# Synthesis and Antitumour Activity of New Derivatives of Flavone-8-acetic Acid (FAA). Part 4[1]: Variation of the Basic Structure ☆

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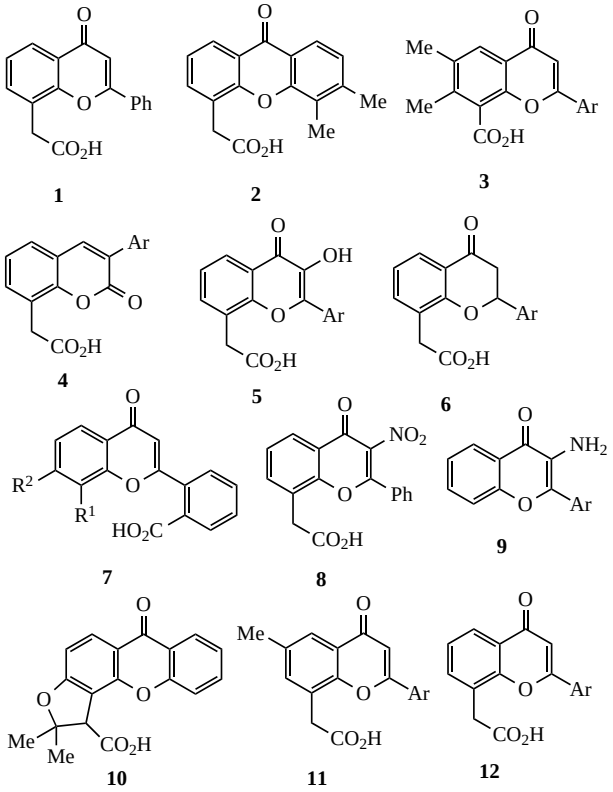
***Key Words:*** *Flavone acetic acid; antitumour activity; structure-activity relationships*

## Summary

A range of 11 derivatives of flavone-8-acetic acid (FAA) in which the structure has been substantially altered in different ways 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. The generally poor activity observed shows that the basic structure cannot be altered much without destroying the activity.

## Introduction

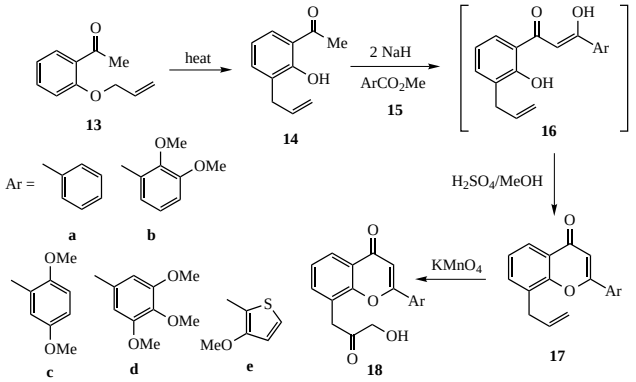
Although flavone-8-acetic acid **1**[1] (FAA, NSC347512, LM975) initially seemed a promising new agent for the treatment of solid tumours, its good activity against murine tumour models[2,3] did not translate into useful clinical activity.[4] In an attempt to clarify the mechanism of action of **1** a number of groups have reported synthesis and anti-tumour evaluation of a variety of structurally related compounds. These include analogues of **1** in which the ring oxygen is replaced by S or NH[5], xanthenone-4-acetic acids such as **2**[6] and flavone-8-carboxylic acids such as **3**[7]. Further examples investigated more recently include the coumarin-, flavonol- and flavanone-acetic acids **4**, **5** and **6**[8], flavone-2′-carboxylic acids **7**[9], the nitro and amino compounds **8** and **9**[10] and conformationally restricted compounds such as **10**[11]. In previous papers in this series, we have described the synthesis and activity of the 6-methyl compounds **11**[12], and a wide range of examples **12** with both subtituted phenyl groups[13] and substituted heteroaromatic groups[14] at the 2-position. In this paper we describe the preparation and *in vitro* and *in vivo* activity of a range of analogues of **1** in which the basic structure has been altered more fundamentally.




## Results and Discussion

### *Synthesis*

The first series of analogues were obtained fortuitously during the previously reported synthesis of compounds **12**. Treatment of 3-allyl-2-hydroxyacetophenone **14** obtained by thermal rearrangement of **13** with the appropriate methyl ester **15** and base followed by *in situ* cyclisation of the condensation product **16** gave the 8-allylflavones **17**. When these were treated with 3 equivalents of $KMnO_4$ rather than the large excess used to obtain **12**, the oxidation proceeded




[1] Part 3: Ref. [14].

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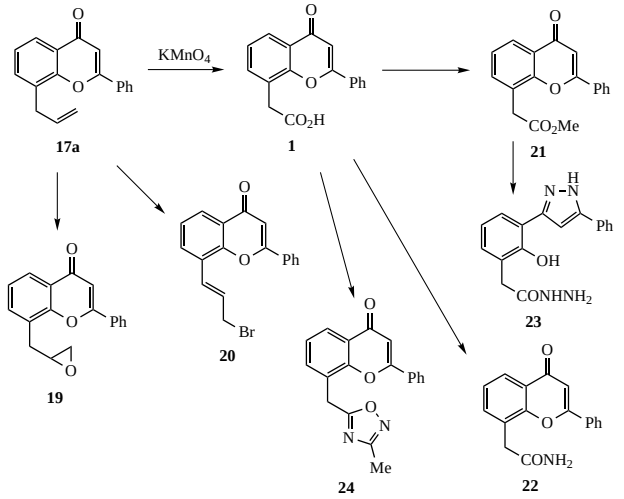
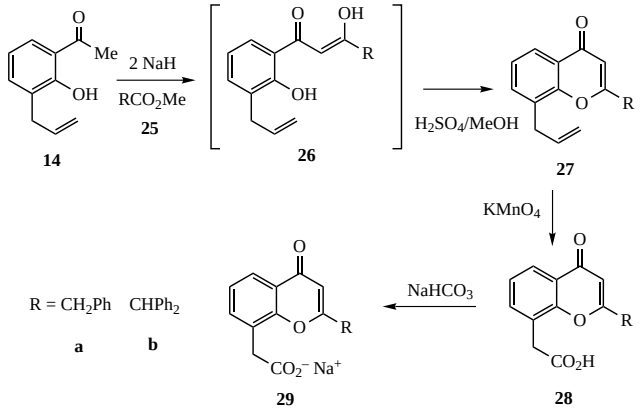
only as far as the stage of the hydroxyketones **18**. As compared to the structure of **12**, these show a considerable similarity, with C=O and acidic OH groups being present in approximately the same location, but the absence of an actual carboxylic acid function is significant and whether or not these compounds are still active will thus be of interest.

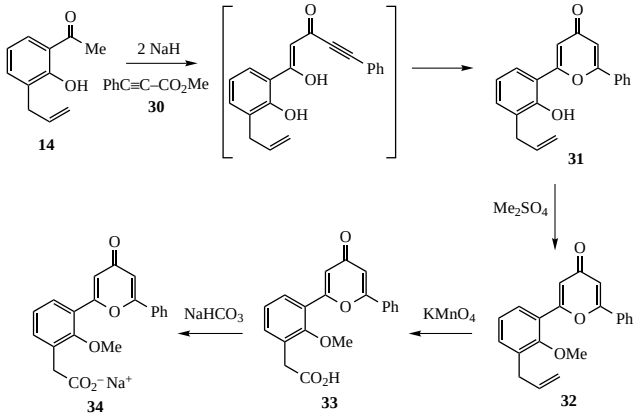



Conventional reactions of both 8-allylflavone **17a** and FAA **1** were used to obtain a further series of analogues: the epoxide **19**, the allylic bromide **20**, the methyl ester **21** and the amide **22**. The last two compounds have been reported[1,15] before but with no anti-tumour activity results. An attempt to prepare the hydrazide by reaction of **21** with hydrazine hydrate led not only to the desired functional group transformation but also to cleavage of the pyran ring to afford the pyrazole **23**. The 1,2,4-oxadiazole **24** was also prepared since this group has been reported to be an effective bio-isostere for a carboxylic ester in various situations[16,17].

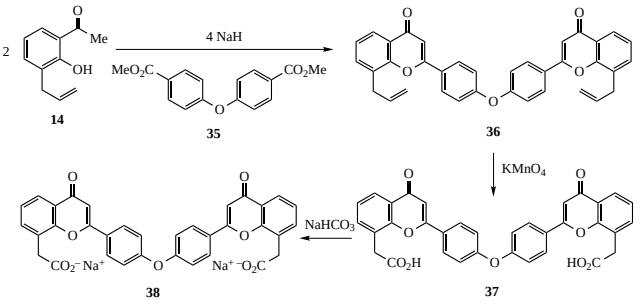



We were also interested to prepare the 2-benzyl compound **29a** since this was reported in the original paper[1] to show good anti-tumour activity against colon adenocarcinoma C38 *in vivo* but the structure differs considerably from all other active analogues reported to date. The diphenylmethyl analogue **29b** was similarly prepared to examine the effect of placing a more bulky and non-planar group at the 2-position.

In an attempt to examine the required spacing between the 8-acetic acid and 2-arylpyranone groups of **1** for activity, we designed an extended analogue **33** in which this spacing is increased. As shown, this was prepared by a variation of the usual synthesis employing the acetylenic methyl ester **30**. Rather than cyclising to give the 2-(phenylethynyl)benzopyranone, the intermediate instead cyclised to give the pyran product **31**. This behaviour of acetylenic 1,3-diketones is well known, having first been reported by Ruhemann in 1908[18] and subsequently applied in a variety of cases[19]. Methylation of the free OH group in **31** followed by permanganate oxidation gave the extended analogue **33** which was tested as its water-soluble sodium salt **34**.




The final analogue prepared was the dimeric bis(FAA) ether **37** and the idea for this came from the work of Breslow and co-workers[20] where a series of bis(amides) gave much greater anti-tumour activity than monoamides, supposedly due to cooperative binding at two proximate receptor sites. When the standard synthetic method was applied to the diphenyl ether 4,4′-diester **35** the target compound was obtained albeit in poor yield.




*Anti-Tumour Activity in Vitro*

Activity of 7 of the compounds was assessed against MAC 15A[3] derived from an ascitic murine adenocarcinoma of the colon. In addition, **18a**, **23** and **29a** were evaluated against a panel of human tumour cell lines. These were the human colon adenocarcinoma DLD-1[21], the human rectal adenocarcinoma HRT-18[22] and the human chronic myelogenous leukaemia with erythroid characteristics K562[23]. In all stud-

**Table 1.** Activity against tumour cell lines *in vitro*.

| Compound | $IC5_0$ value (mM) against | | | |
|---|---|---|---|---|
| | MAC 15A | DLD-1 | HRT-18 | K562 |
| **1** | 23±5.0 | 31±4.3 | 40±12 | 23±4.0 |
| **18d** | 123±34 | 9.4±2.1 | 81±6.5 | >260 |
| **23** | 31±5.8 | 146±15 | 68±24 | 52±9.7 |
| **24** | 79±13 | | | |
| **29a** | 12±4.1 | 22±2.2 | 73±21 | 54±3.2 |
| **29b** | >1280 | | | |
| **34** | 209±50 | | | |
| **38** | 60±11 | | | |

ies the compounds were reconstituted in saline and chemosensitivity was assessed using an MTT assay[24] 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 activity with $IC_{50}$ values for MAC 15A ranging from 12 to over 1200 μM. Although it is clear that all the compounds here have relatively low activity and high concentrations are required for any significant effect, some of the results are interesting. Thus it appears that both the 2-benzyl compound **29a** and the compound **23** with a distinctly non-flavonoid structure have activity comparable to **1**, while the hydroxyketone **18d**, the oxadiazole **24** and the dimeric compound **38** are less active, and both the extended analogue **34** and the diphenylmethyl compound **29b** are essentially inactive.

The results in a broader panel comprising two lines derived from human large bowel tumours, DLD-1 and HRT-18 and a human leukaemia, K562 are also interesting. As shown in Table 1, the hydroxyketone **18d** was much more active than **1** against DLD-1 but essentially inactive against K562. Both **23** and **29a** showed roughly comparable activity to **1** against these three cell lines.

### *Anti-Tumour Activity in Vivo*

The results of evaluation of the compounds against the MAC 15A subcutaneous tumour are shown in Table 2 and these proved to be almost uniformly negative. Only for the oxadiazole compound **24** is there any hint of significant activity. In the case of **29a** which showed good activity *in vitro* this is not entirely unexpected since the benzylic $CH_2$ group is susceptible to biological oxidation and it may be destroyed by metabolism before it can reach the site of action. It seems clear from these results that by introducing the more fundamental alterations to the FAA structure we have removed some of the elements required for activity. However these results do serve to further define the requirements for an active compound of this type and, taken together with the results for compounds **1–12** mentioned earlier, they may contribute to our understanding of the mechanism of action of FAA.

**Table 2.** Activity against MAC 15A *in vivo*.

| Compound | Dose (mg $kg^{-1}$) | Growth delay (d) | Significance (*r*) |
|---|---|---|---|
| **1** | 200 | 2.2 | NS |
| | 300 | 7.25 | < 0.01 |
| **2** | 28 | 13.3 | < 0.01 |
| **18d** | 60 | 0 | NS |
| | 100 | 0.2 | NS |
| **19** | 200 | 0 | NS |
| **20** | 240 | 0 | NS |
| **21** | 212 | 0 | NS |
| **22** | 38 | 1.6 | NS |
| **23** | 30 | 0 | NS |
| **24** | 60 | 0 | NS |
| | 100 | 2.5 | < 0.01 |
| **29a** | 34 | 0 | NS |
| | 40 | 0 | NS |
| | 60 | 0 | NS |
| **29b** | 500 | 0 | NS |
| **34** | 500 | 1.0 | NS |

NS = not significant ($r > 0.05$)

## Acknowledgment

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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 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 8-(3-Hydroxy-2-oxopropyl)flavone Derivatives* **18a–e**

To a stirred solution of the appropriate 8-allylflavone **17** prepared as described previously[13,14] (11 mmol) in a mixture of acetic acid (100 ml), water (100 ml) and acetone (100 ml) was added potassium permanganate (2.6 g, 16.5 mmol) over a period of 1 h. After a further 1 h the mixture was decolourised by the addition of a saturated solution of sodium metabisulfite (approx. 50 ml) and then reduced in volume and extracted with $CH_2Cl_2$ (400 ml). The extract was washed with water (2 × 100 ml), dried and evaporated. The resulting yellow solid was triturated with dry ether at 0 °C and filtered to yield the product as shown in Table 3.–$^1H$ NMR see Table 4.–$^{13}C$ NMR see Table 5.

The compounds **19–24** were prepared as follows and had properties as listed in Table 3.–$^1H$ NMR see Table 4.–$^{13}C$ NMR see Table 5.

**Table 3.** Preparation and properties of flavone derivatives.

| Compound | Yield [%] | Mp [ûC] | Formula or lit. mp (C, H, N)[a] | IR [$cm^{-1}$] | MS [*m/z* (%)] |
|---|---|---|---|---|---|
| **18a** | 66 | 200–202 | $C_{18}H_{14}O_4 + 0.5\ H_2O$ | 3300, 1723, 1641, 1591 | 294 (15) [$M^+$] , 279 (100), 235 (53) |
| **18b** | 66 | 155–157 | $C_{20}H_{18}O_6$ | 3306, 1730, 1624, 1599 | 354 (73) [$M^+$], 324 (21), 295 (63), 133 (100) |
| **18c** | 57 | 182–184 | $C_{20}H_{18}O_6$ | 3440, 1720, 1630, 1581 | 354 (95) [$M^+$], 324 (14), 311 (6), 133 (100) |
| **18d** | 45 | 181–183 | $C_{21}H_{20}O_7 + 0.3\ H_2O$ | 3250, 1710, 1565 | 384 (0.5) [$M^+$], 352 (5), 346 (8), 268 (100) |
| **18e** | 52 | 214–216 | $C_{17}H_{14}O_5S$ | 3322, 3020, 1695, 1550 | 330 (55) [$M^+$], 314 (3), 271 (59), 133 (100) |
| **19** | 62 | 117–118 | $C_{18}H_{14}O_3$ | 1705, 1600, 1560 | 278 (92) [$M^+$], 262 (48), 235 (40), 156 (100) |
| **20** | 75 | 170–172 | $C_{18}H_{13}BrO_2$ | 1620, 1580, 1560 | 342/340 (4) [$^{81/79}$Br–$M^+$], 261 (100) [$M^+$–Br] |
| **21** | 64 | 169–170 | ref.[1] 168–169 | 1720, 1638, 1600 | 294 (90) [$M^+$], 266 (3), 235 (74), 133 (100) |
| **22** | 90 | 235–240 | ref.[14] 232–258 | — | 279 (1) [$M^+$], 262 (1), 247 (5), 105 (100) |
| **23** | 32 | 236–239 | $C_{17}H_{16}N_4O_2 + 0.5\ H_2O$ | 3345, 1620, 1575 | 308 (3) [$M^+$], 276 (100), 248 (68), 220 (16) |
| **24** | 12 | 189–190 | $C_{19}H_{14}N_2O_3$ | 1615, 1570 | 318 (100) [$M^+$], 290 (9), 279 (7), 261 (11) |
| **27a** | 49 | 59–60 | $C_{19}H_{16}O_2$ | 1643, 1599 | 276 (100) [$M^+$], 185 (6), 161 (22), 132 (23) |
| **28a** | 12 | 140–142 | ref.[1] 143–145 | 1716, 1650, 1603, 1590 | 294 (100) [$M^+$], 277 (30), 249 (29), 179 (26) |
| **29a** | 88 | 350 | — | 1610, 1560 | 339 (39) [M+Na]$^+$, 317 (20) [M+H]$^+$, 176 (100)[b] |
| **27b** | 42 | 135–137 | $C_{25}H_{20}O_2$ | 1647, 1598 | 352 (100%) [$M^+$], 274 (12), 191 (16), 161 (84) |
| **28b** | 9 | 171–172 | $C_{24}H_{18}O_4$ | 3400–2800, 1733, 1635 | 370 (23) [$M^+$] , 325 (20), 283 (18), 167 (100) |
| **29b** | 79 | 350 | — | 1610 — | |
| **31** | 74 | 178–180 | $C_{20}H_{16}O_3$ | 2520, 1652, 1585, 1568 | 304 (100) [$M^+$], 276 (83), 157 (52), 129 (44) |
| **32** | 45 | bp 194 | [c] | 1600 | 318 (100) [$M^+$], 290 (83), 275 (9), 258 (14) |
| **33** | 38 | 212–214 | $C_{20}H_{16}O_5$ | 3060–2940, 1720, 1621 | 336 (100) [$M^+$], 308 (75), 292 (13), 265 (44) |
| **34** | 89 | 267–268 | — | 1713, 1612, 1555 | — |
| **36** | 29 | 189–191 | $C_{36}H_{26}O_5 + 0.5\ H_2O$ | 1733, 1634, 1582 | 538 (6) [$M^+$], 298 (3), 256 (5), 91 (100) |
| **37** | 13 | 267–269 | [d] | 3400, 1701, 1640, 1595 | 574 (10) [$M^+$] , 530 (17) [$M^+$–$CO_2$], 486 (100) |
| **38** | 94 | 350 | — | 1571, 1530 | — |

[a] Satisfactory elemental analysis obtained

[b] FAB

[c] HRMS ($C_{21}H_{18}O_3$): calcd, 318.1254; found, 318.1256.

[d] HRMS ($C_{33}H_{22}O_3$ [$M^+$–$CO_2$]): calcd, 530.1366; found, 530.1355.

*8-(2,3-Epoxypropyl)-flavone (**19**)*

A solution of 8-allylflavone (**17a**) (262 mg, 1 mmol) in dichloromethane (10 ml) was stirred at 0–5 °C while *m*-chloroperbenzoic acid (240 mg, 1.1 mmol) was added. The solution was stirred at this temperature for 3 h and for a further 1 h at room temperature before being shaken with 10% aqueous sodium bicarbonate (20 ml). The organic layer was separated, dried and evaporated to give an orange semi-solid. This was triturated with ethanol and the resulting solid recrystallised from ethanol gave the title compound as colourless crystals.

*8-(3-Bromoprop-1-enyl)flavone (**20**)*

8-Allylflavone (**17a**) (3.0 g, 11.5 mmol) was added to a suspension of 1,3-dibromo-5,5-dimethylhydantoin (1.66g, 5.8 mmol) and AIBN (0.18 g, 1.0 mmol) in carbon tetrachloride (75 ml) and the mixture was heated under reflux for 8 h. The resulting suspension was cooled and filtered and the filtrate evaporated. The residue was recrystallised from ethyl acetate to give the title compound as a yellow powder.

*8-(Methoxycarbonylmethyl)flavone (**21**)*

A mixture of flavone-8-acetic acid (**1**) (150 mg, 5.3 mmol) and methanol (15 ml) containing conc. sulfuric acid (0.5 ml) was heated under reflux for 5 h. The reaction mixture was cooled and poured into water (20 ml). The colourless precipitate was filtered off, washed with 10% sodium bicarbonate solution and water, dried and recrystallised from methanol to give the title compound as colourless crystals.

*8-(Carboxamidomethyl)flavone (**22**)*

A solution of flavone-8-acetic acid (**1**) (0.5 g, 1.79 mmol) in thionyl chloride (10 ml) was heated under reflux for 30 min and then evaporated. The residue was stirred with conc. aqueous ammonia (5 ml) for 3 h and then

**Table 4.** $^1$H NMR data for substituted benzopyran-4-ones, $\delta_H$.

| Com-pound | Benzopyranone H-3 | H-5[a] | H-6[b] | H-7[a] | 8-Substituent | 2-Substituent |
|---|---|---|---|---|---|---|
| **18a** | 6.79 (s) | 8.16 (dd) | 7.38 (t) | 7.56 (dd) | 4.41 (s, 2 H), 4.09 (s, 2 H), 3.15 (br s, 1 H) | 7.77 (m, 2 H), 7.52–7.39 (m, 3 H) |
| **18b** | 6.84 (s) | 8.00 (dd) | 7.47 (t) | 7.70 (dd) | 5.40 (br s, 1 H), 4.25 (s, 2 H), 4.18 (s, 2 H) 7 | 31 (m, 3 H), 3.90 (s, 3 H), 3.79 (s, 3 H) |
| **18c** | 7.07 (s) | 8.19 (dd) | 7.39 (t) | 7.57 (dd) | 4.37 (s, 2 H), 4.05 (s, 2 H), 3.20 (br s, 1 H) | 7.27 (m, 1 H), 7.00 (m, 2 H), 3.87 (s, 3 H), 3.85 (s, 3 H) |
| **18d** | 7.14 (s) | 7.98 (dd) | 7.46 (t) | 7.72 (dd) | 5.34 (t, *J* = 5, 1 H), 4.24 (m, 4 H) | 7.29 (s, 2 H), 3.91 (s, 6 H), |
| **18e** | 6.80 (s) | 7.95 (m) | 7.40 (t) | 7.62 (dd) | 5.42 (t, *J* = 6, 1 H), 4.38 (d, *J* = 6, 2 H)[c] | 3.75 (s, 3 H) 7.95 (m, 1 H), 7.26 (d, *J* = 6, 1 H), |
| **19** | 6.88 (s) | 8.17 (dd) | 7.41 (t) | 7.65 (dd) | 3.4–3.25 (m, 3 H), 2.88 (m, 1 H)[c] | 4.06 (s, 3 H) 7.95 (m, 2 H), 7.58 (m, 3 H) |
| **20** | 6.79 (s) | 8.14 (dd) | 7.35 (t) | 7.80 (dd) | 7.18 (d, *J* = 12, 1 H), 4.22 (d, 2 H, *J* = 7)[c] | 7.92–7.85 (m, 2 H), 7.6–7.5 (m, 3 H) |
| **21** | 6.85 (s) | 8.18 (dd) | 7.40 (t) | 7.62 (dd) | 4.02 (s, 2 H), 3.75 (s, 3 H) | 7.95–7.90 (m, 2 H), 7.6–7.5 (m, 3 H) |
| **22** | 7.30 (s) | 7.67 (dd) | 6.90 (t) | 7.14 (dd) | 9.2 (br s, 2H), 4.40 (br s, 2 H) | 7.87 (m, 2H), 7.55–7.38 (m, 3 H) |
| **24** | 6.82 (s) | 8.19 (m) | 7.39 (t) | 7.78 (m) | 4.55 (s, 2 H), 2.35 (s, 3 H) | 7.88 (m, 2 H), 7.57 (m, 3 H) |
| **27a** | 6.13 (s) | 8.03 (dd) | 7.38 (t) | 7.47 (dd) | 5.97–5.83 (m, 1 H), 5.06 (m, 2 H)[c] | 7.38–7.25 (m, 5 H), 3.93 (s, 2 H) |
| **28a** | 6.26 (s) | 7.90 (dd) | 7.27 (t) | 7.67 (dd) | 12.5–11.5 (br s, 1 H), 3.83 (s, 2 H) | 7.40–7.33 (m, 5 H), 3.99 (s, 2 H) |
| **29a**[d] | 7.15 (s) | 7.67 (dd) | 7.50–7.20 (m, 2 H) | | 3.53 (s, 2 H) | 7.50–7.20 (m, 5 H), 3.55 (s, 2 H) |
| **27b** | 6.17 (s) | 8.05 (dd) | 7.30 (m) | 7.45 (dd) | 5.85–5.64 (m, 1 H), 4.95–4.81 (m, 2 H)[c] | 7.40–7.20 (m, 10 H), 5.37 (s, 1 H) |
| **28b** | 6.32 (s) | 8.12 (dd) | 7.40 (m) | 7.52 (dd) | 3.59 (s, 2 H) (acid proton not apparent) | 7.36–7.19 (m, 10 H), 5.38 (s, 1 H) |
| **29b**[d] | 6.25 (s) | 7.58 (dd) | 7.07 (t) | 7.40 (dd) | 3.52 (s, 2 H) | 7.84 (m, 2 H), 7.41–7.22 (m, 8 H), 6.18 (s, 1 H) |
| **36** | 6.81 (s) | 8.12 (dd) | 7.37 (t) | 7.56 (dd) | 6.16–6.03 (m, 2 H), 5.20–5.13 (m, 4 H), 3.76 (d, *J* = 6, 4 H) | 7.96 and 7.21 (AB pattern, *J* = 9, 8 H) |
| **37** | 7.14 (s) | 8.15 (dd) | 7.47 (t) | 7.77 (dd) | 4.04 (s, 4 H) (acid protons not apparent) | 8.01 (m, 4 H), 7.20 (m, 4 H) |
| **38**[c] | 6.71 (s) | 7.73 (dd) | 7.01 (t) | 7.52 (dd) | 3.70 (s, 4 H) | 7.87 and 7.11 (AB pattern, *J* = 9, 8 H) |

[a] *J* = 8, 2 [b] *J* = 8 [c] Additional signals: **18e** 4.16 (s, 2 H); **19** 2.63 (m, 1 H); **20** 6.61 (dt, *J* = 12, 7, 1 H); **27a** 3.54 (d, 2 H, *J* = 8); **27b** , 3.42 (d, *J* = 8, 2 H) [d] in $D_2O$

the mixture evaporated. The residual solid was recrystallised from ethanol to give the product as colourless crystals.

*3-(3-Hydrazidocarbonylmethyl-2-hydroxyphenyl)-5-phenylpyrazole (***23***)*

A solution of 8-(methoxycarbonylmethyl)flavone (**21**) (294 mg, 1.0 mmol) in methanol (10 ml) was stirred at room temperature while hydrazine hydrate (200 mg, 4.0 mmol) in methanol (5 ml) was added. After 8 h, the mixture was poured into water and the resulting precipitate was filtered off and recrystallised from ethanol to give the product.–$^1$H NMR: δ = 11.40 (br s, 31 H), 9.25 (br s, 31 H), 7.90 (m, 2 H), 7.70 (d, *J* = 8, 1 H), 7.59–7.42 (m, 3 H), 7.18 (s, 1 H), 7.16 (d, *J* = 8, 1 H), 6.93 (t, *J* = 8, 1 H), 4.34 (br s, 2 H).–$^{13}$C NMR: δ = 170.3 (CO), 153.7 (4ry), 130.4 (CH), 129.3 (2 CH), 129.1 (4ry), 129.0 (4ry), 128.9 (CH), 128.8 (CH), 125.7 (2 CH, 4ry), 123.8 (CH), 119.2 (4ry), 116.8 (4ry), 100.1 (CH), 35.2 ($CH_2$).

*8-((3-Methyl-1,2,4-oxadiazol-5-yl)methyl)flavone (***24***)*

A solution of acetamide oxime[25] (0.33 g, 4.4 mmol) in dry tetrahydrofuran (15 ml) was heated to reflux in the presence of sodium hydride (0.21 g,

**Table 5.** $^{13}C$ NMR data for substituted benzopyran-4-ones, $\delta_C$.

| Com- | Benzopyranone | | | | | | | | | 8-substituent | | | 2-substituent | | | | Other |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pound | C–2 | C–3 | C–4 | C–4a | C–5 | C–6 | C–7 | C–8 | C–8a | C–1 | C–2 | C–3 | C–1′ | C–2′ | C–3′ | C–4’ | |
| **18a** | 163.4 | 107.9 | 178.3 | 122.7 | 125.6 | 125.2 | 135.5 | 124.2 | 154.3 | 40.2 | 205.9 | 68.0 | 131.7 | 126.2 | 129.3 | 131.8 | |
| **18b** | 160.5 | 111.2 | 177.1 | 123.0 | 124.9 | 123.4 | 135.9 | 124.6 | 154.2 | 40.5 | 207.6 | 67.4 | 125.4 | 152.9 | 147.2 | 115.8 | |
| **18c** | 160.8 | 112.8 | 178.6 | 122.6 | 125.5 | 124.9 | 135.3 | 124.1 | 154.4 | 40.2 | 206.1 | 67.9 | 121.2 | 153.5 | 113.1 | 117.8 | |
| **18d** | 162.3 | 107.0 | 177.5 | 123.5 | 126.7 | 125.1 | 136.2 | 123.9 | 154.3 | 39.9 | 207.5 | 67.6 | 103.9 | 104.0 | 153.5 | 140.7 | |
| **18e** | 159.9 | 105.5 | 176.6 | 123.4 | 125.0 | 124.7 | 135.9 | 123.8 | 153.7 | 39.6 | 207.5 | 67.9 | — | 109.8 | 157.9 | 117.9 | |
| **19** | 163.1 | 107.5 | 178.6 | 124.0 | 125.0 | 124.5 | 134.7 | 126.7 | 154.5 | 32.7 | 51.2 | 46.9 | 131.8 | 126.2 | 129.2 | 131.6 | |
| **20** | 163.2 | 107.6 | 178.1 | 124.3 | 125.7 | 125.0 | 131.1 | 126.0 | 153.2 | 129.1 | 126.6 | 32.9 | 131.8 | 126.3 | 129.2 | 131.7 | |
| **21** | 163.0 | 107.5 | 178.4 | 123.9 | 125.2 | 125.0 | 135.3 | 124.1 | 154.4 | 35.8 | 170.9 | — | 131.7 | 126.2 | 129.1 | 131.7 | 52.4 (OMe) |
| **24** | 163.1 | 107.7 | 178.0 | 123.2 | 125.8 | 125.2 | 134.9 | 124.4 | 154.1 | 27.6 | — | — | 131.6 | 126.3 | 129.2 | 131.8 | |
| **27a** | 167.5 | 110.3 | 178.6 | 123.6 | 127.4 | 123.7 | 135.3 | 124.7 | 154.4 | 33.8 | 133.8 | 116.7 | 134.9 | 128.9 | 129.3 | 129.3 | 40.8 ($CH_2$) |
| **28a** | 167.8 | 109.5 | 176.9 | 123.0 | 124.7 | 123.6 | 135.4 | 125.1 | 154.2 | 34.6 | 171.8 | — | 135.6 | 128.6 | 129.1 | 127.0 | 39.5 ($CH_2$) |
| **29a** | 161.8 | 111.4 | 183.9 | 124.5 | 126.4 | 120.2 | 139.9 | 130.8 | 157.4 | 41.4 | 181.5 | — | 138.8 | 131.4 | 132.5 | 134.7 | 47.2 ($CH_2$) |
| **27b** | 169.2 | 112.1 | 178.6 | 123.8 | 124.8 | 123.7 | 135.1 | 129.6 | 154.4 | 33.9 | 133.9 | 116.6 | 138.9 | 128.8 | 129.0 | 127.5 | 56.1 (CH) |
| **28b** | 169.4 | 112.0 | 178.7 | 123.6 | 125.0 | 123.6 | 135.3 | 125.3 | 154.7 | 34.9 | 174.8 | — | 138.8 | 128.8 | 129.0 | 127.5 | 56.1 (CH) |
| **29b** | 172.8 | 111.5 | 183.4 | 125.0 | 125.4 | 124.7 | 136.1 | 129.6 | 157.3 | 41.1 | 181.4 | — | 137.9 | 131.7 | 132.2 | 138.6 | 57.1 (CH) |
| **36** | 162.3 | 107.0 | 178.6 | 124.0 | 125.0 | 124.0 | 135.3 | 127.7 | 154.2 | 34.0 | 134.2 | 117.0 | 129.4 | 128.3 | 119.6 | 159.2 | |
| **37** | 161.9 | 106.8 | 177.2 | 123.5 | 125.4 | 124.0 | 136.0 | 126.6 | 154.4 | 35.8 | 172.3 | — | 125.2 | 132.1 | 119.0 | 159.1 | |
| **38** | 161.5 | 109.4 | 177.7 | 121.3 | 128.3 | 121.7 | 135.0 | 128.2 | 156.7 | 41.7 | 181.9 | — | 139.1 | 133.8 | 121.1 | 156.7 | |

Additional signals: **18b** 124.5 (C–5′), 120.2 (C–6′), 60.4 (OMe), 55.9 (OMe); **18c** 152.2 (C–5′), 114.3 (C–6′), 56.1 (OMe), 55.9 (OMe); **18d** 60.4 (OMe), 56.3 (2 C, OMe); **18e** 131.1 (C–5′), 59.6 (OMe); **24** 176.7 (C-5′), 167.6 (C-2′), 11.6 (2′-Me)

8.8 mmol). After 0.5 h 8-(methoxycarbonylmethyl)flavone (**20**) (1.29 g, 4.4 mmol) was added and heating continued for a further 4 h. Upon cooling, the mixture was filtered and evaporated. The residue was extracted with dichloromethane which was evaporated to give crude product. This was recrystallised from methanol to give the title compound as colourless needles.

For synthesis of the remaining compounds the required carboxylic acids were commercially available. 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.

*Methyl Phenylacetate (***25a***)*

From phenylacetic acid (84%) as a colourless liquid bp (oven temp.) 81 °C/0.3 Torr (ref.[26] 220 °C).–$^1$H NMR: δ = 7.18 (m, 5 H), 3.67 (s, 3 H), 3.59 (s, 2 H).

*Methyl Diphenylacetate (***25b***)*

From diphenylacetic acid (91%) as cream plates, mp 58–60 °C (ref.[27] 59–62 °C).–$^1$H NMR: δ = 7.31 (m, 10 H), 5.05 (s, 1 H), 3.76 (s, 3 H).

*Dimethyl 4,4′-Oxydibenzoate (***35***)*

From 4,4′-oxydibenzoic acid (60%) as colourless needles, mp 154–155 °C (ref.[28] 156–158 °C).–$^1$H NMR: δ = 8.05 and 7.05 (AB pattern, *J* = 8, 8 H), 3.90 (s, 3 H).

*Methyl Phenylpropiolate (***30***)*

Phenylpropiolic acid was prepared by treatment of a solution of phenylacetylene in dry tetrahydrofuran with butyllithium in hexane followed by $CO_2$ gas. The acid was taken up in the calculated quantity of aqueous 2M sodium hydroxide which was filtered and evaporated to dryness. A suspension of the residual sodium salt in ether and excess thionyl chloride was heated under reflux for 6 h and then evaporated. Distillation of the residue gave the acid chloride which was heated with methanol for 1 h and then evaporated and the residue distilled to give the product as a colourless liquid, bp (oven temp.) 107 °C/0.13 Torr (ref.[29] 132–133 °C/16 Torr).–$^1$H NMR: δ = 7.95 (s, 5 H), 3.80 (s, 3 H).

The compounds **29a,b** and **38** were prepared by the previously reported method[13] involving reaction of 3-allyl-2-hydroxyacetophenone (**14**), sodium hydride and the appropriate methyl ester followed by *in situ* acid-induced cyclisation, permanganate oxidation and conversion into the sodium salts using $NaHCO_3$. The properties of the intermediates and products are listed in Table 3.–$^1$H NMR see Table 4.–$^{13}$C NMR see Table 5.

The preparation of **34** followed the same route save for the addition of the methylation step described below before the permanganate oxidation. The properties of the intermediates and products are listed in Table 3 and the $^1$H and $^{13}$C NMR spectra are given below.

*2-(3-Allyl-2-hydroxyphenyl)-6-phenylpyran-4-one (***31***)*

$^1$H NMR: δ = 9.3 (br s, 1 H, OH), 7.85 (d, *J* = 2, 1 H), 7.83 (d, *J* = 4, 1 H), 7.71 (dd, *J* = 8, 2, 1 H), 7.59 (d, *J* = 2, 1 H), 7.53–7.47 (m, 3 H), 7.29 (dd, *J* = 8, 2, 1 H), 6.98 (t, *J* = 8, 1 H), 6.83 (d, *J* = 6, 1 H), 6.13–6.02 (m, 1 H), 5.27–5.11 (m, 2 H), 3.70 (d, *J* = 6, 2 H).–$^{13}$C NMR: δ = 181.8, 164.1, 162.7, 154.5, 136.2, 133.4, 131.7, 131.5, 129.2, 128.4, 126.7, 126.1, 120.1, 118.9, 116.4, 115.1, 110.8, 34.7.

*2-(3-Allyl-2-methoxyphenyl)-6-phenylpyran-4-one (**32**)*

Sodium hydride (0.24 g, 9.8 mmol) was washed with light petroleum (40 ml) which was decanted off and replaced by THF (200 ml). The suspension was then heated under reflux for 10 min and **31** (3.0 g, 9.8 mmol) was added portionwise over 10 min followed by dimethyl sulfate (3.7 g, 39 mmol)**.** After heating for a further 2 h, the mixture was allowed to cool and concentrated ammonia (40 ml) was added. After stirring for 12 h, water (200 ml) was added and the mixture extracted with methylene chloride which was dried and evaporated to yield a yellow oil**.** Column chromatography (silica, ethyl acetate/petroleum 2:1) gave the product.–$^1$H NMR: δ = 7.88–7.81 (m, 2 H), 7.63 (dd, *J* = 8, 2, 1 H), 7.52–7.49 (m, 3 H), 7.37 (dd, *J* = 8, 2, 1 H), 7.20 (t, *J* = 8, 1 H), 7.00 (d, *J* = 2, 1 H), 6.83 (d, *J* = 2, 1 H), 6.10–5.90 (m, 1 H), 5.20–5.03 (m, 2 H), 3.69 (s, 3 H), 3.50 (d, *J* = 7, 2 H).–$^{13}$C NMR: δ = 180.5, 163.7, 162.0, 156.8, 136.6, 134.8, 133.5, 131.4, 129.1, 129.0, 127.7, 125.9, 125.3, 124.4, 116.5, 115.6, 111.1, 61.3, 33.8.

*2-(3-Carboxymethyl-2-methoxyphenyl)-6-phenylpyran-4-one (**33**)*

$^1$H NMR ($CD_3SOCD_3$): δ = 8.06 (m, 2 H), 7.79 (d, *J* = 8, 1 H), 7.63 (m, 3 H), 7.59 (d, *J* = 8, 1 H), 7.36 (t, *J* = 8, 1 H), 7.11 (s, 1 H), 6.83 (s, 1 H), 3.75 (s, 2 H), 3.69 (s, 3 H) (acid proton not apparent).–$^{13}$C NMR: δ = 184.7, 182.3, 166.7, 164.6, 159.1, 138.3, 135.2, 134.6, 131.9, 131.5, 129.9, 128.0, 127.3, 125.6, 116.2, 111.6, 63.2, 41.4.

*Sodium 2-(3-Carboxylatomethyl-2-methoxyphenyl)-6-phenylpyran-4-one (**34**)*

$^1$H NMR ($D_2O$): δ = 7.51–7.20 (m, 5 H), 7.16–7.01 (m, 3 H), 6.82 (s, 1 H), 6.55 (s, 1 H), 3.57 (m, 5 H).

*In Vitro Chemosensitivity Studies*

The activity 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 $1 \times 10^4$ 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 activity 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. The vehicles used were Arachis oil (**19**, **20**, **21**), 0.9% saline (**18d**, **22**, **23**, **24**) and 0.9% saline/DMSO (9:1) (**29a**, **29b**, **34**). All treatments were administered intraperitoneally to NMRI mice. A minimum of 5 mice were used for each compound. 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. At the dose levels used there was no significant body weight loss.

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[30]. 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.

## References


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