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CYTOTOXICITIES OF SOME FLAVONOID ANALOGUES

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ABSTRACT.—An array of 55 flavones having a variety of substituents was evaluated for cytotoxicity in five cancer cell cultures: A-549 lung carcinoma, MCF-7 breast carcinoma, HT-29 colon adenocarcinoma, SKMEL-5 melanoma, and MLM melanoma. Fifteen of the 55 flavone derivatives were significantly active against at least one of these cell cultures, and 4′-[(*t*-butyldimethylsilyl)oxy]-7,8-dihydroxy-3′,5′-dimethoxyflavone [**40**] was the most active of all. Structure-activity relationships of these compounds are discussed.

Flavones are one of the major classes of natural products with widespread distribution and broad pharmacological profile (1,2), including the ability to inhibit retroviral transcriptases (3–5), protein-tyrosine kinases (6,7), and other enzymes (6). In addition, these compounds have been found to possess anticancer (1,8–18) and chemopreventative activities (9). Our interest in finding more potent and selective inhibitors of protein-tyrosine kinases (19) based on quercetin prompted us to develop new synthetic methods for a wide range of substituted flavones (19–22). Accordingly, a variety of flavone derivatives were synthesized and evaluated for inhibition of protein-tyrosine kinase $p56^{lck}$. A potent inhibitor of this enzyme, 4′-amino-6-hydroxyflavone [**30**], was developed and determined to be highly selective for $p56^{lck}$ over protein-serine/threonine kinases (19). In this context, the flavones synthesized were evaluated in five cancer cell lines and the results are presented here. Five concentrations of each compound were tested in each cell line.

MATERIALS AND METHODS

The synthetic procedures for the flavones described here are reported elsewhere (19–22). For cytotoxicity, an MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) colorimetric assay was employed according to the established procedure (23,24). After the addition of the samples to the cell cultures, the cells were incubated for 6 days before the MTT reagent was added. The assays were performed in the Purdue Cell Culture Laboratory. All of the compounds were initially tested once in each cell line at a starting dose of 100 μg/ml. The active compounds ($ED_{50} < 4$ μg/ml) were tested again, and the values shown for these cytotoxic substances are the average of two determinations.

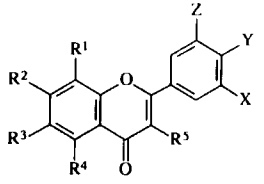
RESULTS AND DISCUSSION

Fifty-five flavones with a variety of substituents including COOMe, COOH, NO_2, NH_2, OBn, $OSi(Me)_2$-*t*-Bu, OH, and OMe were evaluated for cytotoxicity in five cancer cell cultures: A-549 lung carcinoma, MCF-7 breast carcinoma, HT-29 colon adenocarcinoma, SKMEL-5 melanoma, and MLM melanoma. The results are summarized in Table 1.

Among the 55 flavones investigated, 15 compounds (**8**, **28–30**, **32**, **33**, **37–40**, **49–52**, and **55**) were significantly cytotoxic ($ED_{50} < 4$ μg/ml) against at least one of the five cancer cell lines. Eight compounds were significantly active in A-549, six in MCF-7, eleven in HT-29, ten in SK-MEL, and six in MALM-3M. Two compounds (**40** and **49**) were found to have a broad spectrum of cytotoxicity, active against all the five cell lines, and flavone **40** was the most potent of all. It is worth noting that substitution at the 3-position of the flavone with COOMe or COOH groups generally resulted in flavone derivatives (compounds **1–7** and **9–25**) noncytotoxic in all cell lines at 25 μg/ml.

Leaving the 3-substituted flavones, five out of the nine flavones with a 4′-nitro or a 4′-amino substituent were sig-

TABLE 1. Cytotoxicities of Flavones.



Compound	R[1a]	R[2]	R[3]	R[4]	R[5]	X	Y	Z	Cell Line[b]					Reference[c]
									A-549	MCF-7	HT-29	SK-MEL	MALM-3M	
1	H	H	H	H	COOMe	OMe	OMe	OMe	>25	>25	>25	>25	>25	19
2	H	H	H	H	COOMe	OMe	H	OMe	>25	>25	6.99	>25	>25	19
3	H	H	H	H	COOMe	OMe	OMe	H	>25	>25	>25	>25	>25	19
4	H	H	H	H	COOMe	H	OMe	H	>25	>25	>25	>25	>25	19
5	H	H	H	H	COOMe	H	Br	H	>25	>25	>25	>25	>25	19
6	H	H	H	H	COOMe	H	NO_2	H	>25	>25	>25	>25	>25	19
7	H	H	OMe	H	COOMe	H	NO_2	H	>25	>25	>25	8.18	24.09	19
8	H	OMe	H	H	COOMe	H	NO_2	H	>25	>25	>25	2.84	1.12	19
9	H	H	H	H	COOMe	H	NH_2	H	>25	>25	>25	>25	>25	19
10	H	OMe	H	H	COOMe	H	NHAc	H	9.76	9.19	8.21	15.94	9.26	19
11	H	H	H	H	COOMe	H	OBn	H	>25	>25	>25	>25	>25	19
12	H	H	OMe	H	COOMe	H	OBn	H	22.07	>25	15.56	20.88	>25	19
13	H	OMe	H	H	COOMe	H	OBn	H	20.44	5.16	5.42	>25	>25	19
14	H	H	H	H	COOMe	H	OH	H	>25	>25	>25	>25	>25	19
15	H	H	OMe	H	COOMe	H	OH	H	>25	>25	>25	18.69	>25	19
16	H	OMe	H	H	COOMe	H	OH	H	>25	>25	19.23	19.55	20.10	19
17	H	H	H	H	COOH	OMe	OMe	OMe	>25	>25	>25	>25	>25	19
18	H	H	H	H	COOH	OMe	H	OMe	>25	>25	>25	>25	>25	19
19	H	H	H	H	COOH	OMe	OMe	H	>25	>25	>25	>25	>25	19
20	H	H	H	H	COOH	H	OMe	H	>25	>25	>25	>25	>25	19
21	H	H	H	H	COOH	H	Br	H	>25	>25	>25	>25	>25	19
22	H	H	H	H	COOH	H	NO_2	H	>25	>25	>25	>25	>25	19
23	H	H	OMe	H	COOH	H	NO_2	H	>25	15.85	>25	>25	>25	19

TABLE 1. Continued.

Compound	R[1]	R[2]	R[3]	R[4]	R[5]	X	Y	Z	Cell Line[a]					Reference[b]
									A-549	MCF-7	HT-29	SK-MEL	MALM-3M	
24	H	OMe	H	H	COOH	H	NO_2	H	>25	>25	>25	>25	>25	19
25	H	H	OMe	H	COOH	H	OH	H	>25	>25	>25	>25	>25	19
26	H	H	OH	H	H	H	NO_2	H	5.07	>25	>25	21.03	>25	19
27	H	OH	H	H	H	H	NO_2	H	21.78	>25	6.95	>25	>25	19
28	H	OH	H	OH	H	H	NO_2	H	4.19	6.90	3.34	4.87	>25	19
29	OH	OH	H	H	H	H	NO_2	H	2.56	4.69	3.46	3.29	3.61	20
30	H	H	OH	H	H	H	NH_2	H	2.72	>25	2.65	2.03	>25	19
31	H	OH	H	H	H	H	NH_2	H	8.87	>25	4.71	6.59	>25	19
32	H	OH	H	OH	H	H	NH_2	H	2.84	>25	3.44	3.86	>25	19
33	H	OAc	H	OAc	H	H	NHAc	H	21.98	1.78	17.40	13.96	>25	19
34	H	OAc	H	H	H	H	NHAc	H	20.53	19.18	19.79	16.90	>25	19
35	H	H	OH	H	H	OMe	OMe	OMe	9.82	>25	7.85	5.87	>25	22
36	H	OH	H	H	H	OMe	OMe	OMe	>25	>25	>25	>25	>25	22
37	OH	OH	H	H	H	OMe	OMe	OMe	5.27	1.11	3.66	>25	>25	22
38	H	H	OH	H	H	OMe	OR	OMe	3.19	2.01	2.63	2.95	5.30	21
39	H	OH	H	H	H	OMe	OR	OMe	5.30	4.47	1.00	>25	>25	21
40	OH	OH	H	H	H	OMe	OR	OMe	3.31	0.31	0.34	0.11	0.20	21
41	H	H	H	OH	H	OMe	OH	OMe	0.74	0.51	>25	>25	>25	21
42	H	H	OH	H	H	OMe	OH	OMe	>25	>25	>25	5.68	>25	21
43	H	OH	H	H	H	OMe	OH	OMe	>25	>25	6.50	>25	>25	21
44	OH	OH	H	H	H	OMe	OH	OMe	>25	>25	5.12	6.33	>25	21
45	H	OAc	H	H	H	OMe	OH	OMe	>25	>25	>25	>25	>25	21
46	H	H	OMe	H	H	OMe	OMe	OMe	>25	>25	>25	>25	>25	22
47	H	OMe	H	H	H	OMe	OMe	OMe	>25	>25	>25	>25	>25	22
48	OMe	OMe	H	H	H	OMe	OMe	OMe	>25	>25	>25	>25	>25	22
49	H	OAc	H	H	H	OMe	OR	OMe	2.90	1.49	1.94	0.80	2.95	21
50	OAc	OAc	H	H	H	OMe	OR	OMe	>25	>25	>25	0.14	0.31	22
51	H	OH	H	H	H	H	OR	H	6.45	4.66	3.61	5.64	>10	21
52	H	H	H	OH	H	OH	H	H	3.24	6.93	3.47	3.09	>10	22
53	H	H	OH	H	H	OH	H	H	>25	>25	>25	>25	>25	22
54	H	OH	H	H	H	OH	H	H	>25	>25	>25	5.11	>25	22
55	OH	OH	H	H	H	OH	H	H	4.65	3.71	4.18	3.66	4.80	22

[a]R = $Si(Me)_2$-*t*-Bu.
[b]ED_{50} (μg/ml).
[c]References describe the methods of synthesis of the flavones.

nificantly active in at least one of the five cancer cell lines (compounds **28–30**, **32** and **33**). 4′-Amino-6-hydroxyflavone [**30**], a potent protein-tyrosine kinase inhibitor, is significantly active against three cell lines but does not exhibit a markedly greater cytotoxicity in comparison to other 4′-aminoflavones that are less potent inhibitors of protein-tyrosine kinases (19).

Several highly cytotoxic natural products, such as colchicine, steganacin, podophyllotoxin, combretastatin A-4, and several of their synthetic analogues, possess trimethoxyphenyl or 3,5-dimethoxy-4-hydroxyphenyl groups (25–35) as a common structural feature. This characteristic was therefore incorporated in some of the flavones (compounds **1**, **17**, and **35–50**) to determine whether the cytotoxicities of flavone derivatives could be enhanced. Of the eight flavones with 3,4,5-trimethoxy substitution on ring C (compounds **1**, **17**, **35–37**, and **46–48**) only compound **37** showed significant cytotoxicity in MCF-7 and HT-29 cell lines (ED_{50} 1.11 and 3.66 μg/ml, respectively) and all others were not active ($ED_{50} > 4$ μg/ml). Similarly, of the five flavones with 3,5-dimethoxy-4-hydroxy substitution on ring C (compounds **41–45**), 4′,5-dihydroxy-3′,5′-dimethoxyflavone [**41**] was quite active against A-549 and MCF-7 cell lines (ED_{50} 0.74 and 0.51 μg/ml, respectively), and all others were inactive. All five flavones with 4′-[(*t*-butyldimethylsilyl)oxy]-3′,5′-dimethoxy substitution on ring C (flavones **38–40**, **49**, and **50**) were significantly active in at least one of the five cell lines, and flavones **40** and **49** were active in all five cell lines. 4′-[(*t*-Butyldimethylsilyl)oxy]-7,8-dihydroxy-3′,5′-dimethoxyflavone [**40**] was the most active of all. This suggests the possibility of obtaining new cytotoxic flavones with further modifications at the 4′ position. It was also observed that among seven flavones oxygenated at both positions 7 and 8 (compounds **29**, **37**, **40**, **44**, **48**, **50**, and **53**), five (**29**, **37**, **40**, **50**, and **53**) were active in at least two cell lines. The most active flavone (compound **40**) also possesses the 7,8-dihydroxy substitution.

In conclusion, the present study shows that it is possible to develop additional cytotoxic agents based on the flavone structure and to study structure-activity relationships to obtain more potent flavone derivatives. Some of the common features that may be considered while synthesizing such agents are oxygenation at 7,8 positions and appropriate variations at the 4′ position.

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