Synthesis and Antitumour Activity of New Derivatives of Flavone-8-acetic Acid (FAA). Part 2[1]: Ring-Substituted Derivatives

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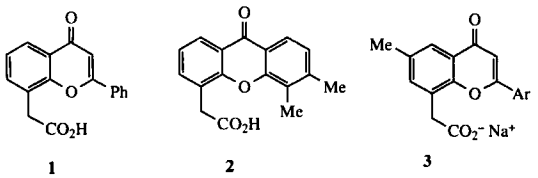
Summary

A range of 18 derivatives of flavone-8-acetic acid (FAA) with substituents on the 2-phenyl group 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. There was no clear-cut relationship between *in vitro* and *in vivo* activity but the activity in each situation was found to be very sensitive to the precise substitution pattern with closely related isomers giving widely different activities. Some of the compounds, notably **10b,c,j**, and **r**, were active *in vivo* and these require further studies in order to evaluate their potential for development.

Introduction

Although flavone-8-acetic acid **1**[1] (FAA, NSC347512, LM975) initially seemed a promising new agent for the treatment of solid tumours, its good activity against murine tumour models[2,3] did not translate into useful clinical activity[4]. The related xanthenone-4-acetic acids also show promising activity and **2** is currently undergoing clinical trials[5]. In Part 1[6] we reported that a range of compounds **3** bearing a 6-methyl substituent showed activity comparable to **1** *in vitro* but were essentially inactive *in vivo* possibly due to metabolic degradation of the methyl group before they could reach the site of action. Of the compounds studied, those with electron-rich aryl groups showed the best *in vitro* activity and so we were interested to examine the activity of analogues without the 6-methyl group. We report here the synthesis of a range of new derivatives **10a-s** and their activity against a panel of human and murine cell lines *in vitro* and *in vivo* against the murine colon tumour line MAC 15A grown subcutaneously which is responsive to FAA[7].



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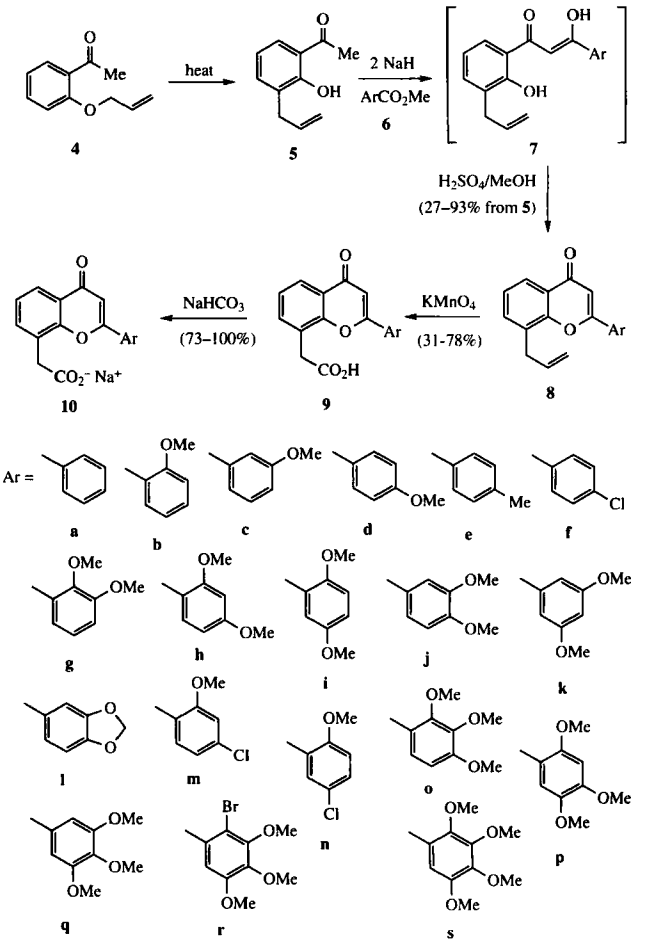
[1] Part 1: Ref. [6].

Results and Discussion

Synthesis

For the synthesis we decided to employ an allyl group as a masked form of the acetic acid in the product, an approach successfully used by Denney and coworkers in the xanthenone series[8]. The required starting material **5** was readily obtained by Claisen rearrangement of 2-allyloxyacetophenone **4** which was in turn obtained from 2-hydroxyacetophenone and allyl bromide[9]. This was then condensed with the required aromatic esters **6** in the presence of sodium hydride using the procedure of Briet and coworkers[10]. To minimise



self-condensation of **5** it was added dropwise to a boiling THF solution of **6** and NaH. In the literature procedure[10] the condensation product **7** was isolated and subsequently cyclised to **8** under acidic conditions. We found however that it was possible to obtain **8** in a convenient one-pot procedure by carefully adding methanol and then sulfuric acid to the solution of **7** and heating it for 3 h. The 19 new allylflavones **8a–s** obtained in this way gave analytical and spectroscopic data in good agreement with expectation (Table 4) and the ^1H and ^{13}C NMR data (Tables 5, 6) formed a highly informative and consistent pattern. The allyl group of these was now oxidised using potassium permanganate in a mixture of acetic acid, water and acetone[8] to give the flavone-8-acetic acids **9a–s** in moderate to good yield. For some of the lower yielding examples, ozonolysis was examined as an alternative but this led to complete breakdown of the flavone nucleus. While our work was in progress, the oxidation of 8-allyl-3-nitroflavone to the corresponding acid using RuO_4 generated *in situ* from $NaIO_4$ and $RuCl_3$ was described[11]. We found this procedure to be unsatisfactory for the compounds **8**, reaction of **8a**, for example, giving a poorer yield of **9** than the permanganate method accompanied by some flavone-8-acetaldehyde. The FAA derivatives **9a–s** gave satisfactory analytical and spectroscopic data (Table 7) and the fully assigned ^1H and ^{13}C NMR spectra (Tables 8, 9) again formed a highly consistent pattern. For evaluation of the anti-tumour activity the acids were converted into their sodium salts **10a–s** by the reported method using sodium bicarbonate[8].

Anti-tumour activity in vitro

Activity of all the compounds **10a–s** was first assessed against MAC 15A[3] derived from an ascitic murine adenocarcinoma of the colon. Based on the results, a limited number, **10a,e,i,l,m** and **n** were selected for evaluation against a panel of human tumour cell lines. These were the human colon adenocarcinoma DLD-1[12], the human rectal adenocarcinoma HRT-18[13], and the human chronic myelogenous leukaemia with erythroid characteristics K562[14]. In all studies the compounds were reconstituted in saline and chemosensitivity was assessed using an MTT assay[15] 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. The results given in Table 1 show a broad spectrum of cytotoxicity with IC_{50} values ranging from 10 to almost 900 μM.

Table 2: Activity against different tumours *in vitro*, IC_{50} values (μM).

	10a	**10e**	**10i**	**10l**	**10m**	**10n**
MAC15A	23±5.0	120±26	24±6.6	49±12	9.6±6.0	33±19
DLD-1	31±4.3	177±29	>276	127±46	65±22	>273
HRT-18	40±12	>316	>276	78±12	128±22	>273
K-562	23±4.0	>316	>276	>289	169±11	>273

A limited number of analogues were tested in a broader panel comprising two lines derived from human large bowel tumours, DLD-1 and HRT-18 and a human leukaemia, K562. As shown in Table 2, compound **10a** proved to be the most active against all the cell lines whereas the other analogues were only active against the murine colon adenocarcinoma

Table 1: Activity against MAC 15A *in vitro*

Compound	Ar	IC_{50} value (μM)	Compound	Ar	IC_{50} value (μM)
1		468±64	**10j**	3,4-$(MeO)_2C_6H_3$	>1380
10a	C_6H_5	23±5.0	**10k**	3,5-$(MeO)_2C_6H_3$	180±28
10b	2-$MeOC_6H_4$	117±72	**10l**	3,4-$(OCH_2O)C_6H_3$	49±12
10c	3-$MeOC_6H_4$	479±111	**10m**	4-Cl-2-$MeOC_6H_3$	9.6±6.0
10d	4-$MeOC_6H_4$	270*	**10n**	5-Cl-2-$MeOC_6H_3$	33±19
10e	4-MeC_6H_4	120±26	**10o**	2,3,4-$(MeO)_3C_6H_2$	41±18
10f	4-ClC_6H_4	131*	**10p**	2,4,5-$(MeO)_3C_6H_2$	314±26
10g	2,3-$(MeO)_2C_6H_3$	898±204	**10q**	3,4,5-$(MeO)_3C_6H_2$	105±23
10h	2,4-$(MeO)_2C_6H_3$	47±9.7	**10r**	2-Br-3,4,5-$(MeO)_3C_6H$	40±4.5
10i	2,5-$(MeO)_2C_6H_3$	24±6.6	**10s**	2,3,4,5-$(MeO)_4C_6H$	71±17

* Only limited testing carried out due to solubility problems.

Table 3: Activity against MAC 15A *in vivo*

Compound	Ar	Dose (mg kg^{-1})	Growth delay (d)	Significance (ρ)
1		200	2.2	NS
		300	7.25	< 0.01
2		28	13.3	< 0.01
10a	C_6H_5	50	0	NS
		100	1.0	0.05
10b	2-$MeOC_6H_4$	300	4.1	< 0.01
		400	3.9	< 0.01
10c	3-$MeOC_6H_4$	294	1.8	< 0.01
10d	4-$MeOC_6H_4$	300	1.6	< 0.05
		500	3.6	< 0.05
10e	4-MeC_6H_4	80	0	NS
10f	4-ClC_6H_4	300	0.8	NS
		450	0	NS
10g	2,3-$(MeO)_2C_6H_3$	500	1.7	NS
10h	2,4-$(MeO)_2C_6H_3$	450	2.8	< 0.05
10i	2,5-$(MeO)_2C_6H_3$	500	0	NS
10j	3,4-$(MeO)_2C_6H_3$	324	1.9	< 0.01
10k	3,5-$(MeO)_2C_6H_3$	400	1.4	NS
10l	3,4-$(OCH_2O)C_6H_3$	37	0	NS
		50	0	NS
10m	4-Cl-2-$MeOC_6H_3$	39	0.8	NS
		50	0	NS
10o	2,3,4-$(MeO)_3C_6H_2$	450	0.5	NS
10p	2,4,5-$(MeO)_3C_6H_2$	500	0	NS
10q	3,4,5-$(MeO)_3C_6H_2$	500	1.7	< 0.05
10r	2-Br-3,4,5-$(MeO)_3C_6H$	500	3.1	< 0.01
10s	2,3,4,5-$(MeO)_4C_6H$	500	1.2	NS

NS = not significant (ρ > 0.05)

cell line (MAC15A), revealing an interesting possible species difference in activity.

Anti-tumour activity in vivo

As shown in Table 3, there was a broad range of efficacy against the MAC15A subcutaneous tumour. Although some analogues were completely inactive, compounds **10b,c,j**, and **r** showed highly significant activity. Compound **10a** which was cytotoxic *in vitro* to all four cell lines proved to be inactive at a tolerated *in vivo* dose. Similarly, **10m** which was highly potent against MAC15A *in vitro* showed no *in vivo* activity. On the other hand examples like **10j** showed good *in vivo* activity despite being inactive *in vitro*. There is, in fact, generally a poor correlation between *in vitro* and *in vivo* activity for this series of compounds. As with previously described flavonoids[1] it is likely that the mechanism of action *in vivo* is highly complex and will result from both direct and indirect effects. In order to discover the real potential of these compounds, further studies will evaluate potential host-mediated effects such as any influence on immune cells and tumour blood flow.

Acknowledgement

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Table 4: Preparation and properties of 8-allylflavones **8a–s.**.

Compound	Yield [%]	Mp [°C]	Formula (C, H)[a]	IR [cm^{-1}]	MS [*m/z* (%)]
8a	74	138	$C_{18}H_{14}O_2$	1637, 1596	262 (100) [M$^+$], 234 (6), 131 (53)
8b	52	88–89	$C_{19}H_{16}O_3$	1732, 1637	292 (35) [M$^+$], 161 (100), 131 (95)
8c	93	109–110	$C_{19}H_{16}O_3$	1735, 1655	292 (100) [M$^+$], 160 (17), 132 (51)
8d	88	113–115	$C_{19}H_{16}O_3$	1718, 1637	292 (100) [M$^+$], 161 (42), 132 (66)
8e	63	108–110	$C_{19}H_{16}O_2$	1637	276 (41) [M$^+$], 131 (43), 115 (100)
8f	83	133–135	$C_{18}H_{13}ClO_2$	1726, 1637	296 (29) [^{35}Cl-M$^+$], 176 (100), 161 (100)
8g	79	87–88	$C_{20}H_{18}O_4$	1636	322 (56) [M$^+$], 239 (22), 161 (100)
8h	54	113–114	$C_{20}H_{18}O_4$	1628	322 (100) [M$^+$], 282 (15), 161 (67)
8i	77	96–98	$C_{20}H_{18}O_4$	1580	322 (64) [M$^+$], 181 (71), 161 (100)
8j	78	141–142	$C_{20}H_{18}O_4$	1645, 1598	322 (100) [M$^+$], 307 (10), 279 (70)
8k	64	137–138	$C_{20}H_{18}O_4$	1732, 1602	322 (100) [M$^+$], 162 (15), 132 (24)
8l	58	178–179	$C_{19}H_{14}O_4$	1732, 1595	306 (100) [M$^+$], 278 (6), 146 (75)
8m	58	140–142	$C_{19}H_{15}ClO_3$	1635, 1590	326 (56) [^{35}Cl-M$^+$], 161 (100), 131 (71)
8n	39	108–110	$C_{19}H_{15}ClO_3$	1637, 1594	326 (57) [^{35}Cl-M$^+$], 161 (100), 131 (52)
8o	63	115–117	$C_{21}H_{20}O_5$	1634	352 (84) [M$^+$], 192 (66), 161 (100)
8p	55	125–128	$C_{21}H_{20}O_5$	1630, 1564	352 (100) [M$^+$], 337 (16), 322 (9)
8q	93	179–181	$C_{21}H_{20}O_5$	1602, 1551	352 (100) [M$^+$], 337 (41), 309 (19)
8r	39	146–147	$C_{21}H_{19}BrO_5$	1632, 1566	430 (100) [^{79}Br-M$^+$], 351 (24) [M$^+$–Br]
8s	27	95–97	$C_{22}H_{22}O_6$	1581, 1551	382 (100) [M$^+$], 367 (24), 336 (9)

[a] Satisfactory elemental analysis obtained

Table 5: ^{1}H NMR data for substituted 8-allylflavones **8** (δ).

Compound	H-3	H-5[a]	H-6[b]	H-7[a]	8-CH_2[c]	$CH=CH_2$	Signals for Ar
8a	6.83 (s)	8.10 (dd)	7.32 (t)	7.53 (dd)	3.75 (d)	6.17–6.00 (m, 1 H)	7.91 (m, 2 H), 7.53 (m, 3 H)
						5.17 (m, 2 H)	
8b	7.16 (s)	8.10 (dd)	7.33 (t)	7.88 (dd)	3.70 (d)	6.13–6.00 (m, 1 H)	7.52 (d, *J* = 6, 1 H), 7.44 (t, *J* = 6, 1 H), 7.09
						5.16–5.10 (m, 2 H)	(t, *J* = 6, 1 H), 7.03 (d, *J* = 6, 1 H), 3.93 (s, 3 H)
8c	6.77 (s)	8.07 (dd)	7.32 (t)	7.52 (dd)	3.71 (d)	6.14–6.00 (m, 1 H)	7.47–7.34 (m, 3 H), 7.05 (d, *J* = 8, 1 H),
						5.18–5.11 (m, 2 H)	3.85 (s, 3 H)
8d	6.83 (s)	8.12 (dd)	7.35 (t)	7.53 (dd)	3.76 (d)	6.13 (m, 1 H)	7.93 and 7.04 (AB pattern, 4 H), 3.89 (s, 3 H)
						5.16 (m, 2 H)	
8e	6.74 (s)	8.06 (dd)	7.29 (t)	7.50 (dd)	3.70 (d)	6.12–5.99 (m, 1 H)	7.74 (half AB pattern, *J* = 8, 2 H),
						5.19–5.09 (m, 2 H)	7.33–7.24 (m, 2 H), 2.37 (s, 3 H)
8f	6.77 (s)	8.07 (dd)	7.33 (t)	7.50 (dd)	3.72 (d)	6.13–5.99 (m, 1 H)	7.82 (half AB pattern, *J* = 8, 2 H),
						5.19–5.09 (m, 2 H)	7.54–7.47 (m, 2 H)
8g	7.04 (m)	8.10 (dd)	7.33 (t)	7.50 (dd)	3.69 (d)	6.10–6.00 (m, 1 H)	7.40–7.27 (m, 1 H), 7.17 (t, *J* = 8,1 H),
						5.14–5.06 (m, 2 H)	7.04 (m, 1 H), 3.90 (s, 3 H), 3.85 (s, 3 H)
8h	7.15 (s)	8.10 (dd)	7.30 (t)	7.50 (dd)	3.70 (d)	6.15–6.01 (m, 1 H)	7.88 (d, *J* = 6, 1 H), 6.63 (dd, *J* = 6, 2, 1 H),
						5.18–5.09 (m, 2 H)	6.53 (d, *J* = 2, 1 H), 3.93 (s, 3 H), 3.88 (s, 3 H)
8i	7.20 (s)	8.11 (dd)	7.34 (t)	7.53 (dd)	3.72 (d)	6.16–6.02 (m, 1 H)	7.46 (d, *J* = 2, 1 H), 7.10–6.95 (m, 2 H),
						5.18–5.06 (m, 2 H)	3.90 (s, 3 H), 3.84 (s, 3 H)
8j	6.77 (s)	8.10 (dd)	7.32 (t)	7.55 (dd)	3.75 (d)	6.19–6.00 (m, 1 H)	7.55 (m, 1 H), 7.39 (d, *J* = 2, 1 H), 6.99 (d,
						5.17–5.09 (m, 2 H)	*J* = 8, 1 H), 3.97 (s, 3 H), 3.96 (s, 3 H)
8k	6.83 (s)	8.05 (dd)	7.38 (t)	7.50 (dd)	3.71 (d)	6.20–6.00 (m, 1 H)	7.07 (d, *J* = 2, 2 H), 6.65 (t, *J* = 2, 1 H),
						5.20–5.10 (m, 2 H)	3.89 (s, 6 H)
8l	6.69 (s)	8.10 (dd)	7.35 (t)	7.52 (dd)	3.73 (d)	6.18–6.00 (m, 1 H)	7.55–7.49 (m, 1 H), 7.36–7.33 (m, 1 H),
						5.20–5.15 (m, 2 H)	6.92 (d, *J* = 8, 1 H), 6.18–6.00 (m, 2 H)
8m	7.13 (s)	8.09 (dd)	7.33 (t)	7.52 (dd)	3.68 (d)	6.12–5.98 (m, 1 H)	7.82 (d, *J* = 8, 1 H), 7.10 (dd, *J* = 8, 2, 1 H),
						5.17–5.10 (m, 2 H)	7.02 (d, *J* = 2, 1 H), 3.94 (s, 3 H)
8n	7.12 (s)	8.08 (dd)	7.33 (t)	7.53 (dd)	3.70 (d)	6.11–5.98 (m, 1 H)	7.87 (d, *J* = 2, 1 H), 7.38 (dd, *J* = 8, 2, 1 H),
						5.20 (m, 2 H)	6.95 (d, *J* = 8, 1 H), 3.93 (s, 3 H)
8o	7.12 (s)	8.12 (dd)	7.38 (t)	7.53 (dd)	3.71 (d)	6.10–6.00 (m, 1 H)	7.61 (d, *J* = 8, 1 H), , 6.83 (d, *J* = 8, 1 H),
						5.19–5.14 (m, 2 H)	3.97 (s, 9 H)
8p	7.19 (s)	8.08 (dd)	7.30 (t)	7.49 (dd)	3.70 (d)	6.17–6.04 (m, 1 H)	7.45 (s, 1 H), 6.58 (s, 1 H), 3.96 (s, 6 H),
						5.15–5.05 (m, 2 H)	3.91 (s, 3 H)
8q	6.76 (s)	8.09 (dd)	7.34 (t)	7.53 (dd)	3.73 (d)	6.19–6.01 (m, 1 H)	7.13 (d, *J* = 2, 2 H), 3.94 (s, 6 H),
						5.18–5.07 (m, 2 H)	3.92 (s, 3 H)
8r	6.91 (s)	8.10 (dd)	7.35 (t)	7.52 (dd)	3.67 (d)	6.12–5.92 (m, 1 H)	6.55 (s, 1 H), 4.00 (s, 3 H), 3.99 (s, 3 H),
						5.13–5.02 (m, 2 H)	3.91 (s, 3 H)
8s	6.91 (s)	8.10 (dd)	7.34 (t)	7.53 (dd)	3.65 (d)	6.10–5.97 (m, 1 H)	6.56 (s, 1 H), 3.95 (s, 6 H), 3.94 (s, 3 H),
						5.11–5.03 (m, 2 H)	3.88 (s, 3 H)

[a] *J* = 8, 2. [b] *J* = 8. [c] *J* = 6–8.

Table 6: ^{13}C NMR data for substituted 8-allylflavones **8** (δ).

Compd.	C-2	C-3	C-4	C-4a	C-5	C-6	C-7	C-8	C-8a	8-CH_2	CH=CH_2	Signals for Ar
8a	163.0	107.4	178.7	124.0	125.0	123.9	135.3	129.5	154.2	34.0	134.1, 117.0	132.1 (C-1′), 126.3 (C-2′,6′), 129.1 (C-3′,5′), 131.6 (C-4′)
8b	160.6	111.8	179.2	123.7	124.6	123.7	135.5	129.4	154.4	34.0	133.8, 116.8	120.9 (C-1′), 158.0 (C-2′), 112.4 (C-3′), 132.4 (C-4′), 120.8 (C-5′), 129.2 (C-6′), 55.6 (OMe)
8c	162.7	107.4	178.6	123.9	124.9	123.8	135.2	129.5	154.1	33.9	134.1, 117.0	133.2 (C-1′), 111.6 (C-2′), 160.0 (C-3′), 117.1 (C-4′), 130.1 (C-5′), 118.5 (C-6′), 55.4 (OMe)
8d	162.5	105.8	178.6	124.1	124.9	123.9	135.3	129.4	154.2	34.0	134.1, 117.0	123.7 (C-1′), 128.1 (C-2′,6′), 114.6 (C-3′,5′), 163.4 (C-4′), 55.5 (OMe)
8e	163.0	106.6	178.6	123.9	124.8	123.8	135.3	129.4	154.1	34.0	133.9, 117.0	129.6 (C-1′), 129.8 (C-2′,6′) 126.1 (C-3′,5′), 142.2 (C-4′), 21.5 (Me)
8f	161.8	107.4	178.5	123.8	125.1	123.9	135.1	129.4	154.1	34.0	134.3, 117.1	130.3 (C-1′), 129.4 (C-2′,6′), 127.4 (C-3′,5′), 137.8 (C-4′)
8g	160.9	112.2	179.0	123.8	124.8	123.8	135.4	129.5	154.4	33.8	133.9, 117.0	126.4 (C-1′), 153.4 (C-2′), 148.1 (C-3′), 114.9 (C-4′), 124.3 (C-5′), 120.6 (C-6′), 60.9, 56.0 (OMe)
8h	160.5	111.1	179.2	123.7	124.4	123.7	135.5	129.2	154.3	34.0	133.6, 116.8	113.7 (C-1′), 163.2 (C-2′), 98.9 (C-3′), 159.7 (C-4′), 105.3 (C-5′), 130.3 (C-6′), 55.6, 55.5 (OMe)
8i	160.1	112.6	179.2	123.8	124.7	123.8	135.4	129.3	154.4	34.0	134.0, 116.8	121.3 (C-1′), 153.4 (C-2′), 113.0 (C-3′), 117.7 (C-4′), 152.4 (C-5′), 114.1 (C-6′), 56.1, 55.8 (OMe)
8j	163.0	106.2	178.7	124.0	124.9	124.0	135.3	129.2	154.2	34.1	134.1, 116.9	124.5 (C-1′), 108.8 (C-2′), 152.1 (C-3′), 149.3 (C-4′), 111.3 (C-5′), 119.9 (C-6′), 56.1, 56.0 (OMe)
8k	162.6	107.5	178.5	123.9	124.9	123.8	135.2	129.4	154.1	34.0	134.1, 117.0	133.7 (C-1′), 104.3 (C-2′, 6′), 161.1 (C-3′, 5′), 103.4 (C-4′), 55.5 (2 OMe)
8l	162.6	106.4	178.5	123.9	124.9	123.9	135.3	129.3	154.1	34.0	134.0, 117.0	125.9 (C-1′), 106.2 (C-2′), 150.6 (C-3′), 148.5 (C-4′), 108.8 (C-5′), 121.3 (C-6′), 101.9 (CH_2)
8m	159.5	112.4	179.0	123.7	124.7	123.8	135.4	129.3	154.3	34.0	134.0, 116.9	119.5 (C-1′), 158.5 (C-2′), 112.5 (C-3′), 138.2 (C-4′), 121.1 (C-5′), 130.0 (C-6′), 56.0 (OMe)
8n	158.8	112.8	179.0	123.8	124.8	123.8	135.4	129.4	154.4	34.2	134.2, 117.0	122.1 (C-1′), 156.6 (C-2′), 113.1 (C-3′), 131.8 (C-4′), 126.0 (C-5′), 128.8 (C-6′), 56.0 (OMe)
8o	160.6	111.0	179.1	123.8	124.6	123.8	135.5	129.3	154.1	33.9	133.8, 116.9	119.0 (C-1′), 153.2 (C-2′),142.6 (C-3′), 156.2 (C-4′), 107.4 (C-5′), 124.0 (C-6′), 61.1, 61.0, 56.1 (OMe)
8p	160.2	111.2	179.0	123.7	124.6	123.8	135.4	128.9	154.2	34.1	134.0, 116.7	111.8 (C-1′), 154.0 (C-2′), 97.1 (C-3′), 143.2 (C-4′), 152.4 (C-5′), 111.5 (C-6′), 56.5, 56.2, 56.1 (OMe)
8q	162.7	107.1	178.6	123.9	125.0	124.0	135.3	129.1	154.2	34.2	134.3, 116.8	127.1 (C-1′), 103.5 (C-2′,6′), 153.5 (C-3′,5′),141.0 (C-4′), 61.1, 56.2 (2 OMe)
8r	163.7	112.6	178.4	123.9	125.0	123.8	135.5	129.4	154.4	33.6	134.2, 116.8	129.7 (C-1′), 109.1 (C-2′), 152.9(C-3′), 145.1 (C-4′), 151.6 (C-5′), 109.9 (C-6′), 61.2, 61.1, 56.4 (OMe)
8s	163.7	112.6	178.4	129.9	125.1	123.9	135.5	129.4	154.5	33.6	134.2, 116.8	129.8 (C-1′), 153.0 (C-2′), 145.2 (C-3′), 151.7 (C-4′), 109.1 (C-5′), 109.9 (C-6′), 61.2, 61.1, 56.4 (2 OMe)

Table 7: Preparation and properties of flavone-8-acetic acids **9a–s.**

Compound	yield [%]	mp [°C]	formula (C, H)[a] and/or lit. mp	IR [cm^{-1}]	MS [*m/z* (%)]
9a	51	238–240	ref.[34] 234; $C_{17}H_{12}O_4$	1716, 1628, 1601, 1583	280 (100) [M^+], 235 (42), 133 (65)
9b	52	203–205	ref.[35] 203–205	1694, 1622, 1566	310 (96) [M^+], 282 (18), 266 (27), 133 (100)
9c	78	253–257	ref.[34] 238–241; $C_{18}H_{14}O_5$	1713, 1627, 1583	310 (100) [M^+], 265 (25), 133 (65)
9d	50	252–254	ref.[34] 228–232; $C_{18}H_{14}O_5$	1718, 1634, 1604, 1586	310 (39) [M^+], 266 (100), 132 (83)
9e	37	250–252	ref.[34] 250–252	1724, 1635, 1600	294 (100) [M^+], 279 (10), 266 (10), 249 (20)
9f	50	234–237	ref.[35] 238–242	1726, 1688, 1624, 1586	314 (100) [^{35}Cl-M^+], 300 (24), 296 (32)
9g	45	188–190	$C_{19}H_{16}O_6$	1729, 1633, 1575	340 (100) [M^+], 326 (41), 311 (6), 294 (14)
9h	71	225–228	ref.[35] 225–227	1709, 1620, 1555	340 (90) [M^+], 300 (7), 162 (100)
9i	63	182–184	$C_{19}H_{16}O_6 + 0.5\ H_2O$	1707, 1620, 1569	340 (17) [M^+], 296 (54), 253 (55), 162 (100)
9j	31	254–256	ref.[34] 250–254	1710, 1624, 1582	340 (13) [M^+], 296 (71), 253 (23), 162 (100)
9k	44	267–269	ref.[34] 261–263	1713, 1627, 1597, 1582	340 (7) [M^+], 296 (100)
9l	43	235–237	$C_{18}H_{12}O_6 + 0.75\ H_2O$	1718, 1642, 1595	324 (100) [M^+], 310 (11), 279 (10)
9m	58	260–262	$C_{18}H_{13}ClO_5$	1729, 1621, 1579, 1556	344 (100) [^{35}Cl-M^+], 300 (57)
9n	46	247–249	$C_{18}H_{13}ClO_5$	1725, 1622, 1585	344 (0.9) [^{35}Cl-M^+], 300 (8), 240 (5), 91 (100)
9o	49	185–186	$C_{20}H_{18}O_7$	1718, 1618, 1577	370 (100) [M^+], 356 (10), 325 (8)
9p	31	262–263	$C_{20}H_{18}O_7$	1715, 1616, 1558	370 (35) [M^+], 341 (16), 309 (17), 77 (100)
9q	74	233–235	$C_{20}H_{18}O_7$	1695, 1610, 1563	370 (1) [M^+], 316 (87), 138 (100)
9r	45	233–236	$C_{20}H_{17}BrO_7$	1713, 1592	450 (10) [^{79}Br-M^+], 400 (48), 375 (30), 77 (100)
9s	47	248–250	$C_{21}H_{20}O_8 + 0.5\ H_2O$	1711, 1589	400 (38) [M^+], 356 (12), 162 (100)

[a] Satisfactory elemental analysis obtained

Experimental

Melting points were determined on a Reichert hot-stage microscope and are uncorrected. NMR spectra were recorded at 300 MHz for ^{1}H and at 75 MHz for ^{13}C on a Bruker AM300 instrument using solutions in $CDCl_3$ unless otherwise stated 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, unless otherwise indicated, on an A.E.I./Kratos MS-50 spectrometer using electron impact (EI) at 70 eV. Fast atom bombardment (FAB) spectra were obtained on a VG Autospec spectrometer using glycerol as the matrix. 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.

Preparation of 2-allyloxyacetophenone (**4**)

Anhydrous potassium carbonate (41.5 g, 0.3 mol) was added to a solution of 2-hydroxyacetophenone (40.9 g, 0.3 mol) and allyl bromide (36.3 g, 0.3 mol) in A.R. acetone (250 ml). The mixture was then heated under reflux with stirring for 4 h after which it was filtered and the filtrate poured into water (300 ml). The mixture was extracted with ether (3 × 25 ml) and the combined extracts washed with 2M sodium hydroxide (100 ml), dried over potassium carbonate and evaporated. The resulting yellow oil was distilled *in vacuo* to afford the title compound (44.0 g, 83%) as a colourless liquid which solidified on storage, mp 19–21 °C, bp 263–265 °C (ref.[8] 263 °C).– IR (melt): ν_{max} = 1667 cm^{-1} (CO), 1595.– ^{1}H NMR: δ = 7.70 (d, *J* = 8, 1 H), 7.38 (m, 1 H), 6.90 (m, 2 H), 6.02 (m, 1 H), 5.45 (d, *J* = 16, 1 H), 5.26 (d, *J* = 8, 1 H), 4.59 (d, *J* = 5, 2 H), 2.61 (s, 3 H).– ^{13}C NMR: δ = 199.5 (4^{ry}), 157.9 (4^{ry}), 133.6 (CH), 132.6 (CH), 130.3 (CH), 128.5 (4^{ry}), 120.7 (CH), 118.1 (CH_2), 112.8 (CH), 69.3 (CH_2), 32.0 (CH_3).– MS; *m/z* (%) = 176 (20) [M^+], 161 (29), 147 (11), 133 (31), 121 (100).

Preparation of 3-allyl-2-hydroxyacetophenone (**5**)

2-Allyloxyacetophenone (20 g, 0.11 mol) was heated under reflux in a nitrogen atmosphere for 5 h. The flask was cooled and the mixture distilled between 140–160 °C at 16 Torr to afford **5** (13.2 g, 66%) as a light yellow liquid, bp 110 °C at 0.3 Torr (ref.[8] 258 °C).– IR: ν_{max} = 3380 cm^{-1} (OH), 1637 (CO).– ^{1}H NMR: δ = 12.61 (s, 1 H), 7.58 (d, *J* = 7, 1 H), 7.32 (d, *J* = 7, 1 H), 6.80 (t, *J* = 7, 1 H), 6.08–5.89 (m, 1 H), 5.11–5.02 (m, 2 H), 3.37 (d, *J* = 7, 2 H), 2.57 (s, 3 H).– ^{13}C NMR: δ = 204.7 (4^{ry}), 160.4 (4^{ry}), 136.4 (CH), 136.1 (CH), 129.3 (4^{ry}), 128.8 (CH), 119.2 (4^{ry}), 118.4 (CH), 116.0 (CH_2), 33.4 (CH_2), 26.7 (CH_3).– MS; *m/z* (%) = 176 (86)[M^+], 161 (100), 143 (11), 133 (28), 77 (26).

Table 8: ^{1}H NMR data for substituted flavone-8-acetic acids **9** (CD_3SOCD_3, δ).

Compd	H-3	H-5[a]	H-6[b]	H-7[a]	8-CH_2	CO_2H	Signals for Ar
9a	7.03 (s)	8.00 (dd)	7.45 (t)	7.78 (dd)	4.03 (s)	13.0–12.0 (br. s)	8.10 (m, 2 H), 7.61 (m, 3 H)
9b	6.94 (s)	7.98 (dd)	7.43 (t)	7.73 (dd)	3.95 (s)	12.50 (br. s)	7.89 (d, *J* = 6, 1 H), 7.57 (t, *J* = 6, 1 H), 7.23 (d, *J* = 6, 1 H), 7.14 (t, *J* = 6, 1 H), 3.95 (s, 3 H)
9c	7.03 (s)	7.98 (dd)	7.46 (m)	7.75 (dd)	4.01 (s)	13.5–12.0 (br. s)	7.64 (d, *J* = 8, 1 H), 7.58 (d, *J* = 2, 1 H), 7.46 (m, 1 H), 7.15 (dd, *J* = 8, 2, 1H), 3.88 (s, 3 H)
9d	6.90 (s)	7.96 (dd)	7.42 (t)	7.72 (dd)	3.99 (s)	12.51 (br. s)	8.03 (d, *J* = 9, 2 H), 7.12 (d, *J* = 9, 2 H), 3.86 (s, 3 H)
9e	6.99 (s)	7.95 (m)	7.40 (t)	7.74 (dd)	4.01 (s)	12.3 (br. s)	7.95 (m, 2 H), 7.47–7.33 (m, 2 H), 2.38 (s, 3 H)
9f	7.04 (s)	7.96 (dd)	7.45 (t)	7.75 (dd)	4.00 (s)	12.12 (br. s)	8.09 and 7.63 (AB pattern, *J* = 8, 4H)
9g	6.87 (s)	8.00 (dd)	7.45 (t)	7.76 (dd)	3.96 (s)	13.0–11.0 (br. s)	7.48–7.42 (m, 1 H), 7.31–7.26 (m, 2 H), 3.90 (s, 3 H), 3.83 (s, 3 H)
9h	6.98 (s)	7.97 (dd)	7.42 (t)	7.72 (dd)	3.98 (s)	13.0–12.6 (br. s)	7.91 (d, *J* = 8, 1 H), 6.79–6.71 (m, 2 H), 3.96 (s, 3 H), 3.90 (s, 3 H)
9i	7.01 (s)	7.96 (dd)	7.43 (t)	7.74 (dd)	3.91 (s)	12.5–12.0 (br. s)	7.46 (d, *J* = 8, 1 H), 7.22–7.11 (m, 2 H), 3.89 (s, 3 H), 3.82 (s, 3 H)
9j	7.06 (s)	8.01 (dd)	7.42 (t)	7.72 (dd)	4.02 (s)	12.49 (br. s)	7.74–7.70 (m, 1 H), 7.61 (d, *J* = 2, 1 H), 7.11 (d, *J* = 8, 1 H), 3.93 (s, 3 H), 3.90 (s, 3 H)
9k	7.09 (s)	7.97 (dd)	7.45 (t)	7.74 (dd)	3.99 (s)	12.50 (br. s)	7.22 (d, *J* = 2, 2 H), 6.70 (t, *J* = 2, 1 H), 3.85 (s, 6 H)
9l	6.98 (s)	7.95 (dd)	7.42 (t)	7.72 (dd)	4.00 (s)	12.7 (br. s)	7.76–7.68 (m, 1 H), 7.61 (s, 1 H), 7.11 (d, *J* = 8, 1 H), 6.17 (s, 2 H)
9m	6.93 (s)	7.92 (dd)	7.43 (t)	7.73 (dd)	3.95 (s)	13.0–12.0 (br. s)	7.87 (dd, *J* = 7, 2, 1 H), 7.33 (d, *J* = 2, 1 H), 7.18 (d, *J* = 7, 1 H), 3.96 (s, 3 H)
9n	6.98 (s)	7.93 (dd)	7.44 (t)	7.74 (dd)	3.98 (s)	12.50 (br. s)	7.97–7.88 (m, 1 H), 7.60 (dd, *J* = 9, 2, 1 H), 7.29 (d, *J* = 9, 1 H), 3.95 (s, 3 H)
9o	6.98 (s)	7.97 (dd)	7.44 (t)	7.73 (dd)	3.97 (s)	12.6 (br. s)	7.65 (d, *J* = 9, 1 H), 7.01 (d, *J* = 9, 1 H), 3.92 (s, 3 H), 3.89 (s, 3 H), 3.83 (s, 3 H)
9p	7.00 (s)	7.94 (dd)	7.40 (t)	7.70 (dd)	3.95 (s)	13.0–12.0 (br. s)	7.48 (s, 1 H), 6.84 (s, 1 H), 3.98 (s, 3 H), 3.91 (s, 3 H), 3.84 (s, 3 H)
9q	7.12 (s)	7.95 (dd)	7.42 (t)	7.73 (dd)	4.01 (s)	13.3–12.0 (br. s)	7.35 (s, 2 H), 3.92 (s, 6 H), 3.79 (s, 3 H)
9r	7.25 (s)	8.04 (dd)	7.51 (t)	7.79 (dd)	3.91 (s)	[not apparent]	6.69 (s, 1 H), 3.88 (s, 3 H), 3.87 (s, 3 H), 3.85 (s, 3 H)
9s	7.23 (s)	8.01 (dd)	7.50 (t)	7.79 (dd)	3.97 (s)	[not apparent]	6.63 (s, 1 H), 3.82 (s, 6 H), 3.80 (s, 3 H), 3.79 (s, 3 H)

[a] *J* = 8, 2. [b] *J* = 8

Substituted methyl benzoates **6**

The substituted benzoic acids were all commercially available with the exception of 2-bromo-3,4,5-trimethoxybenzoic and 2,3,4,5-tetramethoxybenzoic acids which were prepared by the reported method[16]. They were converted into the corresponding methyl esters by treatment with boiling thionyl chloride for 15 min, evaporation, heating the residue with methanol for 1 h, followed by evaporation and distillation. The products **6b-s** were obtained in overall yields of 66–98% and had mp or bp as follows:

6b bp (oven temp.) 230 °C (ref.[17] 228 °C), **6c** bp 88–89 °C/1.0 mmHg (ref.[18] 236–238 °C), **6d** mp 48–49 °C (ref.[19] 49–51 °C), **6e** mp 35–36 °C (ref.[20] 32 °C), **6f** mp 40–43 °C (ref.[21] 42–43 °C), **6g** mp 56–57 °C (ref.[22] 57.5 °C), **6h** bp (oven temp.) 154 °C/1.5 mmHg (ref.[23] 294–296 °C), **6i** bp (oven temp.) 150 °C/0.1 mmHg (ref.[24] 95–98 °C/1 mmHg, **6j** mp 60–61 °C (ref.[25] 62 °C), **6k** mp 40–42 °C (ref.[26] 42–44 °C), **6l** mp 53 °C (ref.[27] 53 °C), **6m** mp 36 °C (ref.[28] 36 °C), **6n** bp (oven temp.) 240 °C (ref.[29] 235–240 °C), **6o** bp (oven temp.) 100–102 °C/1.0 mmHg (ref.[30] 281 °C).,

Table 9: ^{13}C NMR data for substituted flavone-8-acetic acids **9** (CD_3SOCD_3, δ).

Compd	C-2	C-3	C-4	C-4a	C-5	C-6	C-7	C-8	C-8a	8-CH_2	CO_2H	Signals for Ar
9a	162.3	106.9	177.1	123.3	124.9	123.6	135.6	125.6	154.0	35.5	171.6	131.3 (C-1′), 126.3 (C-2′,6′), 129.1 (C-3′,5′), 131.7 (C-4′)
9b	160.2	111.4	177.1	123.1	124.7	123.5	135.5	125.4	154.3	35.2	171.6	120.0 (C-1′), 157.7 (C-2′), 112.6 (C-3′), 132.8 (C-4′), 120.7 (C-5′), 129.0 (C-6′), 55.9 (OMe)
9c	162.1	107.1	177.2	123.3	125.0	123.7	135.7	125.5	154.1	35.6	171.7	132.6 (C-1′), 111.0 (C-2′), 159.8 (C-3′), 118.0 (C-4′), 130.3 (C-5′), 118.6 (C-6′), 55.4 (OMe)
9d	162.3	105.4	176.9	123.4	124.7	123.6	135.4	125.4	153.9	35.5	171.7	123.3(C-1′), 128.1 (C-2′,6′), 114.6 (C-3′,5′), 162.2 (C-4′), 55.5 (OMe)
9e	162.3	106.1	177.0	123.2	124.8	123.6	135.6	125.5	153.9	35.5	172.0	128.4 (C-1′), 126.2 (C-2′,6′), 129.6 (C-3′,5′), 142.0 (C-4′), 21.0 (Me)
9f	161.0	107.2	177.0	123.3	125.0	123.7	135.6	125.5	153.9	35.5	171.7	130.1 (C-1′), 129.1 (C-2′,6′), 128.0 (C-3′,5′), 136.6 (C-4′)
9g	*160.6*	*111.4*	177.2	123.2	124.9	123.6	135.7	125.5	154.4	35.2	171.7	125.5 (C-1′), 153.1 (C-2′), 147.5 (C-3′), 116.1 (C-4′), 124.5 (C-5′), 120.4 (C-6′), 60.5, 56.1 (OMe)
9h	160.2	110.0	177.2	123.1	124.6	123.6	135.4	125.4	154.2	35.5	172.0	112.4 (C-1′), 163.3 (C-2′), 99.1 (C-3′), 159.6 (C-4′), 106.2 (C-5′), 130.2 (C-6′), 56.1, 55.7 (OMe)
9i	159.6	111.5	177.2	123.1	124.7	123.8	135.6	125.5	154.2	35.5	171.6	120.2 (C-1′), 153.2 (C-2′), 113.1 (C-3′), 118.9 (C-4′), 152.0 (C-5′), 114.1 (C-6′), 56.3, 55.6 (OMe)
9j	162.3	105.6	176.9	123.3	124.7	123.6	135.4	125.4	153.9	35.6	171.9	123.2 (C-1′), 111.9 (C-2′), 148.9 (C-3′), 151.8 (C-4′),108.9 (C-5′), 119.9 (C-6′), 55.8, 55.7 (OMe)
9k	161.8	107.2	177.1	123.3	124.9	123.6	135.7	125.5	154.0	35.6	171.6	133.2 (C-1′), 104.1 (C-2′,6′), 160.9 (C-3′,5′), 104.2 (C-4′), 55.5 (2 OMe)
9l	*162.0*	*105.8*	176.9	123.1	124.8	123.5	135.5	125.4	154.0	35.5	171.9	125.0 (C-1′), 106.1 (C-2′), 150.3 (C-3′), 149.1 (C-4′), 109.6 (C-5′), 121.5 (C-6′), 102.0 (CH_2)
9m	159.1	111.4	177.0	122.9	124.8	123.5	135.6	125.4	154.1	35.2	171.8	118.8 (C-1′), 158.3 (C-2′), 113.0 (C-3′), 137.3 (C-4′), 130.2 (C-5′), 120.7 (C-6′), 56.5 (OMe)
9n	158.6	111.9	177.1	123.0	124.9	123.5	135.6	125.5	154.2	35.3	171.6	121.4 (C-1′), 156.5 (C-2′), 114.5 (C-3′), 132.2 (C-4′), 124.9 (C-5′), 128.3 (C-6′), 56.4 (OMe)
9o	160.1	110.0	177.1	123.1	124.8	123.6	135.6	125.4	154.2	35.3	172.0	117.7 (C-1′), 156.2 (C-2′), 142.2 (C-3′), 152.4 (C-4′), 109.2 (C-5′), 123.9 (C-6′), 61.0, 60.5, 56.1 (OMe)
9p	159.8	110.0	177.1	123.5	124.5	123.6	135.3	125.2	154.1	35.5	171.6	110.4 (C-1′), 153.7 (C-2′), 98.4 (C-3′), 143.0 (C-4′), 152.7 (C-5′), 111.3 (C-6′), 56.5, 56.0, 55.9 (OMe)
9q	161.8	106.5	177.1	123.2	124.9	123.6	135.6	125.5	154.0	35.8	171.8	104.3 (C-1′), 103.9 (C-2′,6′), 153.3 (C-3′5′), 140.7 (C-4′), 60.2 (OMe), 56.2 (2 OMe)
9r	163.1	112.4	177.1	123.4	125.8	125.5	136.3	124.0	154.6	35.2	172.0	128.7 (C-1′), 108.1 (C-2′), 153.1 (C-3′), 144.7 (C-4′), 151.1 (C-5′), 111.0 (C-6′), 61.2, 61.1, 56.6 (OMe)
9s	163.0	112.4	177.1	123.2	125.2	123.7	136.1	125.5	154.4	35.0	171.9	111.1 (C-1′), 170.5 (C-2′), 150.9 (C-3′), 165.5 (C-4′), 152.9 (C-5′), 107.5 (C-6′), 62.3 (2 OMe), 56.6, 54.1 (OMe)

6p mp 91–93 °C (ref.[31] 92.5 °C)., **6q** mp 81–82 °C (ref.[30] 81 °C), **6r** bp 200 °C/15 mmHg (ref.[32] 202 °C/16 mmHg)., **6s** bp (oven temp.) 120 °C/1 mmHg (ref.[33] 125–126 °C/1–2 mmHg).

*Preparation of 1-(3-allyl-2-hydroxyphenyl)-3-phenylpropane-1,3-dione (**7a**)*

Sodium hydride (60% dispersion in oil, 0.88 g, 22 mmol) was washed with petroleum (bp 40–60 °C) and the petroleum decanted. A solution of methyl benzoate (1.50 g, 11 mmol) in dry THF (50 ml) was added and the mixture was heated under reflux while 3-allyl-2-hydroxyacetophenone **5** (1.94 g, 11 mmol) in dry THF (50 ml) was added dropwise. After heating under reflux for 5 h the mixture was allowed to cool and dry methanol (200 ml) was added cautiously followed by dropwise addition of concentrated sulphuric acid until pH 4 was reached. The mixture was evaporated and water (50 ml) was added to the residue which was extracted with methylene chloride (2 × 50 ml). The extracts were dried and evaporated to give a yellow solid which was recrystallised from ethanol to give the title compound (2.68 g, 87%) as yellow plates, mp 84–86 °C.– IR: ν_{max} = 1695 cm^{-1}, 1606, 1296, 1235, 1186, 1067, 913, 762, 684.– ^1H NMR: δ = 15.50 (s, 1 H), 12.43 (s, 1 H), 7.95 (m, 2 H), 7.67 (dd, *J* = 8, 2, 1 H), 7.61–7.42 (m, 3 H), 7.38 (dd, *J* = 8, 2, 1 H), 6.85 (m, 2 H), 6.10–5.90 (m, 1 H), 5.11–5.00 (m, 2 H), 3.42 (m, 2 H).– ^{13}C NMR: δ = 196.0 (4^{ry}), 177.2 (4^{ry}), 160.5 (4^{ry}), 136.2 (CH), 135.9 (CH), 133.7 (4^{ry}), 132.3 (CH), 129.7 (4^{ry}), 128.8 (2 × CH), 126.8 (2 × CH), 126.7 (CH), 118.6 (CH), 118.5 (4^{ry}), 116.0 (CH_2), 92.5 (CH), 33.6 (CH_2).– MS; *m/z* (%) = 280 (63) [M^+], 263 (5), 161 (29), 132 (17), 105 (100), 77 (62).– HRMS ($C_{18}H_{16}O_3$): calcd, 280.1099; found, 280.1099.

One-pot preparation of substituted 8-allylflavones **8**

Sodium hydride (60% dispersion in oil, 0.88 g, 22 mmol) was washed with petroleum (bp 40–60 °C) and the petroleum decanted. A solution of the appropriate methyl benzoate (11 mmol) in dry THF (50 ml) was added and the mixture was heated under reflux while **5** (1.94 g, 11 mmol) in dry THF (50 ml) was added dropwise. After heating under reflux for 5 h the mixture was allowed to cool and dry methanol (200 ml) was added cautiously followed by dropwise addition of concentrated sulphuric acid until pH 4 was reached. The mixture was once again heated under reflux for 3 h. Water (20 ml) was added and the solution partly evaporated. Water (100 ml) was added and the mixture extracted with dichloromethane (3 × 50 ml). The extracts were washed with 2M sodium hydroxide to remove starting materials, dried and evaporated. The resulting solid was recrystallised from ethanol to afford the product as shown in Table 4.– ^1H NMR: see Table 5.– ^{13}C NMR: see Table 6.

Preparation of substituted flavone-8-acetic acids **9**

A solution of the 8-allylflavone **8** (20 mmol), acetone (200 ml), glacial acetic acid (200 ml) and water (100 ml) was stirred with cooling to below 5 °C during the addition of potassium permanganate (16.6 g, 110 mmol) over a period of 6 h. Saturated aqueous sodium metabisulphate was added dropwise until the solution turned a cream colour. The solution was evaporated and added to water (200 ml). The precipitate was then filtered off, washed with water and dissolved in a saturated solution of sodium bicarbonate (20 ml). The resulting suspension was filtered and the filtrate carefully acidified to pH 4. The precipitate that formed was filtered off, washed with water and recrystallised from glacial acetic acid and water (2:1) to afford the product as shown in Table 7.– ^1H NMR: see Table 8.– ^{13}C NMR: see Table 9.

Preparation of substituted sodium flavone-8-acetates **10**

A mixture of the appropriate flavone-8-acetic acid **9** (3.2 mmol) and water (10 ml) was heated to 70 °C. Sodium bicarbonate (0.25 g, 3.0 mmol) was added very slowly. After the effervescence had subsided the mixture was heated to 70 °C again with stirring and then allowed to cool to 30 °C and filtered. The filtrate was then added dropwise to acetone (400 ml) and the white precipitate which formed was filtered off and washed with acetone to yield the product in 73–98% yield. Typical experimental data:

*Sodium 2'-methoxyflavone-8-acetate (**10b**)*

Yield 98%. White powder, mp 350 °C.– IR: ν_{max} = 1625 cm^{-1}, 1578.– ^1H NMR (CD_3SOCD_3): δ = 8.09 (dd, *J* = 8, 2, 1 H, H-5), 7.84 (dd, *J* = 8, 2, 1 H, H-7), 7.62 (d, *J* = 8, 1 H, H-6'), 7.54 (t, *J* = 8, 1 H, H-5'), 7.33 (t, *J* = 8, 1 H, H-6), 7.23 (d, *J* = 8, 1 H, H-3'), 7.14 (t, *J* = 8, 1 H, H-4'), 6.95 (s, 1 H, H-3), 3.94 (s, 3 H), 3.60 (s, 2 H).– ^1H NMR (D_2O): δ = 7.57 (d, *J* = 8, 2 H), 7.46 (d, *J* = 8, 1 H), 7.26–7.10 (m, 2 H), 6.89–6.82 (m, 2 H), 6.63 (d, *J* = 8, 1 H), 3.63 (s, 3 H), 3.61 (s, 2 H).– MS (FAB); *m/z* (%) = 687 (31) [$2M^+$ + Na], 355 (100) [M^+ + Na], 333 (26) [M^+ + H], 311 (31) [M^+ + H – Na]$^+$, 288 (14) [$M^+ - CO_2$].

In Vitro Chemosensitivity Studies

The cytotoxicity of each analogue was evaluated *in vitro* in a continuous 96 hour exposure and chemosensitivity assessed using the MTT assay. The MAC15A cell line was used for the primary evaluation. All cells were harvested from subconfluent stocks using 0.25% trypsin, counted on a haemocytometer (Improved Neubauer chamber, Weber UK) and diluted in complete RPMI 1640 for use at a concentration of 1 × 10^4 ml^{-1} MAC15A.

Compounds were dissolved to the appropriate concentration in complete RPMI tissue culture medium immediately prior to use and serially diluted. 100 µl per well of cell suspension was plated in 96 well plates (U bottomed, tissue culture treated, Costar Cat No 3799) and incubated for 3–4 hours before addition of the test solution. To one row of eight wells, 100 µl complete RPMI 1640 was added to serve as the control. Subsequent rows of eight wells received a concentration of test solution over the range 1 mg ml^{-1} to 0.01 µg ml^{-1}. Following the addition of the test compounds, the plates were incubated at 37 °C in an atmosphere of 5% CO_2, 95% air for four days before being assessed.

The tetrazolium dye reduction assay was used in these studies. 150 µl of used medium and compound solution was removed from each of the wells following the 4 day exposure and replaced with 150 µl fresh medium and 20 µl of 5 mg ml^{-1} MTT solution. After 4 hours all of the medium and MTT was removed from all of the wells and replaced with 150 µl DMSO. The formazan crystals produced during the assay were dissolved and mixed by reverse pipetting and the absorbance read at a wavelength of 550 nm using an ELIZA spectrophotometer.

The mean percentage survival of the cells at each compound concentration was calculated relative to the control and cytotoxicity expressed as an IC_{50} value. A limited number of analogues were similarly evaluated against the three human tumour cell lines DLD-1, HRT-18 and K-562.

In Vivo Chemotherapy

For *in vivo* evaluation the compounds were made up immediately prior to use at an appropriate concentration for the desired dose to be administered in 0.1 ml per 10 g body weight. All treatments were administered intraperitoneally to NMRI mice. The activity of the compounds were determined against the subcutaneous MAC15A tumour model by the measurement of tumour growth delay. Wherever possible, activity was tested up to the maximum tolerated dose.

Chemotherapy was administered on day 5 after tumour implantation to allow for vascularization to occur, as determined histologically. Tumour growth was followed by serial calliper measurements and anti-tumour activity assessed by tumour volume. This was calculated by the formula $a^2 \times b/2$ where *a* and *b* were the smaller and larger tumour diameters respectively[36]. Growth delay was determined as the difference in time taken for the median tumours of the analogue treated and solvent control treated mice to reach a relative tumour volume of two. The significance of the growth delay was determined using a Mann-Whitney statistical analysis.

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