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SYNTHESIS AND EVALUATION OF HYDROXYLATED FLAVONES AND RELATED COMPOUNDS AS POTENTIAL INHIBITORS OF THE PROTEIN-TYROSINE KINASE P56^{lck}

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ABSTRACT.—An array of hydroxylated flavones and related compounds was synthesized and evaluated for inhibition of the in vitro protein-tyrosine kinase activity of p56^{lck}, an enzyme that is thought to play a key role in mediating signal transduction from the CD4 receptor during lymphocyte activation. In general, the most active compounds had hydroxyl groups on both the A and C rings. At least two hydroxyl groups were required for good inhibitory activity, and the relative positions of these groups played an important role in determining potency. Compounds without hydroxyl groups were inactive as inhibitors.

Protein-tyrosine kinases (PTKs) are a family of enzymes that catalyze the transfer of phosphate from ATP to tyrosyl residues on protein substrates. Members of this family of enzymes include the receptors for several polypeptide growth factors, the products of several oncogenes encoded by acute transforming retroviruses, and the products of cellular genes (proto-oncogenes) from which these viral oncogenes originated (1,2). The common feature of these enzymes, and the property that makes them the subject of such intensive investigation, is that they play key roles in the regulation of cell proliferation. For example, activation of the tyrosine kinase activity of growth factor receptors by the binding of the growth factor stimulates a round of cell division. Also, the expression of retroviral oncogene-encoded protein-tyrosine kinases leads to the uncontrolled proliferation and malignant transformation of infected cells. Thus, this class of enzyme is of considerable interest to investigators studying the mechanisms by which cell growth and differentiation are regulated. Due to their key role in regulating cell proliferation, these enzymes are potentially important targets for cancer therapeutic agents.

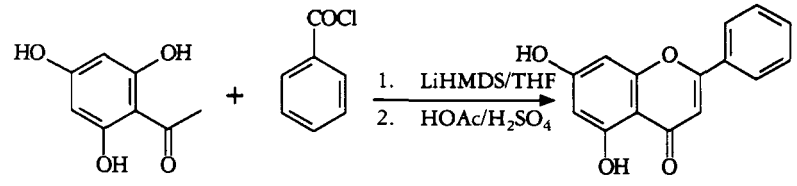
These factors have prompted a search for compounds with inhibitory activity toward PTKs. The identification of inhibitory substances from natural sources has featured prominently in this search. Several inhibitory compounds have been identified, including erbstatin (3), herbimycin A (4), piceatannol (5), the isoflavonoid genistein (6,7), and numerous flavonoids (8–11).

The discovery that a variety of flavonoids were PTK inhibitors generated considerable interest in the relationships between structure and inhibitory potency. Recently, we initiated a program to synthesize analogues of flavones and were successful in identifying new, more potent and selective inhibitors of the protein-tyrosine kinase p56^{lck} (12). This protein-tyrosine kinase is thought to play a key role in mediating signal transduction from the CD4 receptor during lymphocyte activation (13,14) and is found elevated in certain murine lymphomas (15,16) and human colon carcinomas (17). In the present study, we describe the synthesis and evaluation of several hydroxylated flavones as inhibitors of p56^{lck}.

CHEMISTRY

Recently we devised, for the preparation of ring-A hydroxylated flavones, a short and facile synthetic method that effectively avoids the formation of 3-aroylflavones as by-products (18). Adopting this method, reaction of 2′,4′,6′-trihydroxyacetophenone [**1e**] with benzoyl chloride [**2**] in the presence of lithium hexamethyldisilazide (LiHMDS) followed by acid treatment of the intermediate gave 5,7-dihydroxyflavone

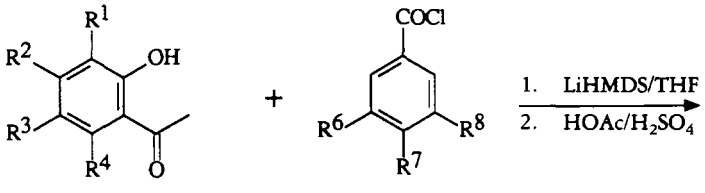
[3] in 84% yield (Scheme 1). Scheme 2 describes a similar preparation of flavones **6a–6c**, **7a–7b** by reaction of the appropriate hydroxylated acetophenones **1a–1d** with 3,4,5-trimethoxybenzoyl chloride [4] and (4-benzyloxy)benzoyl chloride [5] followed by subsequent acid-catalyzed cyclization in 77–92% yields. Methylation of ring-A hydroxylated flavones **6a–6c** with Me_2SO_4 in the presence of K_2CO_3 in boiling Me_2CO gave compounds **8a–8c**.



1e **2** **3**

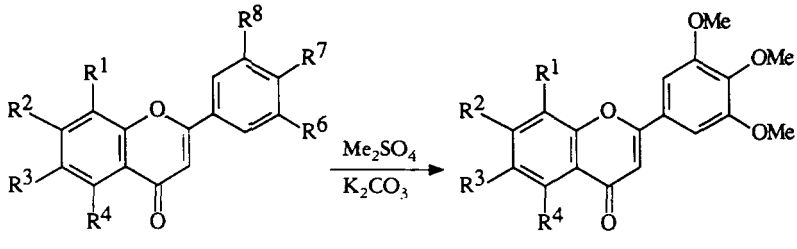
SCHEME 1

Flavones bearing hydroxyl groups on both rings A and C may be prepared through utilization of the *t*-butyldimethylsilyl protecting group in combination with lithium polyanion methodology (19). Reaction of methyl 3-hydroxybenzoate [9] with *t*-butyldimethylsilyl chloride in DMF in the presence of *N,N*-diisopropylethylamine gave



1a–1d

4 $R^6=R^7=R^8=OMe$
5 $R^6=R^8=H$; $R^7=OBn$

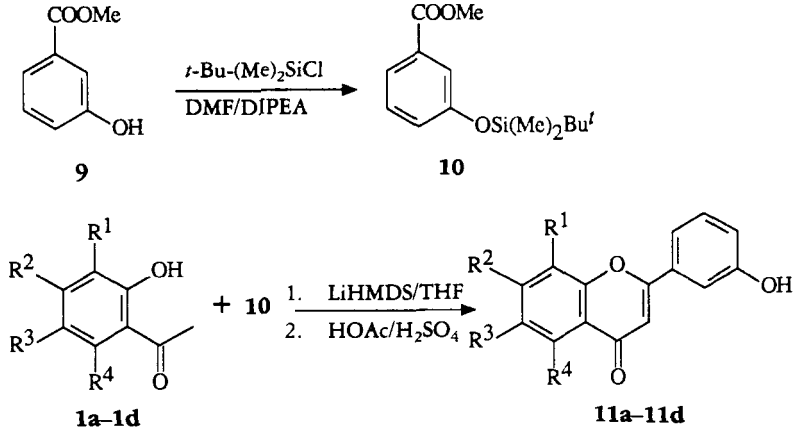


6a–6c, 7a, 7b **8a–8c**

	R^1	R^2	R^3	R^4	R^6	R^7	R^8
1a	H	H	H	OH	—	—	—
1b	H	H	OH	H	—	—	—
1c	H	OH	H	H	—	—	—
1d	OH	OH	H	H	—	—	—
6a	H	H	OH	H	OMe	OMe	OMe
6b	H	OH	H	H	OMe	OMe	OMe
6c	OH	OH	H	H	OMe	OMe	OMe
7a	H	H	H	OH	H	OBn	H
7b	H	OH	H	H	H	OBn	H
8a	H	H	OMe	H	—	—	—
8b	H	OMe	H	H	—	—	—
8c	OMe	OMe	OH	H	—	—	—

SCHEME 2

methyl 3-[(*t*-butyldimethylsilyl)-oxy]-benzoate [**10**] in quantitative yield. Compound **10** on condensation with the polyanions generated from hydroxylated acetophenones **1a–1d** followed by treatment with 0.5% H_2SO_4 in HOAc at 95–100° gave 3′-hydroxyflavones **11a–11d** (Scheme 3). 2-[4-{(*t*-Butyldimethylsilyl)-oxy}-7,8-dihydroxy-3,5-dimethoxyphenyl]-4*H*-benzopyran-4-one [**12**] on acetylation with Ac_2O in the presence of pyridine gave 2-[4-{(*t*-butyldimethylsilyl)-oxy}-7,8-diacetoxy-3,5-dimethoxyphenyl]-4*H*-benzopyran-4-one [**13**] (Scheme 4). Compounds **14a–14g** and **14i–14q** were obtained from our previous work (18).

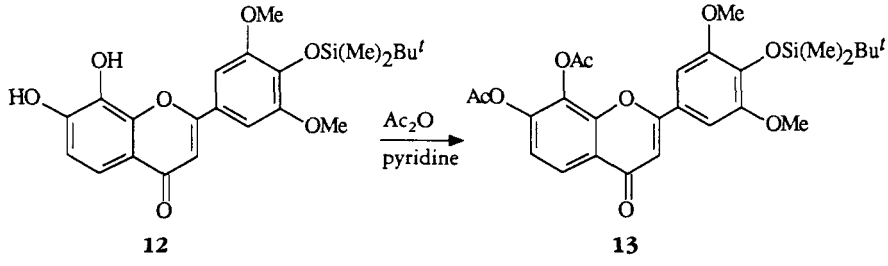


	R^1	R^2	R^3	R^4
1a/11a	H	H	H	OH
1b/11b	H	H	OH	H
1c/11c	H	OH	H	H
1d/11d	OH	OH	H	H

SCHEME 3

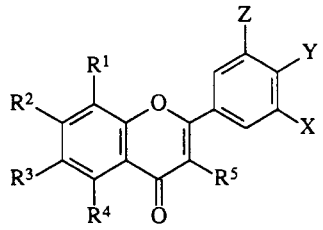
RESULTS AND DISCUSSION

In the present study 36 flavones were tested for their ability to inhibit the phosphorylation of a tyrosine-containing peptide catalyzed by the protein-tyrosine kinase $p56^{lck}$. The results are summarized in Table 1. A close look at the inhibitory data of the compounds tested confirms that flavones are a rich source of new protein-tyrosine kinase inhibitors. Of the 36 flavones tested here, 21 were found to be significantly active ($IC_{50}<100$ μg/ml). As predicted by our earlier findings (8,12), flavones without any free hydroxyl groups were generally inactive ($IC_{50}>800$ μg/ml). Compounds **6c**, **7b**, **14d**, **14i**, **14j**, and **14m** were found to be weak inhibitors of $p56^{lck}$ (IC_{50} = 100–800 μg/ml).



SCHEME 4

TABLE 1. Inhibition of Protein-Tyrosine Kinase Activity of $p56^{lck}$ by Flavone Derivatives.



Compound	R^1	R^2	R^3	R^4	R^5	X	Y	Z	IC_{50} (μg/ml)
3	H	OH	H	OH	H	H	H	H	5
6a	H	H	OH	H	H	OMe	OMe	OMe	20
6b	H	OH	H	H	H	OMe	OMe	OMe	>800
6c	OH	OH	H	H	H	OMe	OMe	OMe	136
7a	H	H	H	OH	H	H	OBn	H	>800
7b	H	OH	H	H	H	H	OBn	H	664
8a	H	H	OMe	H	H	OMe	OMe	OMe	>800
8b	H	OMe	H	H	H	OMe	OMe	OMe	>800
8c	OMe	OMe	H	H	H	OMe	OMe	OMe	>800
11a	H	H	H	OH	H	OH	H	H	80
11b	H	H	OH	H	H	OH	H	H	4
11c	H	OH	H	H	H	OH	H	H	10
11d	OH	OH	H	H	H	OH	H	H	48
12	OH	OH	H	H	H	OMe	OH	OMe	40
13	OAc	OAc	H	H	H	OMe	OR[a]	OMe	>800
14a	H	OH	H	H	H	H	H	H	80
14b	OH	OH	H	H	H	H	H	H	80
14c	H	H	OH	H	H	H	OR[a]	H	44
14d	H	OH	H	H	H	H	OR[a]	H	360
14e	H	H	H	OH	H	H	OH	H	4
14f	H	H	OH	H	H	H	OH	H	30
14g	H	OH	H	H	H	H	OH	H	43
14h	H	OMe	H	OH	H	H	OH	H	80
14i	H	H	OH	H	H	OMe	OR[a]	OMe	160
14j	H	OH	H	H	H	OMe	OR[a]	OMe	656
14k	OH	OH	H	H	H	OMe	OR[a]	OMe	>800
14l	H	H	OH	H	H	OMe	OH	OMe	41
14m	H	OH	H	H	H	OMe	OH	OMe	400
14n	H	OAc	H	H	H	OMe	OH	OMe	>800
14o	H	OH	H	OH	H	H	OH	H	4
14p	H	OH	H	OH	H	H	OH	OH	10
14q	H	OAc	H	H	H	OMe	OR[a]	OMe	>800
15a	H	OH	H	OH	OH	OMe	OH	OMe	24
15b	H	OH	H	OH	OH	OH	H	OH	30
15c	H	OH	H	OH	OH	H	H	H	80
15d	H	OH	H	H	OH	OH	OH	H	4
Quercetin	H	OH	H	OH	OH	OH	OH	H	4

[a]R = Si(*t*-Bu)Me_2.

With the exception of chrysin [**3**], all of the highly active compounds ($IC_{50} \leq 40$ μg/ml) possessed hydroxyl and/or methoxyl groups on both rings A and C (compounds **6a**, **11b**, **11c**, **12**, **14e**, **14f**, **14l**, **14o**, **14p**, **15a**, **15b**, and **15d**). A comparison of the inhibitory activities of the 13 highly active compounds showed that at least two hydroxyl groups are required for enhanced activity and that the relative positions of these

two hydroxyl groups play a key role for optimal interaction of these compounds with the enzyme. This study also showed that compounds with more hydroxyl groups are not necessarily more active, in contrast to the previous finding with natural products (10). A comparison of the inhibitory activities of the 3′-hydroxylated flavones **11a–11d** showed that 3′,5-dihydroxyflavone [**11a**] was significantly active (IC_{50} = 80 μg/ml). However, when the 5-OH group was changed to 6-OH (compound **11b**), a 20-fold increase in the activity was achieved (IC_{50} = 4 μg/ml), equal to the inhibitory activity of quercetin. Similarly, when the 5-OH was changed to 7-OH (compound **11c**), there was an increase in the activity (IC_{50} = 10 μg/ml). Addition of a hydroxyl group to compound **11c** (compound **11d**) decreased the activity.

A comparison of the significantly active flavones possessing a 4′-OH group (compounds **12**, **14e–14h**, **14l–14p**, **15a**, and **15d**) showed that, in general, all of these compounds were more active than the parent compound, 4′-hydroxyflavone (compare with IC_{50} = 120 μg/ml) (12). When a hydroxyl group was added to this compound at the 5-position (compound **14e**), the activity was increased 30-fold and compound **14e** was also as active as quercetin, a highly potent naturally occurring inhibitor (IC_{50} 4 μg/ml for both). While transfer of the 5-OH group to 6-OH or 7-OH (compounds **14f** and **14g**) reduced the protein-tyrosine kinase inhibitory activity, addition of one more OH group at the 7 position or two OH groups at the 3 and 7 positions (compounds **14o** and **15d**) restored the activity at IC_{50} 4 μg/ml. In compound **14o**, addition of a second OH group at the 3 position of ring C (compound **14p**) increased the IC_{50} 2.5-fold, and methylation of the 7-OH group (compound **14h**) reduced the inhibitory activity 20-fold. Our earlier studies showed that the addition of two methoxy groups at the 3′ and 5′ positions of 4′-hydroxyflavone increased the activity by about 15-fold (compare 4′-hydroxyflavone IC_{50} 120 μg/ml; 3′,5′-dimethoxy-4′-hydroxyflavone IC_{50} 7.7 μg/ml) (12). A comparison of the activities of the 4′-hydroxyflavones, **14f** and **14g**, with the corresponding 3′,5′-dimethoxy-4′-hydroxyflavones, **14l** and **14m**, however, showed that protein-tyrosine kinase inhibitory activity did not improve as expected, and in fact, in one case the activity was reduced by about an order of magnitude (compare the IC_{50} of **14g**, 43 μg/ml, to that of **14m**, 400 μg/ml).

Among the flavones with a 3-OH substitution, compound **15d** was found to be the most active and was comparable in potency to quercetin (both with IC_{50} = 4 μg/ml). In a comparison of flavones hydroxylated only on ring A (compounds **3**, **14a**, and **14b**), chrysin [**3**] was about 8 times more potent than compounds **14a** and **14b** and similar in potency (IC_{50} = 5 μg/ml) to quercetin.

In summary, testing of 36 flavones described in the present study has uncovered four new potent inhibitors (compounds **11b**, **14e**, **14o**, and **15d**) of the protein-tyrosine kinase $p56^{lck}$ that are as active as quercetin. This study also uncovered considerable information regarding the structure-activity relationship requirements of the relative positions of the hydroxyl groups for potent inhibitory activity. The combined results of our present and previous (12) studies show that future design efforts involving the substitution of some of the hydroxyl groups with amino groups may provide a route to more potent and selective inhibitors.

EXPERIMENTAL

Melting points were determined in capillary tubes on a Mel-Temp apparatus and are uncorrected. Spectra were obtained as follows: ci mass spectra on a Finnegan 4000 spectrometer; ^{1}H-nmr spectra on a Varian VXR-500S spectrometer with TMS as an internal standard in $CDCl_3$ or DMSO-d_6. Microanalyses were performed at the Purdue Microanalysis Laboratory, and all values were within ±0.4% of the calculated composition. All organic solvents were appropriately dried and/or purified prior to use. Solutions of LiHMDS in THF, 2′-hydroxyacetophenones **1a–1e**, compound **14h**, and 3-hydroxyflavone derivatives **15a–15d** were obtained from commercial sources. Quercetin dihydrate was purchased from Aldrich Chemical Company.

5,7-DIHYDROXY-2-PHENYL-4*H*-BENZOPYRAN-4-ONE [**3**].—A solution of LiHMDS in THF (1 M, 100 ml, 100 mmol) was added to a well-stirred solution of 2′,4′,6′-trihydroxyacetophenone [**1e**] (3.36 g, 20 mmol) in THF under argon atmosphere at −78° in 15 min. The reaction mixture was stirred at −78° for 1 h and at −10° for 2 h. It was cooled again to −78°, and a solution of benzoyl chloride [**2**] (2.80 g, 20 mmol) in THF (15 ml) was added in 10 min. Stirring was continued for 30 min at −78° and at room temperature for 4 h and the reaction mixture was poured into a mixture of ice H_2O (500 ml) and HCl (20 ml). It was extracted with $CHCl_3$ (3 × 50 ml), and the combined extracts were dried (Na_2SO_4). Solvents were evaporated, and the residue was mixed with glacial HOAc (100 ml) and H_2SO_4 (0.5 ml) and heated at 95–100° for 1 h. About 75% of the solvent was removed at reduced pressure, and the residue was poured into ice H_2O (50 ml). The product was filtered, washed with H_2O, and dried. Recrystallization from Me_2CO/hexane gave pure **3** (4.25 g, 84%), mp 290° [lit. (20) mp 289–290°].

COMPOUNDS **6a–6c**.—These substances were prepared essentially as described above for **3**, except four equivalents of the base LiHMDS were employed instead of five.

*6-Hydroxy-2-(3,4,5-trimethoxyphenyl)-4*H*-benzopyran-4-one* [**6a**].—Yield 3.02 g (92%); mp 209–210°; ^{1}H nmr (DMSO-d_6) δ 10.03 (bs, 1H, exchanges with D_2O), 7.70 (d, J = 8.6 Hz, 1H), 7.35 (s, 2H), 7.31 (d, J = 2.3 Hz, 1H), 7.24 (dd, 1H), 7.06 (s, 1H), 3.91 (s, 6H), 3.74 (s, 3H); cims (isobutane) *m/z* $[MH]^+$ 329 (100). *Anal.* calcd for $C_{18}H_{16}O_6$, C 65.86, H 4.91; found C 65.91, H 4.89.

*7-Hydroxy-2-(3,4,5-trimethoxyphenyl)-4*H*-benzopyran-4-one* [**6b**].—Yield 2.92 g (89%); mp 280° [lit. (20) 279–280°]; ^{1}H nmr (DMSO-d_6) δ 10.78 (bs, 1H, exchanges with D_2O), 7.89 (d, J = 8.7 Hz, 1H), 7.33 (s, 2H), 7.09 (s, 1H), 7.06 (d, J = 2.8 Hz, 1H), 6.93 (dd, 1H), 3.91 (s, 6H), 3.75 (s, 3H).

*7,8-Dihydroxy-2-(3,4,5-trimethoxyphenyl)-4*H*-benzopyran-4-one* [**6c**].—Yield 2.96 g (86%); mp 275–276° [lit. (21) 274–275°]; ^{1}H nmr (DMSO-d_6) δ 10.25 (bs, 1H, exchanges with D_2O), 9.54 (bs, 1H, exchanges with D_2O), 7.43 (s, 2H), 7.41 (d, J = 8.5 Hz, 1H), 6.99 (s, 1H), 6.97 (d, J = 8.5 Hz, 1H), 3.91 (s, 6H), 3.75 (s, 3H).

PREPARATION OF COMPOUNDS **7a** and **7b**.—These compounds were prepared utilizing the procedure described above using (4-benzyloxy)benzoyl chloride [**5**] (22) and dihydroxyacetophenones **1a** and **1c** on 10 mmol scale. The crude products were purified by recrystallization from EtOAc/hexane.

*2-(4-Benzyloxyphenyl)-5-hydroxy-4*H*-benzopyran-4-one* [**7a**].—Yield 2.68 g (78%); mp 165–166°; ^{1}H nmr (DMSO-d_6) δ 12.78 (bs, 1H, exchanges with D_2O), 8.12 (d, J = 8.8 Hz, 2H), 7.68 (t, J = 8.4 Hz, 1H), 7.49 (d, J = 8.8 Hz, 2H), 7.42 (t, J = 8.6 Hz, 2H), 7.36 (dd, 1H), 7.23 (d, J = 8.6 Hz, 2H), 7.21 (d, J = 8.1 Hz, 1H), 7.05 (s, 1H), 6.82 (d, J = 8.2 Hz, 1H), 5.24 (s, 2H); cims (isobutane) *m/z* $[MH]^+$ 345 (100). *Anal.* calcd for $C_{22}H_{16}O_4$, C 76.73, H 4.68; found C 76.84, H 4.84.

*2-(4-Benzyloxyphenyl)-7-hydroxy-4*H*-benzopyran-4-one* [**7b**].—Yield 2.67 g (77%); mp 269–271°; ^{1}H nmr (DMSO-d_6) δ 10.41 (bs, 1H, exchanges with D_2O), 8.03 (d, J = 9.0 Hz, 2H), 7.87 (d, J = 8.7 Hz, 1H), 7.49 (d, J = 7.4 Hz, 2H), 7.41 (t, J = 7.2 Hz, 2H), 7.37 (t, J = 7.3 Hz, 1H), 7.19 (d, J = 9.0 Hz, 2H), 6.99 (d, J = 3.0 Hz, 1H), 6.93 (dd, 1H), 6.82 (s, 1H), 5.22 (s, 2H); cims (isobutane) *m/z* $[MH]^+$ 345 (100). *Anal.* calcd for $C_{22}H_{16}O_4$, C 76.73, H 4.68; found C 76.92, H 4.91.

GENERAL PROCEDURE FOR THE PREPARATION OF COMPOUNDS **8a–8c**.—Me_2SO_4 (8 ml) and anhydrous K_2CO_3 (5 g) were added to solutions of compounds **6a–6c** (3 mmol) in Me_2CO (100 ml), and the mixtures were heated under reflux for 6 h. The solids were removed by filtration. Solvents were evaporated from the filtrate, and the residue was crystallized from Me_2CO/hexane.

*6-Methoxy-2-(3,4,5-trimethoxyphenyl)-4*H*-benzopyran-4-one* [**8a**].—Yield 0.96 g (94%); mp 264–266°; ^{1}H nmr (DMSO-d_6) δ 7.81 (dd, 1H), 7.45 (d, J = 2.8 Hz, 1H), 7.42 (d, J = 8.7 Hz, 1H), 7.39 (s, 2H), 7.15 (s, 1H), 3.91 (s, 6H), 3.88 (s, 3H), 3.75 (s, 3H); cims (isobutane) *m/z* $[MH]^+$ 343 (100). *Anal.* calcd for $C_{19}H_{18}O_6$, C 66.66, H 5.30; found C 66.49, H 5.50.

*7-Methoxy-2-(3,4,5-trimethoxyphenyl)-4*H*-benzopyran-4-one* [**8b**].—Yield 0.94 g (92%); mp 191° [lit. (23) 190–191°]; ^{1}H nmr (DMSO-d_6) δ 7.95 (d, J = 8.7 Hz, 1H), 7.38 (s, 2H), 7.39 (s, 1H), 7.02 (d, J = 2.7 Hz, 1H), 7.06 (dd, 1H), 3.94 (s, 3H), 3.92 (s, 6H), 3.76 (s, 3H).

*7,8-Dimethoxy-2-(3,4,5-trimethoxyphenyl)-4*H*-benzopyran-4-one* [**8c**].—Yield 0.99 g (89%); mp 194–195°; ^{1}H nmr (DMSO-d_6) δ 7.78 (d, J = 8.8 Hz, 1H), 7.36 (s, 2H), 7.29 (d, J = 8.8 Hz, 1H), 7.10 (s, 1H), 3.98 (s, 3H), 3.97 (s, 3H), 3.91 (s, 6H), 3.76 (s, 3H); cims (isobutane) *m/z* $[MH]^+$ 373 (100). *Anal.* calcd for $C_{20}H_{20}O_7$, C 64.51, H 5.41; found C 64.73, H 5.62.

METHYL 3-[(*t*-BUTYLDIMETHYLSILYL)-OXY]-BENZOATE [**10**].—A solution of methyl 3-hydroxybenzoate [**9**] (3.80 g, 25 mmol) in dry DMF (150 ml) under argon atmosphere was cooled to 0°, and *N,N*-diisopropylethylamine (6.50 g, 50 mmol) was added in 5 min. *t*-Butyldimethylsilyl chloride (4.22 g, 28 mmol) was added in 30 min and the reaction mixture was allowed to warm to room temperature over

a period of 1 h. After 4 h, the reaction mixture was poured into ice H_2O (500 ml) and extracted with Et_2O (3 × 50 ml). The combined extracts were washed with brine (3 × 50 ml) and dried (Na_2SO_4). Evaporation of solvents and drying the residue at reduced pressure for 24 h at room temperature gave methyl 3-[(*t*-butyldimethylsilyl)-oxy]-benzoate [**10**] (6.67 g, 100%) as a single product. An analytical sample was prepared by passing a solution of a small amount of it in hexane through a bed of neutral alumina, yielding an oil: ^{1}H nmr ($CDCl_3$) δ 7.64 (ddd, 1H), 7.49 (dd, $J_1 = J_2 = 3$ Hz, 1H), 7.29 (dd, $J_1 = 8.0$ Hz, $J_2 = 7.9$ Hz, 1H), 7.03 (ddd, 1H), 3.91 (s, 3H), 0.99 (s, 9H), 0.21 (s, 6H); cims (isobutane) *m/z* $[MH]^+$ 267 (100). *Anal.* calcd for $C_{14}H_{22}O_3Si$, C 63.12, H 8.32; found C 63.24, H 8.44.

HYDROXYFLAVONES **11a–11d**.—These compounds were prepared as indicated above for **6a–6c**, except a methyl ester **10** was employed instead of an acid chloride.

5-Hydroxy-2-(3-hydroxyphenyl)-4H-benzopyran-4-one [**11a**].—Yield 2.18 g (86%); mp >400°; ^{1}H nmr (DMSO-d_6) δ 12.68 (bs, 1H, exchanges with D_2O), 9.97 (bs, 1H, exchanges with D_2O), 7.69 (t, $J = 8.2$ Hz, 1H), 7.57 (d, $J = 7.6$ Hz, 1H), 7.46 (s, 1H), 7.39 (t, $J = 7.9$ Hz, 1H), 7.21 (d, $J = 8.2$ Hz, 1H), 7.04 (m, 2H), 6.84 (d, $J = 8.2$ Hz, 1H); cims (isobutane) *m/z* $[MH]^+$ 255 (100). *Anal.* calcd for $C_{15}H_{10}O_4$, C 70.80, H 3.96; found C 70.98, H 3.93.

6-Hydroxy-2-(3-hydroxyphenyl)-4H-benzopyran-4-one [**11b**].—Yield 1.98 g (78%); mp 300–302°; ^{1}H nmr (DMSO-d_6) δ 10.05 (bs, 1H, exchanges with D_2O), 9.89 (bs, 1H, exchanges with D_2O), 7.65 (d, $J = 8.9$ Hz, 1H), 7.51 (m, 1H), 7.41 (d, $J = 1.8$ Hz, 1H), 7.37 (t, $J = 8.0$ Hz, 1H), 7.32 (d, $J = 2.9$ Hz, 1H), 7.27 (dd, 1H), 7.00 (m, 1H), 6.85 (s, 1H); cims (isobutane) *m/z* $[MH]^+$ 255 (100). *Anal.* calcd for $C_{15}H_{10}O_4$, C 70.80, H 3.96; found C 71.01, H 3.99.

7-Hydroxy-2-(3-hydroxyphenyl)-4H-benzopyran-4-one [**11c**].—Yield 2.31 g (91%); mp 285–286°; ^{1}H nmr (DMSO-d_6) δ 10.85 (bs, 1H, exchanges with D_2O), 9.89 (bs, 1H, exchanges with D_2O), 7.90 (d, $J = 8.7$ Hz, 1H), 7.49 (dd, 1H), 7.40 (d, $J = 1.5$ Hz, 1H), 7.36 (t, $J = 8.0$ Hz, 1H), 6.98 (m, 2H), 6.94 (dd, 1H), 6.79 (s, 1H); cims (siobutane) *m/z* $[MH]^+$ 255 (100). *Anal.* calcd for $C_{15}H_{10}O_4$, C 70.80, H 3.96; found C 70.82, H 3.86.

7,8-Dihydroxy-2-(3-hydroxyphenyl)-4H-benzopyran-4-one [**11d**].—Yield 2.37 g (88%); mp 305–306°; ^{1}H nmr (DMSO-d_6) δ 10.41 (bs, 1H, exchanges with D_2O), 9.90 (bs, 1H, exchanges with D_2O), 9.49 (bs, 1H, exchanges with D_2O), 7.56 (d, $J = 7.6$ Hz, 1H), 7.51 (t, $J = 2.0$ Hz, 1H), 7.41 (d, $J = 8.7$ Hz, 1H), 7.37 (t, $J = 7.5$ Hz, 1H), 6.99 (dd, 1H), 6.96 (d, $J = 8.7$ Hz, 1H), 6.78 (s, 1H); cims (isobutane) *m/z* $[MH]^+$ 271 (100). *Anal.* calcd for $C_{15}H_{10}O_5$, C 66.67, H 3.73; found C 66.81, H 3.75.

2-[4-{ (*t*-BUTYLDIMETHYLSILYL)-OXY }7,8-DIACETOXY-3,5-DIMETHOXYPHENYL]-4*H*-BENZOPYRAN-4-ONE [**13**].—2[4-{(*t*-Butyldimethylsilyl)-oxy}-7,8-dihydroxy-3,5-dimethoxyphenyl]-4*H*-benzopyran-4-one [**12**] (19) (0.89 g, 2 mmol) was mixed with Ac_2O (5 ml) and pyridine (10 ml), and the mixture was stirred at room temperature for 3 h. Excess Ac_2O and pyridine were distilled off at reduced pressure, and the residue was treated with H_2O (20 ml). The solid (0.99 g, 94%) was filtered, washed with H_2O, and dried: mp 192–193°; ^{1}H nmr ($CDCl_3$) δ 8.14 (d, $J = 8.8$ Hz, 1H), 7.26 (d, $J = 8.8$ Hz, 1H), 7.02 (s, 2H), 6.73 (s, 1H), 3.87 (s, 6H), 2.44 (s, 3H), 2.38 (s, 3H), 1.03 (s, 9H), 0.17 (s, 6H); cims (isobutane) *m/z* $[MH]^+$ 529 (73). *Anal.* calcd for $C_{27}H_{32}O_9Si$, C 61.35, H 6.10; found C 61.40, H 6.23.

PROTEIN-TYROSINE KINASE ASSAYS.—In vitro assays of protein-tyrosine kinase activity were carried out using angiotensin I (1.2 mM) and [γ-^{32}P]ATP (50 μM) as described previously for the routine assay of the p40 protein-tyrosine kinase (24) except that reactions contained 8% DMSO, which was used as a carrier for the inhibitors. Control reactions run in the absence of inhibitor also contained 8% DMSO. Angiotensin I was prepared by the Purdue Peptide Synthesis Facility. The $p56^{lck}$ was partially purified from bovine thymus by sequential chromatography on columns of DEAE-cellulose, heparin-agarose and butyl-agarose (12). Analogues were screened for inhibition of $p56^{lck}$ at seven concentrations ranging from 0.8 to 800 μg/ml. IC_{50} values were determined graphically, and represented the concentration of inhibitor that gives half-maximal inhibition as compared to control assays carried out in the absence of inhibitor but in the presence of DMSO carrier.

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