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The Total Synthesis of an Aurone Isolated from *Uvaria hamiltonii*: Aurones and Flavones as Anticancer Agents

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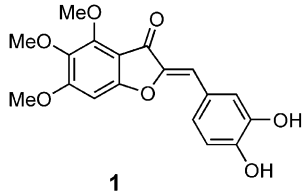
Abstract—The naturally occurring aurone **1**, isolated from *Uvaria hamiltonii*, and a series of aurones analogues based structurally on known tubulin binding agents were prepared and evaluated for anticancer activity. Aurone **20** was the most active (IC_{50} K562 50 nM) and caused significant G_2/M cell-cycle arrest.

Interest in flavonoids as pharmacological agents has been considerable since these natural products have been shown to constitute the active ingredient of many folk medicines.[1] Aurones have been reported to display analgesic activity,[2] while aurone derivatives from plant extracts have been used in the treatment of thyroid diseases.[3] Flavones are known to possess anticancer activity,[4] along with selective inhibition of both cyclin-dependent kinases (CDK's)[5] and tyrosine kinases.[6]

Aurone **1** is one of several constituents isolated from the extracts of *Uvaria hamiltonii* by Wani and co-workers.[7] The constituents were isolated by way of a bioassay-guided fractionation based on DNA strand-scission[8] and 9KB assays. While aurone **1** appeared to be inactive in the 9KB assay it offered the greatest potency, of the compounds isolated, in the DNA strand-scission assay. It should be noted that in comparison to a bleomycin standard, compounds of this nature actually offer weak DNA strand-scission activity.

The resemblance of aurone **1** to the tumour vascular targeting agent combretastatin A-4,[9–11] made it an appealing target for total synthesis.



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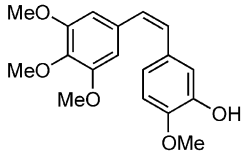
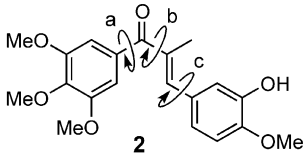


Combretastatin A-4



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The development of a flexible synthesis of **1** would also provide access to analogues to support our programme of research into tumour vascular targeting agents.[12] The spatial relationship between the two aromatic rings of combretastatin A-4, colchicine and similar drugs is an important structural feature that determines their ability to bind to tubulin.[13] The chalcone **2** is a potent inhibitor of tubulin polymerisation,[14,15] which we believe adopts an s-*trans* conformation.[16] The aurones and flavones would effectively provide conformationally restricted analogues of **2**. We hoped that this would present an insight into the importance of aryl ring orientation about the rotatable bonds a and c in influencing cytotoxicity and tubulin-binding properties.

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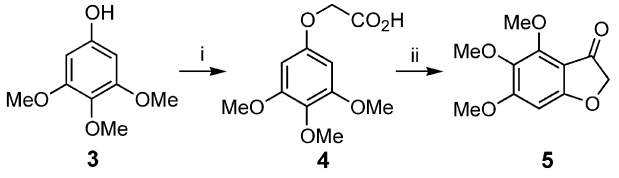
Not widely distributed in nature, aurones are one of the least common types of flavonoid, and so have received little attention in comparison to the structurally related and widely investigated flavones and isoflavones. Therefore, not surprisingly, general methods for their synthesis are not so common. The most general route to aurones involves the acid or base catalyzed condensation of substituted 3(2*H*)-benzofuranones with aromatic aldehydes. We therefore needed to develop a route to the appropriate benzofuranones.

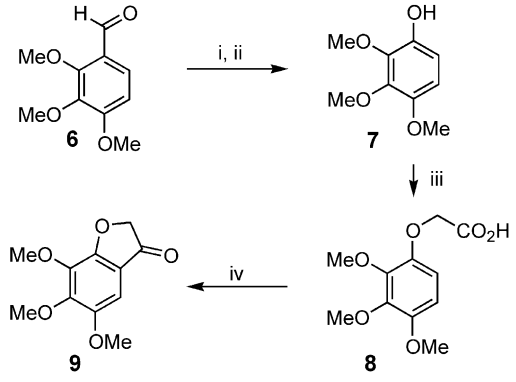
Benzofuranone **5** was prepared in two steps from 3,4,5-trimethoxyphenol (**3**) in 51% overall yield, via the reaction with chloroacetic acid and excess sodium hydride, followed by polyphosphoric acid (PPA) mediated cyclization of the first-formed phenoxyacetic acid **4** (Scheme 1).

Benzofuranone **9** was prepared in the same way as **5** by PPA promoted intramolecular Friedel–Crafts reaction of the phenoxyacetic acid **8**. Phenol **7** was prepared by sequential Baeyer–Villiger oxidation of the benzaldehyde **6**, using *m*-chloroperbenzoic acid, and base-catalysed methanolysis of the resulting formate (Scheme 2). The overall yield of benzofuranone **9** from **6** was 38%.

The synthesis of aurone **1** and its regioisomer **14** required the use of the TBDMS-protected benzaldehyde **11**, prepared in high yield from 3,4-dihydroxybenzaldehyde (**10**) using *tert*-butyldimethylsilyl chloride in the presence of imidazole.[17] On reaction with the benzofuranones **5** and **9** in the presence of neutral alumina—a protocol developed by Varma and Varma[18]—the TBDMS-protected aurones **12** and **13** were obtained in good yield. Subsequent treatment with tetrabutylammonium fluoride afforded aurones **1** and **14** (Scheme 3). The aurone **1** was fully characterised and



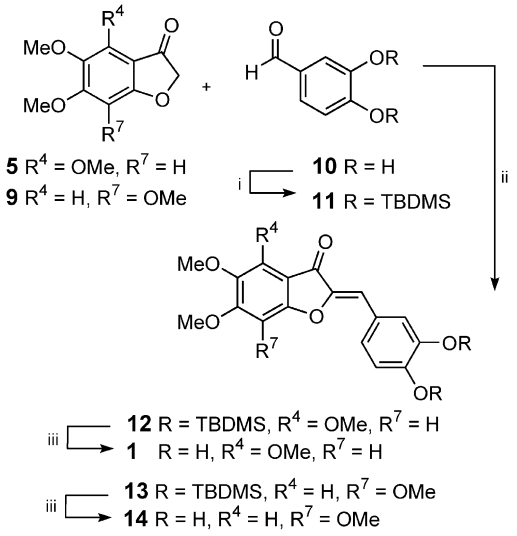
Scheme 1. Reagents and conditions: (i) $ClCH_2CO_2H$, NaH, DMF, rt, overnight, 85%; (ii) PPA, 80 °C, 8 h, 60%.



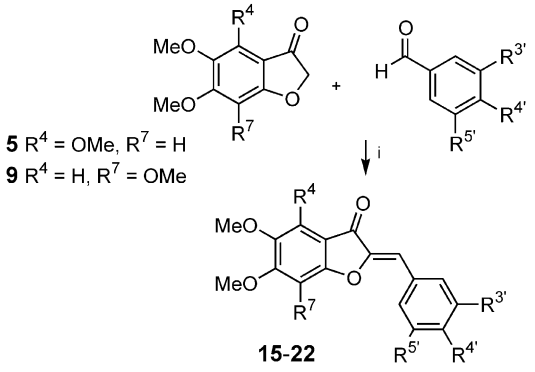
Scheme 2. Reagents and conditions: (i) *m*-CPBA, DCM, rt, 18 h, 93%; (ii) NaOMe, MeOH, rt, 4 h, 88%; (iii) $ClCH_2CO_2H$, NaH, DMF, rt, overnight, 81%; (iv) PPA, 80 °C, 8 h, 58%.

deemed to be identical to the natural product originally disclosed by Wani and co-workers by ^1H and ^{13}C NMR.[7] The total synthesis of **1** was therefore achieved in four steps in 37% overall yield.

With the benzofuranones **5** and **9** in hand, a series of substituted aurones was prepared (Scheme 4). The yields were generally lower when a deactivated (electron-rich) benzaldehyde was used (Table 1). Fluorinated aurones are included in the series since we wish to investigate the ability of combretastatin derivatives to



Scheme 3. Reagents and conditions: (i) TBDMSCl, imidazole, DCM, rt, overnight, 92%; (ii) Al_2O_3, DCM, rt, overnight, 74% (**12**) and 97% (**13**); (iii) TBAF, DCM, rt, 30 min, 98% (**12** to **1**) or 78% (**13** to **14**).



Scheme 4. Reagents and conditions: (i) Al_2O_3, DCM, rt, 1–3 days, yields (see Table 1).

Table 1. Synthesis of substituted aurones

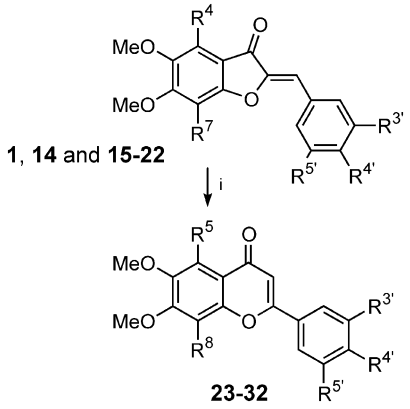
	R^4	R^7	$R^{3'}$	$R^{4'}$	$R^{5'}$	Yield (%)
15	OMe	H	H	OMe	H	71
16	OMe	H	OH	OMe	H	31
17	OMe	H	F	OMe	H	62
18	OMe	H	F	OMe	F	71
19	H	OMe	H	OMe	H	78
20	H	OMe	OH	OMe	H	48
21	H	OMe	F	OMe	H	80
22	H	OMe	F	OMe	F	82

induce tumour vasculature damage via ^{18}F positron emission tomography (PET).[12]

In all cases a single geometric isomer (*Z*) was obtained, this generally being more thermodynamically stable than the (*E*)-isomer.[19] Although an unambiguous assignment of our aurone series was not made, the values are consistent with those present in the literature for known (*Z*)-aurones.[20] In a single step—using the protocol developed by Wheeler and co-workers[21]—the aurones were transformed into their corresponding flavones[22] by simply heating in the presence of potassium cyanide (Scheme 5 and Table 2). The reaction is impeded by the presence of a phenol group. The rearrangement is also clearly complicated by the presence of two fluorine atoms.

The cell growth inhibitory properties of the substituted aurones and flavones (summarised in Table 3) were determined in the K562 human chronic myelogenous leukaemia cell line using the MTT assay as detailed by Edmondson et al.[23] The IC_{50} value represents the concentration which results in a 50% inhibition in cell growth after 5 days incubation.

It is clear that the 5,6,7-trimethoxyaurones (**19**–**22**) offer greater cell growth inhibitory properties than their corresponding 4,5,6-trimethoxy isomers. It would appear that either the presence of a methoxyl group at the 7-position of the A-ring or the lack of a methoxyl group at the 4-position is responsible for the differences in cytotoxicity. Natural product aurone **1** (IC_{50} 12 μM) and its regioisomer **14** (IC_{50} 9.7 μM) display similarly poor cell-growth inhibitory properties. The aurone **20** (IC_{50} 50 nM) bearing the greatest resemblance to combretastatin A-4 displays the highest activity, along with the monofluorinated aurone **21** (IC_{50} 0.11 μM). Again, as with the corresponding 5,6,7-trimethoxyaurones, it is clear that the structurally related flavones bearing a 6,7,8-trimethoxyphenyl A-ring arrangement display the greatest cytotoxicity, with flavone **30** (IC_{50} 40 nM) being equipotent to aurone **20** (IC_{50} 50 nM).



Scheme 5. Reagents and conditions: (i) KCN, EtOH/DCM, reflux, 12 h, yields (see Table 2).

Table 2. Synthesis of substituted flavones

	R^5	R^8	$R^{3'}$	$R^{4'}$	$R^{5'}$	Yield (%)
23	OMe	H	OH	OH	H	0
24	OMe	H	H	OMe	H	73
25	OMe	H	OH	OMe	H	20
26	OMe	H	F	OMe	H	57
27	OMe	H	F	OMe	F	0
28	H	OMe	OH	OH	H	0
29	H	OMe	H	OMe	H	80
30	H	OMe	OH	OMe	H	32
31	H	OMe	F	OMe	H	64
32	H	OMe	F	OMe	F	0

Preliminary results from modeling of the structures are shown in Figure 1.[24] Superposition of energy-minimized structures of **20** and **30** onto the chalcone **2**, indicates that a reasonable degree of overlap can be achieved in both cases. The positions of the A-ring and linker are similar. The main difference in both cases seems to be the tilt of the B-ring, which arises in **2** from the presence of the α-methyl group.

The effects of the most active compounds upon the cell cycle were measured by flow cytometry. The results show aurones **20** and **21** to cause significant arrest of the cell cycle at the G_2/M point, relative to the untreated control, at this stage consistent with the behaviour of tubulin binding agents. As expected (from the lack of cytotoxicity), the natural product aurone **1** and its regioisomer **14** fail to induce significant levels of G_2/M block, along with flavone **30** (Table 4).

Table 3. Cell growth inhibition[a] against the K562 cell line

	IC_{50} (μM)		IC_{50} (μM)		IC_{50} (μM)
1	12	**14**	9.7	**24**	42
15	> 50	**19**	0.15	**25**	22
16	18	**20**	0.05	**26**	> 50
17	> 50	**21**	0.11	**29**	0.83
18	> 50	**22**	0.15	**30**	0.04
2	0.0002			**31**	0.59

[a]As measured by the MTT assay after 5 days incubation of the drug with the cells cultured at 37 °C.

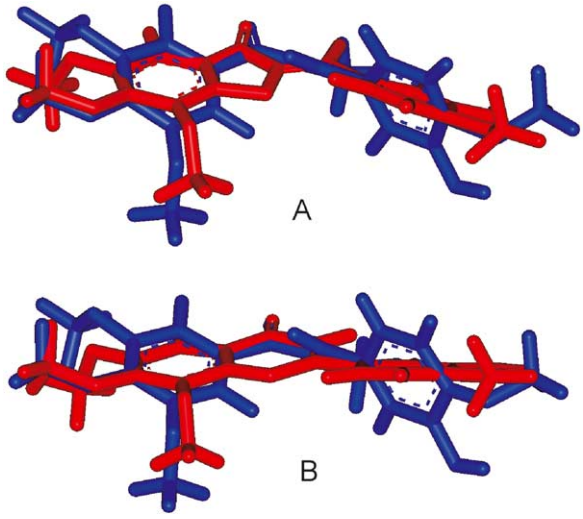


Figure 1. Superposition of the structures aurone **20** (A) and flavone **30** (B) onto chalcone **2**. Structures **20** and **30** are shown in red, while **2** is shown in blue.

Table 4. Effects upon the cell cycle[a]

	G_0–G_1	S	G_2/M
Control	48	45	7
1	57	18	25
14	45	16	39
20	17	14	69
21	12	11	77
30	52	11	38
2	10	7	83

[a]As measured by flow cytometry.[25]

Table 5. Effects upon tubulin binding

	Microtubule assembly[a] IC_{50} (μM)		Microtubule assembly[a] IC_{50} (μM)
1	> 50	**21**	> 50
16	> 50	**25**	> 50
17	> 50	**26**	> 50
14	> 50	**30**	25
20	22	**31**	> 50
2	0.5		
	Colchicine displacement[b] IC_{50} (μM)		
20	60		
30	40		

[a]Microtubule assembly assay was carried out as previously described.[26] The IC_{50} values shown represent the concentrations that cause a 50% inhibition in tubulin assembly as measured by an increase in turbidity at 350 nm.
[b]Competition for the colchicine binding site was carried out as previously described.[19] The IC_{50} values shown represent the concentrations which cause 50% decrease in the binding of ^{3}H-colchicine from purified porcine tubulin.

With the exception of aurone **20** and flavone **30** (which interact at the colchicine binding site) all compounds were inactive against tubulin polymerisation, generally reflecting their lack of cytotoxicity and antimitotic properties (Table 5). These observations provide remarkable evidence that the conformational restriction of **2** about bonds a and b results in lower cytotoxic and antitubulin activity.

In conclusion we have developed an efficient synthesis of the natural aurone from the key benzofuranone **5**. A series of significantly more active aurones were prepared from this aurone and its isomer **9**. The reduced activity of the aurones and flavones compared to that of the chalcone **2** indicates the importance of conformational flexibility in influencing the anticancer properties of the chalcones.

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