**)

To a solution of **6** (59.4 g, 0.3 mol) in anhydrous methanol (250 ml) were added concentrated HCl (30 ml) and iron powder (55 g, 0.32 mol). The mixture was heated under reflux for 4.5 h and then cooled and filtered. The filtrate was evaporated and the residue taken up in dichloromethane (250 ml) and washed well with 10% aqueous $NaHCO_3$. Drying and evaporation gave a yellow semisolid which was extracted with hexane (3 × 200 ml). Evaporation of the extracts followed by Kugelrohr distillation gave **7** as an oil (49 g, 82%) which formed light yellow crystals upon standing; bp (oven temp.) 160–165 °C/20 Torr (ref.[15] 95–103 °C/0.6 Torr), mp 36–38 °C.– IR 3380–3440 cm^{-1} (br, OH), 1640 (C=O).– ^1H NMR ($CDCl_3$) δ = 2.30 (s, 3 H, 5-CH_3), 2.60 (s, 3 H, $COCH_3$), 3.45 (s, 3 H, CH_2OCH_3), 4.52 (s, 2 H, CH_2OCH_3), 7.42 (d, J = 2, 1 H, 4-H), 7.46 (d, J = 2, 1 H, 6-H), 12.44 (s, 1 H, exchangeable with D_2O, 2-OH).– ^{13}C NMR ($CDCl_3$) 204.6 (C=O), 158.0 (C-2), 136.5 (C-4), 129.7 (C-6), 127.6 (C-5), 127.2 (C-3), 119.0 (C-1), 68.7 (CH_2), 58.6 (CH_2OCH_3), 26.7 ($COCH_3$), 20.6 (5-CH_3).

Preparation of 1-Aryl-3-(2′-hydroxy-3′-methoxymethyl-5′-methylphenyl)-propane-1,3-diones **8**

Sodium hydride dispersion in oil (14.4 g NaH, 0.6 mol) was thoroughly washed by decantation with dry petroleum ether and suspended in dry THF (500 ml). The mixture was heated at 60–70 °C while a solution of **7** (48.5 g, 0.25 mol) and the appropriate aromatic methyl ester (0.25 mol) in dry tetrahydrofuran (50 ml) was added dropwise with stirring. The mixture was heated under reflux for 4–8 h and then cooled at 0 °C while anhydrous methanol (200 ml) was cautiously added dropwise. After 2 h at 20 °C the solution was evaporated and the residue dissolved in dichloromethane (1 l). The solution was washed with 1M HCl (2 × 250 ml) dried, and evaporated to give the crude product. Recrystallisation from methanol afforded **8** as light yellow crystals.

1-(2′-Hydroxy-3′-methoxymethyl-5′-methylphenyl)-3-phenylpropane-1,3-dione (**8a**)

Yield 80%. Mp 53–54 °C.– IR 3340 cm^{-1} (br, OH), 1635 (C=O), 1610 (C=C), 1590 (C=C).– ^1H NMR ($CDCl_3$) δ = 2.38 (s, 3 H, 5′-CH_3), 3.49 (s, 3 H, CH_2OCH_3), 4.58 (s, 2 H, CH_2OCH_3), 6.82 (s, 1 H, α-H), 7.39–7.62 (m, 4 H), 7.93 (d, 1 H), 8.02 (s, 1 H, 4′-H), 8.13 (s, 1 H, 6′-H), 12.25 (s, 1 H, 2′-OH), 15.53 (s, 1 H, β-OH).– MS; *m/z* (%) = 298 (18) [M^+], 281 (9), 266 (11), 250 (10), 149 (50), 105 (100).

1-(2′-Hydroxy-3′-methoxymethyl-5′-methylphenyl)-3-(3-methoxyphenyl)-propane-1,3-dione (**8b**)

Yield 73%. Mp 72–74 °C.– IR 3440–3360 cm^{-1} (br, OH), 1615 (C=O), 1600 (C=C).– ^1H NMR ($CDCl_3$) δ = 2.32 (s, 3 H, 5′-CH_3), 3.46 (s, 3 H, CH_2OCH_3), 3.92 (s, 3 H, 3′-OCH_3), 4.62 (s, 2 H, CH_2OCH_3), 6.91 (s, 1 H, α-H), 7.15–7.24 (m, 2H), 7.58–7.64 (m, 4H), 12.40 (br s, 1 H, 2′-OH), 15.62 (br s, 1 H, β-OH).– MS; *m/z* (%) 310 (40) [M^+–H_2O], 295 (29), 279 (23), 267 (6), 250 (21), 147 (100).

1-(3,5-Dimethoxyphenyl)-3-(2′-hydroxy-3′-methoxymethyl-5′-methylphenyl)propane-1,3-dione (**8c**)

Yield 65%. Mp 104–105 °C.– IR 3400 cm^{-1} (br, OH), 1610 (C=O), 1600, 1580 (C=C).– ^1H NMR ($CDCl_3$) δ = 2.37 (s, 3 H, 5′-CH_3), 3.48 (s, 3 H, CH_2OCH_3), 3.85 (s, 6 H, 3, 5-OCH_3), 4.54 (s, 2 H, CH_2OCH_3), 6.64 (t, J = 2, 1 H, 4-H), 6.76 (s, 1 H, α-H), 7.03 (d, J = 2, 2 H, 2, 6-H), 7.40 (d, J = 3, 1 H, 4′-H), 7.48 (d, J = 3, 1 H, 6′-H), 12.20 (br s, 1 H, 2′-OH), 15.55 (s, 1 H, β-OH).– ^{13}C NMR ($CDCl_3$) 195.6 (C=O), 177.1 (β-*C*-OH), 160.9 (C-3, 5), 158.1 (C-2′), 136.0 (C-4′), 135.8 (C-5′), 127.9 (C-3′), 127.5 (C-6′), 127.4 (C-1), 118.2 (C-1′), 104.9 (C-2, 6), 104.2 (C-4), 92.8 (α-=CH), 68.9 (CH_2OCH_3), 58.6 (CH_2OCH_3), 55.6 (3, 5-OCH_3), 20.6 (5′-CH_3).– MS; *m/z* (%) = 358 (3) [M^+], 340 (4), 310 (6), 296 (3), 165 (39), 82 (93), 80 (100).

1-(4-Chlorophenyl)-3-(2′-hydroxy-3′-methoxymethyl-5′-methylphenyl)-propane-1,3-dione (**8d**)

Yield 88%. Mp 118–120 °C.– IR 3420 cm^{-1} (OH), 1610 (C=O), 1590 (C=C).– ^1H NMR ($CDCl_3$) δ = 2.30 (s, 3 H, 5′-CH_3), 3.46 (s, 3 H, CH_2OCH_3), 4.38 (s, 2 H, CH_2OCH_3), 6.56 (s, 1 H, α-H), 7.25–7.40 (m, 5 H), 8.02 (d, 1 H, 6′-H), 12.7 (br s, 1 H, 2′-OH), 15.2 (s, 1 H, β-OH).

*1-(2′-Hydroxy-3′-methoxymethyl-5′-methylphenyl)-3-(2-thienyl)-propane-1,3-dione (**8e**)*

Yield 85%. Mp 82–84 °C.– ^{1}H NMR ($CDCl_3$) δ = 2.24 (s, 3 H, 5′-CH_3), 3.38 (s, 3 H, CH_2OCH_3), 4.48 (s, 2 H, CH_2OCH_3), 6.51(s, 1 H, α-H), 7.16–7.30 (m, 4 H), 7.92 (m, 1 H, 6′-H), 13.06 (br s, 1 H, 2′-OH), 15.82 (s, 1 H, β-OH).– MS; *m/z* (%) = 304 (13) [M^+], 289 (2), 273 (12), 260 (4), 147 (25), 111 (100).

*1-(2′-Hydroxy-3′-methoxymethyl-5′-methylphenyl)-3-(3-thienyl)-propane-1,3-dione (**8f**)*

Yield 84%. Mp 77–78 °C.– IR 3360–3450 cm^{-1} (br, OH), 1615, 1610.– ^{1}H NMR ($CDCl_3$) δ = 2.34 (s, 3 H, 5′-CH_3), 3.47 (s, 3 H, CH_2OCH_3), 4.56 (s, 2 H, CH_2OCH_3), 6.71 (s, 1 H, α-H), 6.32–7.64 (m, 4 H), 8.18 (br s, 1 H, 6′-H), 12.40 (br s, 1 H, 2′-OH), 15.52 (s, 1 H, β-OH).– MS; *m/z* (%) = 304 (34) [M^+], 273 (11), 272 (23), 230 (22), 147 (78), 111 (100).

*1-(3-Furyl)-3-(2′-hydroxy-3′-methoxymethyl-5′-methylphenyl)propane-1,3-dione (**8g**)*

Yield 80%. Mp 69–70 °C.– IR 3400–3300 cm^{-1} (br, OH), 1615 (C=O), 1590 (C=C).– ^{1}H NMR ($CDCl_3$) δ = 2.28 (s, 3 H, 5′-CH_3), 3.36 (s, 3 H, CH_2OCH_3), 4.40 (s, 2 H, CH_2OCH_3), 6.35 (s. 1 H, α-H), 6.78 (m, 1 H), 7.42–7.6 (m, 4 H), 8.05 (d, 1 H, 6′-H) [2′-OH and β-OH not apparent].– MS; *m/z* (%) 288 (14) [M^+], 256 (12), 147 (19), 95 (100).

Preparation of 2-Aryl-8-methoxymethyl-6-methyl-4H-1-benzopyran-4-ones **9**

A solution of **8** (125 mmol) in anhydrous methanol (300 ml) containing conc. H_2SO_4 (0.5 ml) was heated under reflux for 3–4.5 h. Excess methanol was removed under reduced pressure and the residue added to cold water (250 ml). The resulting precipitate was filtered off, washed with cold water, dried, and recrystallized from methanol to afford **9** as colourless crystals.

*8-Methoxymethyl-6-methyl-2-phenyl-4H-1-benzopyran-4-one (**9a**)*

Yield 95%. Mp 122–123 °C.– IR 1630 cm^{-1} (C=O), 1600 (C=C).– ^{1}H NMR ($CDCl_3$) δ = 2.45 (s, 3 H, 6-CH_3), 3.55 (s, 3 H, CH_2OCH_3), 4.83 (s, 2 H, CH_2OCH_3), 6.85 (s, 1 H, 3-H), 7.46–7.54 (m, 3 H, 3′, 4′, 5′-H), 7.58 (d, *J* = 3, 1 H, 7-H), 7.87–7.92 (m, 2 H, 2′, 6′-H), 7.96 (d, *J* = 3, 1 H, 5-H).– ^{13}C NMR ($CDCl_3$) see Table 3.– MS; *m/z* (%) = 280 (86) [M^+], 265 (50), 249 (63), 236 (48), 220 (69), 147(100). Anal. ($C_{18}H_{16}O_3$) C, H.

*8-Methoxymethyl-6-methyl-2-(2-thienyl)-4H-1-benzopyran-4-one (**9e**)*

Yield 86%. Mp 149–150 °C.– IR 1650 cm^{-1} (C=O), 1615 (C=C), 1585.– ^{1}H NMR ($CDCl_3$) δ = 2.45 (s, 3 H, 6-CH_3), 3.54 (s, 3 H, CH_2OCH_3), 4.79 (s, 2 H, CH_2OCH_3), 6.69 (s, 1 H, 3-H), 7.18 (dd, *J* 7, 6, 1 H, 4′-H), 7.56 (d, *J* 3, 1 H, 7-H), 7.58 (dd, *J* 6, 2, 1 H, 5′-H), 7.69 (dd, *J* 7, 2, 1 H, 3′-H), 7.94 (d, *J* 3, 1 H, 5-H).– ^{13}C NMR ($CDCl_3$) see Table 3.– MS; *m/z* (%) = 286 (7) [M^+], 271 (4), 255 (4), 226 (4), 203 (17), 162 (65), 161 (100). Anal. ($C_{16}H_{14}O_3S$) C, H.

*8-Methoxymethyl-6-methyl-2-(3-thienyl)-4H-1-benzopyran-4-one (**9f**)*

Yield 89%. Mp 148 °C.– IR 1640 cm^{-1} (C=O), 1605 (C=C).– ^{1}H NMR ($CDCl_3$) δ = 2.46 (s, 3 H, 6-CH_3), 3.54 (s, 3 H, CH_2OCH_3), 4.80 (s, 2 H, CH_2OCH_3), 6.65 (s, 1 H, 3-H), 7.45–7.48 (m, 2 H, 4′, 5′-H), 7.56 (d, *J* 3, 1 H, 7-H), 7.94 (d, *J* 3, 1 H, 5-H), 7.97 (m, 1 H, 2′-H).– ^{13}C NMR ($CDCl_3$) see Table 3.– MS; *m/z* (%) = 286 (100) [M^+], 271 (42), 255 (37), 226 (38), 147 (51). Anal. ($C_{16}H_{14}O_3S$) C, H.

*2-(3-Furyl)-8-methoxymethyl-6-methyl-4H-1-benzopyran-4-one (**9g**)*

Yield 90%. Mp 150–2 °C.– IR 1640 cm^{-1} (C=O), 1610 (C=C), 1590.– ^{1}H NMR ($CDCl_3$) δ = 2.42 (s, 3 H, 6-CH_3), 3.51 (s, 3 H, CH_2OCH_3), 4.73 (s, 2 H, CH_2OCH_3), 6.49 (s, 1 H, 3-H), 6.73 (m, 1H, 4′-H), 7.02–7.04 (m, 2 H, 2′, 5′-H), 7.92 (d, 1 H, 7-H), 8.04 (d, 1 H, 5-H).– ^{13}C NMR ($CDCl_3$) see Table 3.– MS; *m/z* (%) = 270 (100) [M^+], 255 (55), 240 (54.), 239 (52), 210 (53), 147 (85). Anal. ($C_{16}H_{14}O_4$) C, H.

Preparation of 2-Aryl-8-bromomethyl-6-methyl-4H-1-benzopyran-4-ones **10** *(Method A)*

A solution of **9** (0.1 mol) in a mixture of glacial acetic acid (60 ml) and 48% hydrobromic acid (50 ml) was heated under reflux for 8 h. The mixture was poured into ice cold water (500 ml) and the resulting gray precipitate was filtered off and washed thoroughly with cold water. The product was dissolved in acetone (150 ml) and boiled for 15 minutes with charcoal. The solution was filtered, the filtrate evaporated, and the residue recrystallised from methanol to give **10** as colourless crystals.

*8-Bromomethyl-6-methyl-2-(2-thienyl)-4H-1-benzopyran-4-one (**10e**)*

Yield 74%. Mp 175–177 °C.– IR 1650 cm^{-1} (C=O), 1615 (C=C), 1585.– ^{1}H NMR ($CDCl_3$) δ = 2.44 (s, 3 H, 6-CH_3), 4.76 (s, 2 H, CH_2Br), 6.70 (s, 1 H, 3-H), 7.19 (dd, *J* = 8, 6, 1H, 4′-H), 7.51 (d, *J* =3, 1 H, 7-H), 7.59 (d, *J* = 8, 1 H, 5′-H), 7.78 (d, *J* = 6, 1 H, 3′-H), 7.96 (d, *J* = 3, 1 H, 5-H).– ^{13}C NMR ($CDCl_3$) see Table 3.– MS; *m/z* (%) = 336 (9) [^{81}Br–M^+], 334 (9) [^{79}Br–M^+], 256 (22), 255 (52) [M^+–Br], 242 (24), 147 (100). Anal. ($C_{15}H_{11}BrO_2S$) C, H.

*8-Bromomethyl-6-methyl-2-(3-thienyl)-4H-1-benzopyran-4-one (**10f**)*

Yield 77%. Mp 189–190 °C.– IR 1650 cm^{-1} (C=O), 1605 (C=C), 1580.– ^{1}H NMR ($CDCl_3$) δ = 2.40 (s, 3 H, 6-CH_3), 4.74 (s, 2 H, CH_2Br), 6.66 (s, 1 H, 3-H), 7.44 (dd, 1 H, 4′-H), 7.50 (d, *J* = 3, 71H, -H), 7.52 (d, 1 H, 5′-H), 7.92 (d, *J* = 3, 1 H, 5-H), 8.19 (d, 1H, 2′-H).– ^{13}C NMR ($CDCl_3$) see Table 3.– MS; *m/z* (%) = 336 (18) [^{81}Br–M^+], 334 (17) [^{79}Br-M^+], 256 (19), 255 (100) [M^+–Br], 147 (98). Anal. ($C_{15}H_{11}BrO_2S$) C, H.

*8-Bromomethyl-2-(3-furyl)-6-methyl-4H-1-benzopyran-4-one (**10g**)*

Yield 70%. Mp 179–180 °C.– IR 1640 cm^{-1} (C=O), 1610 (C=C), 1590.– ^{1}H NMR ($CDCl_3$) δ = 2.45 (s, 3 H, 6-CH_3), 4.73 (s, 2 H, CH_2Br), 6.58 (s, 1 H, 3-H), 6.79 (dd, 1 H, 4′-H), 7.51 (d, *J* = 3, 1 H, 7-H), 7.56 (d, 1 H, 5′-H), 7.96 (d, *J* = 3, 1 H, 5-H), 8.17 (d, 1 H, 2′-H).– ^{13}C NMR ($CDCl_3$) see Table 3.– MS; *m/z* (%) = 320 (9) [^{81}Br–M^+], 318 (11) [^{79}Br–M^+], 240 (19), 239 (77) [M^+–Br], 147 (100). HRMS ($C_{15}H_{11}{}^{79}BrO_3$): calcd, 317.9892; found, 317.9888.

Preparation of 2-Aryl-8-bromomethyl-6-methyl-4H-1-benzopyran-4-ones **10** *(Method B)*

A solution of **8** (0.1 mol) in glacial acetic acid (60 ml) and 48% hydrobromic acid (50 ml) was heated at 80 °C for 4 h. After cooling the mixture was poured into ice cold water (500 ml) and the resulting grey precipitate was filtered off and washed thoroughly with cold water. The product was dissolved in acetone (150 ml) and boiled for 15 minutes with charcoal. The solution was filtered, the filtrate evaporated, and the residue recrystallised from methanol to give **10** as colourless crystals.

*8-Bromomethyl-6-methyl-2-phenyl-4H-1-benzopyran-4-one (**10a**)*

Yield 68%. Mp 172–173 °C.– IR 1640 cm^{-1} (C=O).– ^{1}H NMR ($CDCl_3$) δ = 2.46 (s, 3 H, 6-CH_3), 4.79 (s, 2 H, CH_2Br), 6.82 (s, 1 H, 3-H), 7.50–7.58 (m, 4 H), 7.98 (m, 3 H).– ^{13}C NMR ($CDCl_3$) see Table 3.– MS; *m/z* (%) = 330 (4) [^{81}Br–M^+], 328 (4) [^{79}Br–M^+], 250 (11), 249 (58) [M^+–Br], 147 (100). Anal. ($C_{17}H_{13}BrO_2$) C, H.

Table 3: ^{13}C NMR data for flavone derivatives **9–15** (δ).

Compound	C-2	C-3	C-4	C-4a	C-5	C-6	C-7	C-8	C-8a	6-Me	8-CH_2	8-CH_2X	Signals for Ar
9a	162.9	107.2	178.7	123.5	124.6	134.9	134.5	127.3	152.2	21.0	68.9	58.7	131.9 (C-1′), 126.2 (C-2′,6′), 129.1 (C-3′,5′), 131.6 (C-4′)
9e	158.5	105.9	177.9	123.6	124.6	135.4	134.2	127.1	151.8	21.0	68.7	58.8	134.9 (C-2′), 130.1 (C-3′), 128.5 (C-4′), 128.1(C-5′)
9f	159.0	106.9	178.5	123.6	124.6	134.8	134.4	127.0	151.9	21.0	68.9	58.7	127.4 (C-2′), 134.4 (C-3′), 126.5 (C-5′), 124.9 (C-4′)
9g	158.1	107.0	178.1	123.6	124.6	134.8	134.4	126.9	151.9	20.9	68.9	58.8	144.7 (C-2′), 120.5 (C-3′), 107.7 (C-4′), 142.8 (C-5′)
10a	163.0	107.5	178.1	124.2	126.1	135.1	135.8	126.9	152.3	21.0	26.5		131.8 (C-1′), 126.4 (C-2′,6′), 129.2 (C-3′,5′), 131.7 (C-4′)
10b	162.8	107.7	178.1	124.2	126.1	135.1	135.7	126.8	152.3	20.8	26.7		133.1 (C-1′), 111.3 (C-2′), 160.1(C-3′)117.9 (C-4′), 130.2(C-5′), 118.7(C-6′), 55.5
10d	160.9	107.2	176.6	123.4	124.9	134.9	136.1	127.4	151.7	20.2	27.6		130.0 (C-1′), 129.2 (C-2′,6′), 128.1 (C-3′,5′), 136.7 (C-4′)
10e	158.7	105.9	177.5	124.0	126.0	135.1	135.8	126.6	151.8	20.9	26.3		135.0 (C-2′), 130.6 (C-3′), 128.7 (C-4′), 128.7 (C-5′)
10f	159.2	106.8	178.0	123.9	125.0	134.9	135.7	126.6	152.0	20.8	26.6		127.5 (C-2′), 134.0 (C-3′), 125.9 (C-4′), 127.3 (C-5′)
10g	158.5	107.0	177.9	124.0	126.1	135.0	135.7	126.5	152.1	20.8	26.4		144.8 (C-2′), 120.3 (C-3′), 107.6 (C-4′), 143.4 (C-5′)
11e	158.6	106.3	177.3	119.2	125.7	135.6	134.7	124.1	151.5	20.9	34.4	116.6	134.9 (C-2′), 130.5 (C-3′), 128.7 (C-5′), 128.6 (C-4′)
12a	162.0	106.6	177.1	122.9	122.9	134.5	136.7	125.2	152.2	20.3	35.4	171.8	131.1 (C-1′), 126.1 (C-2′,6′), 129.1 (C-3′,5′), 131.7 (C-4′)
12b	161.8	106.9	177.1	123.1	123.0	134.3	136.7	125.2	152.3	20.4	35.6	171.6	132.7 (C-1′), 110.9 (C-2′), 159.7 (C-3′), 117.9 (C-4′), 130.1 (C-5′), 118.5(C-6′), 55.4
12c	161.6	107.0	177.1	123.0	122.9	134.4	136.7	125.2	152.3	20.3	35.6	171.6	133.2 (C-1′), 104.0 (C-2′,6′). 160.9 (C-3′,5′), 104.0 (C-4′), 55.5 (2 × OMe)
12e	158.0	104.8	176.5	122.9	122.9	134.4	136.6	124.9	151.9	20.3	35.1	171.6	134.2 (C-2′), 131.8 (C-3′), 129.3 (C-4′), 128.9 (C-5′)
12f	158.6	106.2	177.0	123.0	122.9	134.2	136.5	125.1	152.1	20.3	35.5	171.8	128.3 (C-2′), 133.8 (C-3′), 125.3 (C-4′), 127.7 (C-5′)
12g	157.8	106.4	176.8	123.1	123.0	134.3	136.5	125.1	152.1	20.4	35.6	172.0	145.4 (C-2′), 120.1 (C-3′), 107.8 (C-4′), 143.8 (C-5′)
13a	162.2	106.2	177.6	122.9	121.1	133.9	136.3	130.2	152.5	20.5	39.7	173.4	131.7 (C-1′), 126.4 (C-2′,6′), 129.1 (C-3′,5′), 131.5 (C-4′)
13b	161.8	106.4	177.5	122.8	121.0	133.7	136.2	130.3	152.5	20.5	40.4	173.9	133.0 (C-1′), 111.2 (C-2′), 159.7 (C-3′), 117.7 (C-4′), 130.1 (C-5′), 118.6(C-6′), 55.5
13e	158.0	104.6	176.9	122.8	120.0	134.7	136.2	129.8	152.0	20.5	39.2	173.2	133.7 (C-2′), 131.3 (C-3′), 129.4 (C-5′), 128.8 (C-4′)
13f	158.8	105.9	177.8	122.9	121.1	134.3	136.4	130.3	152.5	20.7	40.5	173.4	128.5 (C-2′), 133.8 (C-3′), 125.7 (C-4′), 128.2 (C-5′)
13g	157.9	105.8	177.2	122.8	121.0	133.6	136.2	129.9	152.3	20.5	39.9	173.6	145.0 (C-2′), 120.2 (C-3′), 107.8 (C-4′), 144.4 (C-5′)
14a	162.9	104.5	178.4	124.0	124.7	135.0	136.5	123.7	152.8	20.9	35.7	171.0, 52.2	132.1 (C-1′), 126.2 (C-2′,6′), 129.1 (C-3′,5′), 131.5 (C-4′)
14b	162.8	107.5	178.6	123.8	124.5	135.0	136.7	123.6	152.6	20.9	35.9	171.0, 52.3	133.1 (C-1′), 111.5 (C-2′), 160.1 (C-3′), 117.4 (C-4′), 130.1 (C-5′), 118.6(C-6′), 55.5
14e	158.3	106.2	177.9	124.0	124.9	135.0	136.8	124.4	152.4	20.8	35.9	171.1, 52.4	134.7 (C-2′), 130.1 (C-3′), 128.9 (C-5′), 128.5 (C-4′)
15a	162.4	107.1	176.9	123.6	124.6	134.9	135.9	124.4	152.0	20.5	29.5	168.9	131.1 (C-1′), 126.5 (C-2′,6′), 129.3 (C-3′,5′), 132.0 (C-4′)
15d	161.0	107.3	176.6	123.4	124.4	134.8	135.7	124.5	151.8	20.4	29.4	168.6	130.0 (C-1′), 129.2 (C-2′,6′), 128.2 (C-3′,5′), 136.7 (C-4′)
15e	158.0	105.2	176.1	123.5	124.5	134.8	135.7	124.0	151.4	20.4	29.3	168.8	134.0 (C-2′), 132.0 (C-3′), 129.7 (C-5′), 129.0 (C-4′)

8-Bromomethyl-2-(3′-methoxyphenyl)-6-methyl-4H-1-benzopyran-4-one (**10b**)

Yield 55%. Mp 144–145 °C.– IR 1645 cm^{-1} (C=O), 1615 (C=C).– ^1H NMR ($CDCl_3$) δ = 2.46 (s, 3 H, 6-CH_3), 3.90 (s, 3 H, 3′-OCH_3), 4.77 (s, 2 H, CH_2Br), 6.81 (s, 1 H, 3-H), 7.08 (dd, 1 H, 5′-H), 7.45–7.52 (m, 3 H), 7.56 (s, 1 H, 7-H), 7.98 (s, 1 H, 5-H).– ^{13}C NMR ($CDCl_3$) see Table 3.– MS; *m/z* (%) = 360 (6) [^{81}Br–M^+], 358 (6) [^{79}Br-M^+], 279 (46) [M^+–Br], 147 (100). Anal. ($C_{18}H_{15}BrO_3$) C, H.

8-Bromomethyl-2-(3′,5′-dimethoxyphenyl)-6-methyl-4H-1-benzopyran-4-one (**10c**)

Yield 44%. Mp 190–191 °C.– IR 1630 cm^{-1} (C=O), 1600 (C=C), 1575.– ^1H NMR ($CDCl_3$) δ = 2.40 (s, 3 H, 6-CH_3), 3.84 (s, 6 H, 3′, 5′-OCH_3), 4.70 (s, 2 H, CH_2Br), 6.58 (m, 1 H, 4′-H), 6.71 (s, 1 H, 3-H), 7.10 (m, 2 H, 2′, 6′-H), 7.44 (d, 1 H, 7-H), 7.92 (d, 1 H, 5-H).– MS; *m/z* (%) = 390 (10) [^{81}Br–M^+], 388 (9) [^{79}Br-M^+], 309 (40) [M^+–Br], 147 (100).

8-Bromomethyl-2-(4′-chlorophenyl)-6-methyl-4H-1-benzopyran-4-one (**10d**)

Yield 83%. Mp 210–212 °C.– IR 1640 cm^{-1} (C=O), 1590 (C=C).– ^1H NMR (CD_3SOCD_3) δ = 2.47 (s, 3 H, 6-CH_3), 5.02 (s, 2 H, CH_2Br), 7.02 (s, 1 H, 3-H), 7.65 and 8.18 (AB pattern, *J* = 12, 4 H), 7.73 (d, 1 H, 7-H), 7.82 (d, 1 H, 5-H).– ^{13}C NMR ($CDCl_3$) see Table 3.– MS; *m/z* (%) 364 (9) [M^+], 362 (5) [$^{79}Br^{35}Cl$–M^+], 283 (37) [M^+–Br], 147 (90), 43 (100). HRMS ($C_{17}H_{12}{}^{35}ClO_2$) [M^+–Br]: calcd, 283.0526; found, 283.0511.

Preparation of 2-Aryl-8-carboxymethyl-6-methyl-4H-1-benzopyran-4-ones **12**

(i)A suspension of **10** (75 mmol) in boiling ethanol (100 ml) was added in 3–4 portions to a stirred solution of KCN (7.0 g, 0.1 mol) in water (100 ml) maintained at 70 °C. The mixture was heated under reflux for 12 h, the ethanol was removed by evaporation and the residual solution stored at 0 °C for 12 h. The precipitate so formed was filtered off, washed well with ice cold water, dried, and dissolved in hot acetone. Treatment with decolourising charcoal followed by filtration and evaporation gave **11** as colourless crystals which were recrystallised from methanol. The crude products were generally used directly for hydrolysis to **12**.

8-Cyanomethyl-6-methyl-2-(2-thienyl)-4H-1-benzopyran-4-one (**11e**)

Mp 194–196 °C.– IR 1650 cm^{-1} (C=O) and 1590 (C=C).– ^1H NMR (CD_3SOCD_3) δ = 2.47 (s, 3 H, 6-CH_3), 4.02 (s, 2 H, CH_2CN), 6.67 (s, 1 H, 3-H), 7.18 (dd, 1 H, 4′-H), 7.55 (d, *J* = 3, 1 H, 7-H), 7.59 (d, 1 H, 5′-H), 7.72 (d, 1 H, 3′-H), 7.97 (d, *J* = 3, 1 H, 5-H).– ^{13}C NMR ($CDCl_3$) see Table 3.– MS; *m/z* (%) 281 (70) [M^+], 280 (34), 256 (46) [M^+–HCN], 242 (68), 147 (88), 134 (100).

(ii) Concentrated H_2SO_4 (20 ml) was added slowly to a stirred suspension of **11** (50 mmol) in glacial acetic acid (20 ml) and water (20 ml) with cooling. After the addition the mixture was heated under reflux for 5 h then cooled and poured into ice cold water (50 ml). The resulting grey precipitate was filtered off, washed thoroughly with ice cold water, and then dissolved in 10% aqueous $NaHCO_3$ by heating at 70–80 °C. The solution was filtered and acidified with conc. HCl to afford a white precipitate. This was filtered off, washed well with ice cold water, dried, and recrystallised from methanol to give **12** as colourless crystals.

8-Carboxymethyl-6-methyl-2-phenyl-4H-1-benzopyran-4-one (**12a**)

Yield 70%. Mp 223–235 °C.– IR 3400 cm^{-1} (O–H), 1715 (C=O), 1630 (C=O), 1600 (C=C).– ^1H NMR (CD_3SOCD_3) δ = 2.38 (s, 3 H, 6-CH_3), 3.93 (s, 2 H, CH_2CO_2H), 6.93 (s, 1 H, 3-H), 7.47–7.59 (m, 4 H), 7.74 (d, 1 H, 4′-H), 8.00 (d, *J* = 3, 1 H,7-H), 8.03 (d, *J* = 3, 1 H, 5-H).– ^{13}C NMR (CD_3SOCD_3) see Table 3.– MS; *m/z* (%) = 294 (26) [M^+], 249 (18), 148 (40), 147 (42), 69 (100). Anal. ($C_{18}H_{14}O_4.0.2H_2O$) C, H.

8-Carboxymethyl-2-(3′-methoxyphenyl)-6-methyl-4H-1-benzopyran-4-one (**12b**)

Yield 48%. Mp 227–229 °C.– IR 3410 cm^{-1} (O–H), 1720 (C=O), 1630 (C=O), 1590 (C=C).– ^1H NMR (CD_3SOCD_3) δ = 2.38 (s, 3 H, 6-CH_3), 3.86 (s, 3 H, 3′-OCH_3), 3.95 (s, 2 H, CH_2CO_2H), 6.96 (s, 1 H, 3-H), 7.13 (d, *J* = 10, 1 H, 4′-H), 7.46 (t, *J* = 10, 1 H, 5′-H), 7.5–7.58 (m, 2 H, 2′, 7-H), 7.59 (d, *J* = 10, 1 H, 6′-H), 7.76 (d, *J* = 3, 1 H, 5-H).– ^{13}C NMR (CD_3SOCD_3) see Table 3.– MS; *m/z* (%) = 324 (78) [M^+], 296 (15) [M^+–CO], 279 (27), 148 (100), 147 (87). HRMS ($C_{19}H_{16}O_5$): calcd, 324.0998; found, 324.0975.

8-Carboxymethyl-2-(3′,5′-dimethoxyphenyl)-6-methyl-4H-1-benzopyran-4-one (**12c**)

Yield 32%. Mp 231–233 °C.– IR 3420 cm^{-1} (O–H), 1715 (C=O), 1625 (C=O), 1600 (C=C).– ^1H NMR (CD_3SOCD_3) δ = 2.44 (s, 3 H, 6-CH_3), 3.87 (s, 6 H, 2 × OCH_3), 3.96 (s, 2 H, CH_2CO_2H), 6.70 (s, 1 H, 4′-H), 7.03 (s, 1 H, 3-H), 7.21 (s, 2 H, 2′, 6′-H), 7.59 (d, *J* = 3, 1 H, 7-H), 7.78 (d, *J* = 3, 1 H, 5-H).– ^{13}C NMR (CD_3SOCD_3) see Table 3.– MS; *m/z* (%) = 354 (100) [M^+], 309 (15), 162 (36), 147 (48). HRMS ($C_{20}H_{18}O_6$): calcd, 354.1103; found, 354.1121.

8-Carboxymethyl-6-methyl-2-(2-thienyl)-4H-1-benzopyran-4-one (**12e**)

Yield 61%. Mp 238–240 °C.– IR 3420 cm^{-1} (O–H), 1720 (C=O), 1630 (C=O), 1585 (C=C).– ^1H NMR (CD_3SOCD_3) δ = 2.37 (s, 3 H, 6-CH_3), 3.88 (s, 2 H, CH_2CO_2H), 6.80 (s, 1 H, 3-H), 7.26 (dd, 1 H, 4′-H), 7.52 (d, 1 H, 5′-H), 7.70 (d, 1H, 3′-H), 7.93 (d, 1 H, 7-H), 7.96 (d, 1 H, 5-H).– ^{13}C NMR (CD_3SOCD_3) see Table 3.– MS; *m/z* (%) = 300 (100) [M^+], 272 (8), 255 (32), 148 (64), 147 (55). Anal. ($C_{16}H_{12}O_4S$) C, H.

8-Carboxymethyl-6-methyl-2-(3-thienyl)-4H-1-benzopyran-4-one (**12f**)

Yield 63%. Mp 235–236 °C.– IR 3440 cm^{-1} (O–H), 1710 (C=O), 1625 (C=O), 1595 (C=C).– ^1H NMR (CD_3SOCD_3) δ = 2.35 (s, 3 H, 6-CH_3), 3.91 (s, 2 H, CH_2CO_2H), 6.81 (s, 1 H, 3-H), 7.50 (d, *J* = 3, 1 H, 7-H), 7.77–7.79 (m, 3 H), 8.36 (d, *J* = 3, 1 H, 5-H).– ^{13}C NMR (CD_3SOCD_3) see Table 3.– MS; *m/z* (%) = 300 (35) [M^+], 272 (4), 256 (8), 255 (15), 148 (100), 147 (84). HRMS ($C_{16}H_{12}O_4S$): calcd, 300.0456; found, 300.0463.

8-Carboxymethyl-2-(3-furyl)-6-methyl-4H-1-benzopyran-4-one (**12g**)

Yield 52%. Mp 236–238 °C.– IR 3440 cm^{-1} (O–H), 1710 (C=O), 1640 (C=O), 1600 (C=C).– ^1H NMR (CD_3SOCD_3) δ = 2.37 (s, 3 H, 6-CH_3), 3.86 (s, 2 H, CH_2CO_2H), 6.66 (s, 1 H, 3-H), 7.06 (d, 1 H, 4′-H), 7.45 (d, 1 H, 7-H), 7.64 (d, 1 H, 5′-H), 7.85 (d, 1 H, 2′-H), 8.40 (d, 1 H, 5-H).– ^{13}C NMR (CD_3SOCD_3) see Table 3.– MS; *m/z* (%) = 284 (38) [M^+], 256 (6), 239 (29), 211 (5), 148 (100), 147 (90). Anal ($C_{16}H_{12}O_5$) C, H.

Preparation of Sodium 2-Aryl-8-carboxylatomethyl-6-methyl-4H-1-benzopyran-4-ones **13**

The acid **12** (10 mmol) was added to 1.0M sodium hydroxide solution (10 ml) and heated at 70–80 °C until the solid had completely dissolved. The solution was evaporated and the dark brown solid so obtained was dissolved in ice cold water. Filtration and evaporation gave crude **13** as a yellow to brown coloured solid which was purified by reprecipitation from water by addition of acetone.

Sodium 8-carboxylatomethyl-6-methyl-2-phenyl-4H-1-benzopyran-4-one (**13a**)

Yield 96%. Mp 265–267 °C (dec.).– IR 1635 cm^{-1} (C=O), 1585 (C=C).– ^1H NMR (CD_3SOCD_3) δ = 2.49 (s, 3 H, 6-CH_3), 3.63 (s, 2 H, CH_2CO_2Na), 6.92 (s, 1 H, 3-H), 7.46 (d, 1 H, 7-H), 7.53–7.64 (m, 3 H), 7.64 (d, 1 H, 5-H), 8.15 (m, 2 H).– ^{13}C NMR (CD_3SOCD_3) see Table 3.

Sodium 8-carboxylatomethyl-2-(3′-methoxyphenyl)-6-methyl-4H-1-benzopyran-4-one (**13b**)

Yield 94%. Mp 265–266 °C (dec.).– IR 1728 cm^{-1} (C=O), 1630 (C=O), 1600 (C=C), 1575.– ^1H NMR (CD_3SOCD_3) δ = 2.40 (s, 3 H, 6-CH_3), 3.31 (s, 3 H, 3′-OCH_3), 3.86 (s, 2 H, CH_2CO_2Na), 6.96 (s, 1 H, 3-H), 7.17 (dd, 1 H, 5′-H), 7.45 (m, 2 H, 4′, 6′-H), 7.64 (s, 1 H, 7-H), 7.68 (s, 1 H, 2′-H), 7.72 (s, 1 H, 5-H).– ^{13}C NMR (CD_3SOCD_3) see Table 3.

Sodium 8-carboxylatomethyl-6-methyl-2-(2-thienyl)-4H-1-benzopyran-4-one (**13e**)

Yield 94%. Mp 251–253 °C (dec.).– IR 1710 cm^{-1} (C=O), 1630 (C=O), 1585–1575 (C=C).– ^1H NMR (CD_3SOCD_3) δ = 2.36 (s, 3 H, 6-CH_3), 3.57 (s, 2 H, CH_2CO_2Na), 6.73 (s, 1 H, 3-H), 7.26 (d, 1 H, 4′-H), 7.46 (d, 1 H, 7-H), 7.63 (d, 1 H, 5′-H), 7.89 (d, 1 H, 3′-H), 8.02 (d, 1 H, 5-H).– ^{13}C NMR (CD_3SOCD_3) see Table 3.

Sodium 8-carboxylatomethyl-6-methyl-2-(3-thienyl)-4H-1-benzopyran-4-one (**13f**)

Yield 92%. Mp 258–260 °C (dec.).– IR 1635 cm^{-1} (C=O) 1600 (C=C), 1575.– ^1H NMR (CD_3SOCD_3) δ = 2.35 (s, 3 H, 6-CH_3), 3.53 (s, 2 H, CH_2CO_2Na), 6.84 (s, 1 H, 3-H), 7.40 (d, 1 H, 7-H), 7.58 (d, 1 H, 5′-H), 7.71 (d, 1 H, 4′-H), 7.78 (d, 1 H, 5-H), 8.52 (d, 1 H, 2′-H).– ^{13}C NMR (CD_3SOCD_3) see Table 3.

Sodium 8-carboxylatomethyl-2-(3-furyl)-6-methyl-4H-1-benzopyran-4-one (**13g**)

Yield 91%. Mp 273–275 °C (dec.).– IR 1728 cm^{-1} (C=O), 1635 (C=O), 1575 (C=C).– ^1H NMR (CD_3SOCD_3) δ = 2.36 (s, 3 H, 6-CH_3), 3.56 (s, 2 H, CH_2CO_2Na), 6.66 (s, 1 H, 3-H), 7.12 (d, 1 H, 4′-H), 7.41 (d, 1 H, 7-H), 7.62 (d, 1 H, 5′-H), 7.83 (d, 1 H, 5-H), 8.55 (d, 1 H, 2′-H).– ^{13}C NMR (CD_3SOCD_3) see Table 3.

Preparation of 2-Aryl-8-(methoxycarbonylmethyl)-6-methyl-4H-1-benzopyran-4-ones **14**

A solution of **12** (5 mmol) in anhydrous methanol (20 ml) containing conc. H_2SO_4 (0.1 ml) was heated under reflux for 3 h. After cooling the volume was reduced to 5 ml by evaporation and the residue was poured in to ice cold water (50 ml). The resulting precipitate was filtered off and washed well with 10% aqueous $NaHCO_3$ and ice cold water then recrystallised from methanol to give **14** as colourless crystals.

8-(Methoxycarbonylmethyl)-6-methyl-2-phenyl-4H-1-benzopyran-4-one (**14a**)

Yield 91%. Mp 180–181 °C.– IR 1730 cm^{-1} (C=O), 1640 (C=O), 1610 (C=C).– ^1H NMR ($CDCl_3$) δ = 2.46 (s, 3 H, 6-CH_3), 3.76 (s, 3 H, CO_2CH_3), 3.96 (s, 2 H, $CH_2CO_2CH_3$), 6.80 (s, 1 H, 3-H), 7.41(d, *J* = 3, 1H, 7-H), 7.46-7.54 (m, 3 H), 7.89 (m, 2 H), 7.98 (d, *J* = 3, 1 H, 5-H).– ^{13}C NMR ($CDCl_3$) see Table 3.– MS; *m/z* (%) = 308 (48) [M^+], 280 (3), 250 (11), 249 (51), 147 (100). Anal. ($C_{19}H_{16}O_4.0.3\ H_2O$) C, H.

8-(Methoxycarbonylmethyl)-2-(3′-methoxyphenyl)-6-methyl-4H-1-benzopyran-4-one (**14b**)

Yield 87%. Mp 113–115 °C.– IR 1730 cm^{-1} (C=O), 1640(C=O), 1610 (C=C), 1580.– ^1H NMR ($CDCl_3$) δ = 2.45 (s, 3 H, 6-CH_3), 3.73 (s, 3 H, CO_2CH_3), 3.89 (s, 3 H, 3′-OCH_3), 3.96 (s, 2 H, $CH_2CO_2CH_3$), 6.82 (s, 1 H, 3-H), 7.06 (dd, 1H, 5′-H), 7.40–7.51 (m, 4 H), 7.94 (d, *J* = 3, 1 H, 5-H).– ^{13}C NMR ($CDCl_3$) see Table 3.– MS; *m/z* (%) 338 (100) [M^+], 310 (5), 279 (67), 147 (90). Anal. ($C_{20}H_{18}O_5.0.5\ H_2O$) C, H.

8-(Methoxycarbonylmethyl)-6-methyl-2-(2-thienyl)-4H-1-benzopyran-4-one (**14e**)

Yield 90%. Mp 165–166 °C.– IR 1720 cm^{-1} (C=O), 1640 (C=O), 1610 (C=C), 1580.– ^1H NMR ($CDCl_3$) δ = 2.46 (s, 3 H, 6-CH_3), 3.74 (s, 3 H, CO_2CH_3), 3.93 (s, 2 H, $CH_2CO_2CH_3$), 6.66 (s, 1 H, 3-H), 7.17 (dd, *J* = 8, 5, 1 H, 4′-H), 7.40 (d, *J* = 3, 1 H, 7-H), 7.56 (dd, *J* = 8, 2, 1 H, 5′-H), 7.70 (dd, *J* = 5, 2, 1 H, 3′-H), 7.93 (d, *J* = 3, 1 H, 5-H).– ^{13}C NMR ($CDCl_3$) see Table 3.– MS; *m/z* (%) = 314 (84) [M^+], 286 (8), 255 (60), 174 (43), 147 (100). Anal. ($C_{17}H_{14}O_4S$): calcd, C, 64.95; H, 4.49; found, C, 64.57; H, 3.94. HRMS ($C_{17}H_{14}O_4S$): calcd, 314.0613; found, 314.0628.

Preparation of 2-Aryl-8-carboxamidinylthio-6-methyl-4H-1-benzopyran-4-one hydrobromides **15**

A solution of **10** (10 mmol) in absolute ethanol (40 ml) was stirred while a solution of thiourea (15 mmol) in absolute ethanol (20 ml) was added dropwise. The mixture was heated under reflux for 5 h, cooled, and evaporated. Trituration of the resulting semi-solid with dry ether gave a light yellow precipitate which was filtered off, washed well with ether, and recrystallized from methanol-water (1:3) to give **15** as colourless crystals.

8-(Carboxamidinylthio)methyl-6-methyl-2-phenyl-4H-1-benzopyran-4-one hydrobromide (**15a**)

Yield 89%. Mp 254–255 °C.– IR 3400–3260 cm^{-1} (NH), 1650 (C=O), 1620, 1585.– ^1H NMR (CD_3SOCD_3) δ = 2.43 (s, 3 H, 6-CH_3), 4.88 (s, 2 H, CH_2S), 7.02 (s, 1 H, 3-H), 7.54–7.68 (m, 3 H), 7.76 (d, *J* = 3, 1 H, 7-H), 7.92 (d, *J* = 3, 1 H, 5-H), 8.11–8.16 (m, 2 H), 9.20–9.30 (br s, 4 H, NH).– ^{13}C NMR (CD_3SOCD_3) see Table 3.– MS; *m/z* (%) = 324 (18) [M^+–HBr], 282 (13), 249 (45), 147 (100). Anal. ($C_{18}H_{17}BrN_2O_2S$) C, H, N.

8-(Carboxamidinylthio)methyl-2-(4′-chlorophenyl)-6-methyl-4H-1-benzopyran-4-one hydrobromide (**15d**)

Yield 92%. Mp 244–245 °C.– IR 3300 cm^{-1} (NH), 1630, 1590.– ^1H NMR ($CDCl_3$) δ = 2.44 (s, 3 H, 6-CH_3), 4.89 (s, 2 H, CH_2S), 7.08 (s, 1 H, 3-H), 7.68 and 8.16 (AB pattern, *J* = 12, 4 H), 7.75 (d, *J* = 3, 1 H, 7-H), 7.85 (d, *J* = 3, 1 H, 5-H), 9.18 (br s, 4 H, NH).– ^{13}C NMR ($CDCl_3$) see Table 3.– MS; *m/z* (%) 358 (7) [^{35}Cl–M^+–HBr], 283 (48), 219 (3), 179 (7), 147 (100). Anal. ($C_{18}H_{16}BrClN_2O_2S$) C, H, N.

8-(Carboxamidinylthio)methyl-6-methyl-2-(2-thienyl)-4H-1-benzopyran-4-one hydrobromide (**15e**)

Yield 93%. Mp 235–237 °C.– IR 3410 cm^{-1} (NH), 1655 (C=O), 1620, 1590.– ^1H NMR ($CDCl_3$) δ = 2.42 (s, 3 H, 6-CH_3), 4.83 (s, 2 H, CH_2S), 6.88 (s, 1 H, 3-H), 7.34 (dd, *J* = 8, 5, 1 H, 4′-H), 7.76 (d, *J* = 3, 1H, 7-H), 7.82 (d, *J* = 3, 1 H, 5-H), 8.02 (dd, *J* = 8, 2, 1 H, 3′-H), 8.10 (dd, *J* = 5, 2, 1 H, 5′-H), 9.20 (br s, 4 H, NH).– ^{13}C NMR ($CDCl_3$) see Table 3.– MS; *m/z* (%) 330 (1) [M^+–HBr], 313 (3), 288 (17), 255 (58), 179 (6), 147 (100). Anal. ($C_{16}H_{15}BrN_2O_2S_2.1.5\ H_2O$) C, H, N.

In Vitro Chemosensitivity

A panel of human and murine tumour cell lines were employed as described in the Results and Discussion section. All cell lines with the exception of WEHI-3B and K562 were routinely maintained as monolayer cultures in RPMI 1640 medium supplemented with 10% foetal calf serum, sodium pyruvate (1 mM), penicillin/streptomycin (50 IU ml^{-1}/50 μg ml^{-1}) and buffered with HEPES (25 mM). WEHI-3B and K562 cell lines were maintained as suspension cultures in RPMI 1640 as above. Primary bone marrow cultures were set up as follows; Bone marrow cells were obtained from the femurs of non tumour bearing NMRI mice and collected in RPMI 1640 at 4 °C. Cells were cultured in 96 well plates containing RPMI 1640 supplemented with 20% foetal calf serum and 10% WEHI-3B conditioned medium immediately prior to chemosensitivity testing.

Chemosensitivity was assessed using an MTT assay[22] following the continuous (96 hours) exposure of cell lines to each compound as describe below. Between 0.5 and 1×10^4 viable cells (BM cells were plated out at 5×10^5 cells per well) were plated into 96 well culture vessels containing 180 μl of RPMI 1640 medium. To each well 20 μl of drug solution was added to give a final concentration range up to 500 μg ml^{-1} (8 wells per drug exposure were used). Following a 4 day incubation at 37 °C in an atmosphere containing 5% CO_2, 150 μl of old medium was replaced with 150 μl of fresh RPMI 1640 immediately prior to the addition (20 μl) of MTT solution (5 mg ml^{-1}). Following a further 4 hour incubation at 37 °C, 180 μl of

medium was removed and discarded from each well and the formazan crystals dissolved in 150 µl of DMSO. Absorbance of the resulting solution was read at 550 nm using an ELISA spectrophotometer. All results were expressed in terms of % survival taking the control absorbance values to represent 100% survival. From the dose response curves constructed, IC_{50} (the concentration required to reduce cell survival by 50%) values were estimated.

Anti-Tumour Activity in Vivo

Pure strain NMRI mice were used from the Bradford Clinical Oncology Unit inbred colony. NMRI mice were housed in cages in an air conditioned room where regular alternate 12 hr cycles of light and darkness were maintained. Animals were supplied with pellet diet (CRM Labsure, Croydon, UK) and water *ad libitum*.

The development of several adenocarcinoma of the colon in NMRI mice from primary tumours induced by the prolonged administration of 1,2-dimethylhydrazine has been described elsewhere[23]. Chemotherapy began when tumours had reached a size that could be accurately measured and had an established vasculature. Anti-tumour activity was assessed by tumour weights and all tumours were of comparable size. All drugs were administered intraperitoneally at comparable doses to FAA. Drug vehicles differed depending on the analogue used, brief details of which are outlined below; **12a,e,f,g** were administered in 20% Cremophor/saline; **12b,c** were administered in 10% NaOH (0.1 M)/saline; **14a,b,e**, **15a,d,e** were administered in arachis oil, and finally **13a,b,e,f,g** were administered in saline. FAA **1** was administered in 20% Cremophor/saline, saline + NaOH, and arachis oil as positive controls.

Statistical analysis was performed using one way analysis of variance on tumour weights[24]. Where significant differences between mean tumour weights were obtained Tukeys test[24] was performed to determine whether or not treated tumour weights were significantly different from control tumour weights.

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