Structure−Activity Requirements for Flavone Cytotoxicity and Binding to Tubulin

John A. Beutler, Ernest Hamel, Arnold J. Vlietinck,† Achiel Haemers,† Padinchare Rajan,† James N. Roitman,‡ John H. Cardellina II, and Michael R. Boyd*

Laboratory of Drug Discovery Research and Development, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Frederick, Maryland 21702-1201

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A series of 79 flavones related to centaureidin (3,6,4′-trimethoxy-5,7,3′-trihydroxyflavone, **1**) was screened for cytotoxicity in the NCI in vitro 60-cell line human tumor screen. The resulting cytotoxicity profiles of these flavones were compared for degree of similarity to the profile of **1**. Selected compounds were further evaluated with in vitro assays of tubulin polymerization and [^{3}H]colchicine binding to tubulin. Maximum potencies for tubulin interaction and production of differential cytotoxicity profiles characteristic of **1** were observed only with compounds containing hydroxyl substituents at C-3′ and C-5 and methoxyl groups at C-3 and C-4′.

Introduction

Compounds such as vincristine, vinblastine, and taxol, which interfere with tubulin polymerization and cause mitotic arrest, are of proven clinical utility.[1] In addition to these established clinical agents, other structurally diverse organic compounds are known to interfere with tubulin polymerization. For example, colchicine,[2] stilbenes,[3] and chalcones[4] all appear to bind to a common site on tubulin which partially overlaps a binding site for podophyllotoxin.[5] The *Vinca* alkaloids,[6] dolastatin 10,[7,8] the spongistatins,[9] and taxol[10] have also been reported to bind to several apparently distinct sites on tubulin.

We have explored the use of the NCI 60-cell line in vitro screen, in conjunction with a pattern-matching algorithm, Compare,[11,12] as a means of identifying natural product extracts potentially containing new tubulin-interactive antimitotic leads.[13,14] Stemming from that effort, we recently reported the identification of the flavone centaureidin, **1**, as a cytotoxic principle of the plant *Polymnia fruticosa* and further demonstrated inhibitory effects of **1** on tubulin polymerization.[13] That first report was followed shortly thereafter by two independent reports of a second flavone, **2**, with similar properties.[15,16]

The goal of the present study was to explore the structural requirements for flavone interactions with tubulin, both to ascertain potential directions for synthetic lead-optimization studies, as well as to identify an optimal candidate among currently available compounds for in vivo xenograft studies. We report here results of comparative in vitro antitumor screening of 79 flavones and subsequent tubulin polymerization studies with 20 of those compounds.

Results and Discussion

For this study, we selected a set of flavones from the NCI repository, augmented by additional natural products and synthetic and commercially available compounds, all of which had either hydrogen, hydroxyl, or methoxyl substituents at C-3. The compounds were tested in the NCI primary in vitro human tumor cell screen for cytotoxicity, and the resulting dose−response curves were analyzed for the differential cytotoxicity pattern[12] characteristic of **1** and other antimitotic standard agents. Selected compounds were further examined in biochemical assays for inhibition of tubulin polymerization and inhibition of [^{3}H]colchicine binding to tubulin.

The cytotoxic potencies (mean panel GI_{50}) of 26 3-methoxyflavones in the NCI human tumor cell line screen are reported in Table 1. Only compounds **1**−**3** showed significant cytotoxicity (GI_{50} < 2 μM) and TGI (net total growth inhibition[12])−Compare correlation coefficients of >0.7. Several other compounds (e.g., **4**−**7**) showed modest cytotoxicity (GI_{50} 7−10 μM) but did not yield TGI−Compare correlation coefficients > 0.5 in reference to **1**.

Results from testing a second series, 15 compounds with a 3-hydroxyl substituent, are summarized in Table 2. While many showed modest cytotoxicity (the GI_{50}'s of **27**−**33** were 4−10 μM), none yielded TGI−Compare correlation coefficients of >0.5 in reference to **1**.

Finally, the results for a third series of 38 flavones with no substitution at C-3 are listed in Table 3. The majority of the 3-unsubstituted flavones showed minimal cytotoxicity in the NCI in vitro human tumor cell assay, and none yielded TGI−Compare correlation coefficients > 0.5. In this group, only compounds **42** and **43** exhibited GI_{50} < 10 μM.

Selected compounds were then evaluated for inhibition of tubulin polymerization in vitro (Table 4). Compounds **1**−**3** significantly inhibited tubulin assembly at low drug concentrations (substoichiometric to the assay tubulin concentration of 10 μM). The IC_{50} values obtained with **1**−**3** ranged from 0.8 to 3 μM, similar to that obtained for colchicine (1.4 μM). Compound **3** was essentially equipotent with **1** and only slightly less potent than **2** in both tubulin assays, even though its potency in the NCI primary screen was one-tenth that

† Department of Pharmaceutical Sciences, University of Antwerp, B-2610 Antwerpen, Belgium.
‡ Agricultural Research Service, USDA, Pacific West Area Regional Research Center, Albany, CA 94710.

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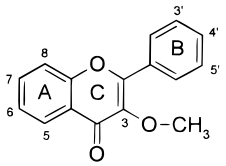
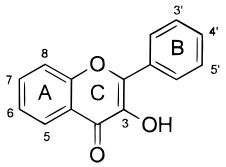
Table 1. Structure and Cytotoxicity Data for 3-Methoxyflavone Derivatives



compd	C-5	C-6	C-7	C-8	C-3′	C-4′	C-5′	mean GI_{50} (μM)
1	HO	MeO	HO	H	HO	MeO	H	0.24
2	HO	MeO	MeO	MeO	HO	MeO	H	0.13
3	HO	H	HO	H	HO	MeO	H	1.7
4	HO	MeO	MeO	MeO	MeO	MeO	H	7.1
5	HO	MeO	MeO	MeO	MeO	HO	H	7.6
6	HO	H	HO	H	BnO	MeO	H	8.3
7	HO	H	MeO	MeO	MeO	HO	H	9.5
8	HO	H	EtO	H	HO	MeO	H	12
9	Me	Me	HO	H	HO	MeO	H	12
10	AcO	H	AcO	H	H	MeO	H	15
11	HO	MeO	H	MeO	MeO	MeO	MeO	19
12	H	MeO	HO	H	HO	MeO	H	19
13	HO	MeO	HO	MeO	HO	HO	H	20
14	HO	H	HO	H	H	MeO	H	24
15	HO	MeO	HO	MeO	MeO	HO	H	27
16	HO	H	MeO	H	HO	MeO	H	32
17	HO	MeO	HO	MeO	HO	MeO	MeO	32
18	Me	Me	MeO	H	HO	MeO	H	44
19	HO	MeO	HO	MeO	MeO	MeO	MeO	54
20	HO	H	MeO	H	H	MeO	H	54
21	MeO	HO	MeO	H	HO	MeO	H	54
22	HO	H	MeO	H	MeO	MeO	H	65
23	HO	H	MeO	HO	MeO	MeO	H	>100
24	MeO	H	MeO	H	HO	MeO	H	>100
25	MeO	MeO	MeO	MeO	MeO	MeO	H	>27
26	HO	MeO	MeO	H	MeO	HO	H	>33

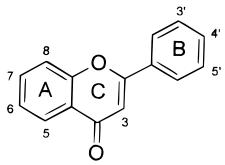
Table 2. Structure and Cytotoxicity Data for 3-Hydroxyflavone Derivatives



compd	C-5	C-6	C-7	C-8	C-3′	C-4′	C-5′	mean GI_{50} (μM)
27	HO	MeO	MeO	H	HO	MeO	H	4
28	HO	MeO	HO	H	H	MeO	H	4.1
29	H	H	MeO	H	H	MeO	H	4.2
30	H	H	MeO	H	MeO	MeO	H	5.5
31	H	H	H	H	2′,4′,6′-tri-MeO			5.9
32	MeO	H	MeO	H	MeO	MeO	H	6.6
33	HO	H	MeO	H	HO	HO	H	8.9
34	MeO	MeO	MeO	H	HO	MeO	H	14
35	H	H	HO	H	HO	HO	H	18
36	H	H	H	H	MeO	MeO	H	32
37	HO	H	HO	H	HO	HO	HO	36
38	HO	H	MeO	H	HO	MeO	H	37
39	HO	H	HO	H	HO	HO	H	60
40	H	Me	H	H	H	MeO	H	76
41	HO	H	HO	H	2′,4′-di-HO			>100

of **1**. Only one other compound (**31**) showed any inhibition of tubulin polymerization, albeit at an IC_{50} of 31 μM, or only slightly less than the highest test concentration of 40 μM. The same subset of compounds was also evaluated for inhibition of the binding of [^{3}H]-colchicine to tubulin. Only **1**−**3** had significant activity, even when the flavone (at 50 μM) was present in 10-fold molar excess to colchicine.

Table 3. Structure and Cytotoxicity Data for Flavone Derivatives Unsubstituted at Position 3



compd	C-5	C-6	C-7	C-8	C-3′	C-4′	C-5′	mean GI_{50} (μM)
42	HO	MeO	MeO	MeO	HO	HO	H	4
43	HO	MeO	HO	H	HO	MeO	H	6
44	MeO	MeO	MeO	MeO	H	*i*-PrO	H	11
45	HO	MeO	HO	H	MeO	MeO	H	15
46	HO	MeO	MeO	MeO	H	MeO	MeO	17
47	H	H	H	H	MeO	MeO	MeO	18
48	HO	MeO	MeO	MeO	MeO	MeO	MeO	19
49	HO	MeO	MeO	MeO	MeO	HO	H	22
50	MeO	MeO	MeO	MeO	2′,5′-di-MeO			24
51	MeO	MeO	MeO	MeO	2′,4′-di-MeO			26
52	MeO	MeO	MeO	H	H	MeO	H	28
53	H	HO	H	H	2′,3′,4′-tri-MeO			30
54	MeO	MeO	MeO	MeO	MeO	H	MeO	30
55	MeO	MeO	MeO	MeO	MeO	MeO	MeO	32
56	HO	MeO	HO	H	H	HO	H	33
57	MeO	MeO	MeO	MeO	penta-MeO			36
58	MeO	MeO	MeO	MeO	MeO	MeO	H	40
59	H	MeO	H	H	2′,3′,4′-tri-MeO			42
60	MeO	HO	MeO	H	H	MeO	H	43
61	HO	H	H	H	2′,3′,4′-tri-MeO			45
62	HO	H	HO	H	MeO	MeO	H	48
63	MeO	MeO	MeO	MeO	H	MeO	H	54
64	H	H	MeO	MeO	2′-MeO			55
65	H	H	HO	H	2′,3′,4′-tri-MeO			58
66	HO	MeO	HO	MeO	H	MeO	H	59
67	HO	MeO	MeO	MeO	2′,4′,5′-tri-MeO			60
68	MeO	HO	MeO	H	H	HO	H	62
69	HO	H	HO	H	2′,3′,4′-tri-MeO			68
70	H	H	MeO	H	2′,3′,4′-tri-MeO			78
71	MeO	H	MeO	H	2′,3′,4′-tri-MeO			79
72	MeO	H	H	H	2′,3′,4′-tri-MeO			89
73	HO	MeO	HO	H	H	MeO	H	>31
74	HO	HO	MeO	H	HO	MeO	H	>100
75	HO	Me	MeO	H	H	H	H	>100
76	HO	Me	MeO	Me	H	H	H	>100
77	MeO	MeO	MeO	H	H	HO	H	>100
78	HO	H	Me	H	MeO	MeO	H	>100
79	MeO	MeO	MeO	MeO	H	AcNH	H	>100

Some structure−activity relationships for cytotoxicity and associated inhibitory effects on tubulin polymerization are apparent from these results. The requirements for the B ring may be quite stringent. For example, compounds **1**−**3** all have 3′-hydroxyl-4′-methoxyl substituents, whereas compounds **5**, **7**, **15**, and **26**, in which these substituents were reversed, were weakly cytotoxic and showed no detectable effect on tubulin polymerization or colchicine binding (Tables 1 and 4). The case of **5** is striking because it differs from **2** only in the reversal of B-ring substituents. The same was true for compounds with 3′,4′-dimethoxyl substitution (**4**, **22**, **23**), even though they had a substitution pattern on the A and C rings identical to those of the more active agents. The 3-methoxyl functionality also appears to be essential for interference with tubulin polymerization, as compounds with a 3-hydroxyl (**27**, **30**−**32**, **34**, Table 2) or hydrogen (**45**−**48**, **73**, Table 3) substituent had no significant effect on in vitro tubulin polymerization or colchicine binding. The substitution on most of the A ring is apparently not critical for activity, as compounds **1**−**3** vary from 5,7-dihydroxy substitution

Table 4. Effects of Selected Flavones on in Vitro Tubulin Polymerization and [^{3}H]Colchicine Binding

compd	tubulin polymerization IC_{50} (μM)	percent inhibition of [^{3}H]colchicine binding @5mM	@50 mM
1	2.0 ± 0.5	35	76
2	0.83 ± 0.2	59	89
3	3.0 ± 0.4	43	75
4	>40		4
5	>40		0
7	>40		0
15	>40		6
16	>40		0
19	>40		6
25	>40		1
27	>40		9
30	>40		0
31	31 ± 5		22
32	>40		0
34	>40		2
45	>40		6
46	>40		5
47	>40		0
48	>40		0
73	>40		11
colchicine	1.4 ± 0.3		
podophyllotoxin		82	

to 5-hydroxy-6,7,8-trimethoxy, without major differences in activity in the tubulin assays. However, the importance of a 5-hydroxyl was suggested by comparison of **12** with **1**, where the only difference between the two compounds is the lack of a 5-hydroxy group in **12**.

Flavones having 3-methoxyl substitution have previously been reported as cytotoxic. In addition, antiviral activity has been reported for certain members of this group.[17] Compounds **1** and **3** were identified as weakly cytotoxic to KB cells by Kupchan.[18] Other 3-methoxyflavones have also been reported as cytotoxic to KB cells[19−24] (ED_{50} values of 1−10 μg/mL) or to P388 leukemia[23,25,26] (ED_{50} values of 1−5 μg/mL). In contrast, relatively few 3-hydroxyflavones have been found to be cytotoxic. Kupchan identified compounds **25** and **32** as cytotoxic to KB cells,[27,28] while 3,5-dihydroxy-3′,4′,6,7-tetramethoxyflavone was cytotoxic to five cell lines with ED_{50}'s of 0.5−2.5 μg/mL.[29] Flavonoids unsubstituted at the 3 position are more common in nature and have been commonly reported as weak cytotoxins in a variety of systems.[19,21,27,28,30−39] Recently, Zahir et al.[40] reported two cytotoxic flavones which were inhibitory to topoisomerase I. Cushman and co-workers have synthesized a variety of flavones as potential tyrosine kinase inhibitors.[41] Of an array of 55 flavone derivatives tested for cytotoxicity,[42] only 15 had an ED_{50} of <4 μg/mL in a test panel of five cell lines. Other recent studies of cytotoxic flavones have focused on polymethoxylated flavones from citrus peels[43] and synthetic 3-aminoflavones.[44]

Edwards et al.[45] reviewed NCI in vivo and in vitro screening data for 139 flavones, 15 isoflavones, and 62 flavanones in 1979. On the basis of the data available at that time, they concluded that flavonoids were not a promising group of antitumor compounds. It was striking, however, that they suggested that "It is also possible that some of the compounds may inhibit mitotic spindle formation, owing to their possession of contiguous alkoxy-groups deviating from strict coplanarity."[45,46] While their prediction was not consistent with the structure−activity observations in the present work, the prediction of an antimitotic mechanism nonetheless has proven true for a very limited set of flavone structures.

On the basis of the studies reported herein and on the ready availability of **1** from a natural source,[47] centaureidin has been selected for further evaluation in the in vivo hollow fiber assay.[48]

Experimental Section

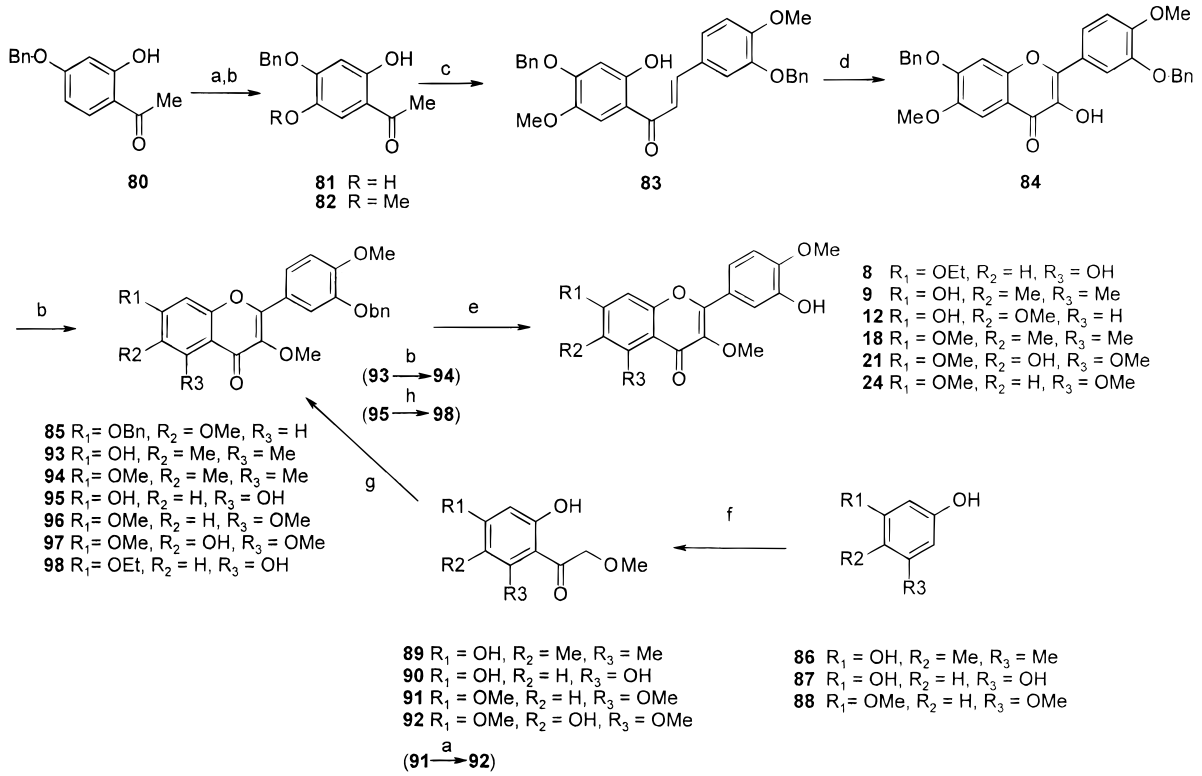
Compounds. Centaureidin (**1**) (NSC #106969) was isolated from *P. fruticosa* as previously described.[13] Compound **2** (NSC #642321) was submitted to the NCI by K. H. Lee.[16] Compounds **3**, **6**, **22**, and **23** were purchased from Indofine Chemicals. Eupatin (**27**, NSC #122412) was received from the NCI repository, found to be a mixture of related flavones by ^{1}H NMR, and resolved into three components, **27**, **34**, and **73**, by C_{18} HPLC on a Rainin Dynamax column (2.1 × 25 cm), using a linear gradient of 60−100% MeOH at 16 mL/min. The sample of eupatoretin (NSC #122414) was similarly resolved into **27** and **45**. All compounds thus obtained matched literature values for UV, NMR, and MS data.[18,27,28] Compounds **13**, **15**, and **17** were isolated from *Gutierrezia microcephala* (Compositae) as previously reported.[49] Compound **4** was prepared by refluxing **13** with CH_3I, K_2CO_3, and acetone as described in the same paper. Compounds **20** and **38** were prepared in like manner from kaempferol and quercetin, respectively, and were purified by preparative centrifugal circular TLC. Compound **25** was isolated from extracts of Fairchild tangerine peels and purified chromatographically to homogeneity. Sideritoflavone (**42**) was isolated from *Hyptis pectinata* (Lamiaceae) and identified by comparison to literature data.[50] Compounds **8**, **9**, **12**, **18**, **21**, and **24** were synthesized as detailed below. These novel compounds were isolated as glassy solids and gave either satisfactory elemental analysis or purity of ≥94% in two HPLC systems. All other compounds were obtained directly from the NCI repository. The structures of the compounds were all further confirmed by ^{1}H and ^{13}C NMR, UV, and HRMS.

Synthesis of 3′-Hydroxy-3,4′-methoxyflavonoids. Summary (See Scheme 1). Flavone **12**, lacking a C-5 substituent, was prepared from 2,4-dihydroxyacetophenone (Acros). Benzylation with benzyl bromide afforded phenol **80**. The latter was oxidized in an Elbs reaction into the parahydroxy analogue **81** and methylated selectively to afford hydroxyacetophenone **82**. Compound **82** was used to prepare chalcone **83** in alkaline medium with 3-(benzyloxy)-4-methoxybenzaldehyde. This chalcone gave rise to the 3-hydroxyflavonoid **84** in an AFO oxidation with hydrogen peroxide. Methylation of the 3-hydroxy group and debenzylation by hydrogenolysis afforded the target **12**, 3′-hydroxy-3,4′-methoxyflavone.

Flavones with 5-substitution (**8**, **9**, **18**, **21**, **24**) were synthesized from the corresponding phenols (**86**−**88**). Phenol **86** was prepared by aromatization of 5,5-dimethyl-1,3-cyclohexanedione. Phenols **87** and **88** were commercially available (Acros). Compounds **86**−**88** were converted in a Houben−Hoesch reaction into the corresponding methoxy ketones **89**−**91**. Compound **91** was further oxidized in an Elbs oxidation with potassium persulfate into the parahydroxy analogue **92**. Compounds **89**−**92** were treated with 3-(benzyloxy)-4-methoxybenzoic anhydride and the potassium salt of 3-(benzyloxy)-4-methoxybenzoic acid (Allan−Robinson condensation) to give rise to the corresponding 3-methoxyflavones **93** and **95**−**97**. Compound **93** was further methylated into the methoxy analogue **94**. Ethylation of **95** under analogous conditions yielded the monoethyl derivative **98**. Intermediates **93**−**98** were debenzylated by hydrogenolysis to the desired 3′-hydroxy-3,4′-dimethoxyflavones (**8**, **9**, **18**, **21**, **24**).

4-(Benzyloxy)-2-hydroxyacetophenone (80). 2′,4′-Dihydroxyacetophenone (10 g, 66 mmol) was dissolved in 50 mL of dry acetone and refluxed for 3 h with 8 g of powdered anhydrous K_2CO_3 and 9.4 mL (13.5 g, 79 mmol) of benzyl bromide. The mixture was cooled, and the acetone was removed under reduced pressure; 150 mL of H_2O was added,

Scheme 1[a]



[a] Reaction conditions: (a) $K_2S_2O_8$, NaOH, aq pyridine; (b) $(CH_3O)_2SO_2$, K_2CO_3, acetone; (c) 3-(benzyloxy)-4-methoxybenzaldehyde, NaOH, EtOH; (d) H_2O_2, NaOH, MeOH; (e) H_2, Pd/C, MeOH; (f) $MeOCH_2CN$, HCl, $ZnCl_2$, ether; (g) 3-(benzyloxy)-4-methoxybenzoic anhydride, potassium 3-(benzyloxy)-4-methoxybenzoate; (h) C_2H_5I, K_2CO_3, acetone, DMF.

and the products were acidified and extracted with CH_2Cl_2. The extract was dried over $MgSO_4$, filtered, and evaporated. The residue was purified through a silica column (EtOAc−petroleum ether) to obtain 13.5 g (85%) of the benzyloxy derivative **80**.

4-(Benzyloxy)-2,5-dihydroxyacetophenone (81). Compound **81** was prepared by the oxidation of **80** as described[50,51] (39% yield).

4-(Benzyloxy)-2-hydroxy-5-methoxyacetophenone (82). Compound **81** (5 g, 18.4 mmol) was dissolved in 50 mL of dry acetone and refluxed with 6 g of powdered anhydrous K_2CO_3 and 2.6 g (20.5 mmol) of dimethyl sulfate for 15 h. The acetone was removed under reduced pressure, and the residue was taken up in EtOAc (150 mL) and washed with dilute NaOH. The aqueous layer was acidified and extracted with CH_2Cl_2. The extract was dried over $MgSO_4$, filtered, and evaporated. The residue was purified on silica (EtOAc−petroleum ether) to yield 3.7 g (70%) of crystalline **82**.

2′-Hydroxychalcone (83). The chalcone **83** was prepared from **82** and 3-(benzyloxy)-4-methoxybenzaldehyde following published procedures[17] (80% yield).

7,3′-Bis(benzyloxy)-3-hydroxy-4′,6′-dimethoxyflavone (84). The 3-hydroxyflavone **84** was prepared by the Algar−Flynn−Oyamada (AFO) oxidation of the chalcone **83** as described[17] (66% yield).

7,3′-Bis(benzyloxy)-3,6,4′-trimethoxyflavone (85). Methylation of **84** with dimethyl sulfate in dry acetone following the procedure described[17] gave **85** in 76% yield.

1,3-Dihydroxy-4,5-dimethylbenzene (86). Compound **86** was prepared as described[51] from 5,5-dimethyl-1,3-cyclohexanedione.

2,4-Dihydroxy-*β*-methoxy-5,6-dimethylacetophenone (89). Compound **89** was prepared from **86** and methoxyacetonitrile following the Houben−Hoesch procedure as described[52] (71% yield).

2,4,6-Trihydroxy-*β*-methoxyacetophenone (90). The Houben−Hoesch acylation of phloroglucinol with methoxyacetonitrile as described[52] gave **90** in 79% yield.

2-Hydroxy-*β*,4,6-trimethoxyacetophenone (91). Compound **91** was prepared by the acylation of 3,5-dimethoxyphenol as described.[52] Silica column separation (EtOAc−petroleum ether) gave 45% yield of the unsymmetrical product **91**.

2,5-Dihydroxy-*β*,4,6-trimethoxyacetophenone (92). Compound **92** was prepared by the Elbs oxidation of **91** as described[53,54] (22% yield).

3′-(Benzyloxy)-7-hydroxy-3,4′-dimethoxy-5,6-dimethylflavone (93). Compound **93** was synthesized by the Allan−Robinson reaction involving **84**, 3-(benzyloxy)-4-methoxybenzoic anhydride and potassium 3-(benzyloxy)-4-methoxybenzoate as described[17] (85% yield).

3′-(Benzyloxy)-3,7,4′-trimethoxy-5,6-dimethylflavone (94). Compound **94** was obtained by methylation of **93** using dimethyl sulfate as described for the preparation of **82** (80% yield).

3′-(Benzyloxy)-5,7-dihydroxy-3,4′-dimethoxyflavone (95). Allan−Robinson reaction of (methoxyacetyl)phloroglucinol **90** with 3-(benzyloxy)-4-methoxybenzoic anhydride and potassium 3-(benzyloxy)-4-methoxybenzoate as described[17] gave 33% yield of **95**.

3′-(Benzyloxy)-3,5,7,4′-tetramethoxyflavone (96). Compound **96** was prepared by the Allan−Robinson method from **91**, 3-(benzyloxy)-4-methoxybenzoic anhydride, and potassium 3-(benzyloxy)-4-methoxybenzoate as described[17] (68% yield).

3′-(Benzyloxy)-6-hydroxy-3,5,7,4′-tetramethoxyflavone (97). Compound **97** was prepared by the Allan−Robinson procedure as described[17] (52% yield).

3′-(Benzyloxy)-7-ethoxy-5-hydroxy-3,4′-dimethoxyflavone (98). Compound **95** (0.5 g, 1.2 mmol) was dissolved in 50 mL of dry acetone and 10 mL of dry *N*,*N*-dimethylformamide and stirred with 5 g of powdered anhydrous K_2CO_3. Ethyl iodide (0.25 g, 1.6 mmol) was added, and the mixture was refluxed for 2 h. The acetone was removed under reduced pressure and the residue dissolved in EtOAc. The solution was washed with H_2O, dried over $MgSO_4$, and evaporated. The product was purified through a silica column (EtOAc−toluene) to obtain 0.33 g (60%) of crystalline **98**.

7,3′-Dihydroxy-3,6,4′-trimethoxyflavone (12). The bis(benzyloxy)flavone **85** (0.5 g, 1 mmol), dissolved in 50 mL of methanol, was stirred with 0.5 g of Pd/C catalyst (10%). A steady stream of H_2 gas was passed over the suspension for 90 min. The catalyst was removed by filtration and the filtrate evaporated. The residue was crystallized from methanol (yield 0.28 g, 85%). ^{1}H NMR: δ 3.80 (3H, s, OCH_3), 3.93 (6H, s, 2 × OCH_3), 5.70 (1H, s, OH), 6.40 (1H, br s, OH), 6.90 (2H, s), 7.45−7.75 (3H, m). HREIMS: *m/z* 344.0899; calcd for $C_{18}H_{16}O_7$, 344.0896.

7,3′-Dihydroxy-3,4′-dimethoxy-5,6-dimethylflavone (9). Flavone **9** was prepared from **93** by hydrogenolysis, as described for the preparation of **12** (91% yield). ^{1}H NMR: δ 2.20 (3H, s), 2.82 (3H, s), 3.78 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 6.80 (1H, s), 7.06 (1H, d, J = 10 Hz), 7.60 (1H, d, J = 10 Hz), 7.95 (1H, s). HREIMS: *m/z* 342.1106; calcd for $C_{19}H_{18}O_6$, 342.1103.

3′-Hydroxy-3,7,4′-trimethoxy-5,6-dimethylflavone (18). Compound **94** was debenzylated by hydrogenolysis following the procedure described for the preparation of **12** (92% yield). ^{1}H NMR: δ 2.17 (3H, s), 2.78 (3H, s), 3.25 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 6.80 (1H, s), 7.00 (1H, d, J = 10 Hz), 7.55, 7.65 (2H, m). HREIMS: *m/z* 356.1263; calcd for $C_{20}H_{20}O_6$, 356.1259.

3′-Hydroxy-3,5,7,4′-tetramethoxyflavone (24). Debenzylation of **96** by hydrogenolysis as described for the preparation of **12** gave **24** (yield 80%). ^{1}H NMR: δ 3.77 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 6.35 (1H, s), 6.55 (1H, s), 6.95 (1H, d, J = 9 Hz), 7.50−7.65 (2H, m). HREIMS: *m/z* 358.1052; calcd for $C_{19}H_{18}O_7$, 358.1052.

6,3′-Dihydroxy-3,5,7,4′-tetramethoxyflavone (21). Debenzylation of **97** by hydrogenolysis in the presence of Pd/C (10%) catalyst, as described for the preparation of **12**, yielded 89% of **21**. ^{1}H NMR: δ 3.75 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 6.90 (1H, s, arom), 7.45−7.65 (3H, m). HREIMS: *m/z* 374.1007; calcd for $C_{19}H_{18}O_8$, 374.1002.

7-Ethoxy-5,3′-dihydroxy-3.4′-dimethoxyflavone (8). Flavone **8** was prepared by the debenzylation of **98** by hydrogenolysis in the presence of Pd/C (10%) catalyst, as described for the preparation of **12** (82% yield). ^{1}H NMR: δ 1.35 (3H, t, J = 8.5 Hz), 3.72 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 4.01 (2H, q, J = 8.5 Hz), 6.21 (1H, s), 6.36 (1H, s), 6.88 (1H, d, J = 8.5 Hz), 7.45−7.60 (2H, m). HREIMS: *m/z* 358.1041; calcd for $C_{19}H_{18}O_7$, 358.1052.

Biological Assays. In vitro tubulin polymerization and colchicine binding assays were performed as previously reported.[2,55] Tubulin polymerization results shown in Table 4 are averages ± SD from three determinations, using a tubulin concentration of 10 μM; the colchicine inhibition assay results were derived from three determinations. The 60-cell cytotoxicity screen has been described previously.[12] Due to limited compound availability, results shown in Tables 1−3 were derived from single tests of each compound in the full 60-cell panel.

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