Synthesis and Protein-Tyrosine Kinase Inhibitory Activities of Flavonoid Analogues

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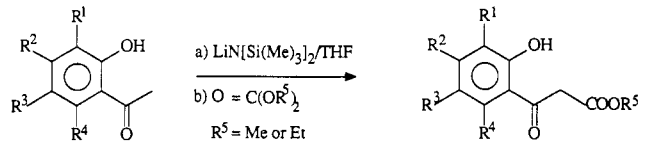
Treatment of *o*-hydroxyacetophenones **2a-e** with excess lithium bis(trimethylsilyl)amide followed by dialkyl carbonates gave alkyl 3-(2-hydroxyaryl)-3-oxopropanoates **3a-e**. The latter substances were transformed through the reaction of their magnesium chelates with benzoyl chlorides into a series of 3-(alkoxycarbonyl)-2-arylflavones, which were subsequently elaborated into a variety of flavonoids. These compounds were tested for their abilities to inhibit the in vitro protein-tyrosine kinase activity of $p56^{lck}$, an enzyme which is thought to play a key role in mediating signal transduction from the CD4 receptor during lymphocyte activation. All of the active compounds had either an amino or a hydroxyl substituent at the 4'-position of the 2-aryl ring. The most active substance prepared in this study is compound **17c**, which is approximately 1 order of magnitude more potent than the natural product quercetin (**1**). Compound **17c** was a competitive inhibitor of $p56^{lck}$ with respect to ATP and was highly selective for the inhibition of protein-tyrosine over protein-serine/threonine kinases.

The discovery of activated protein-tyrosine kinases as the products of dominant viral-transforming genes (oncogenes) first established the connection between protein-tyrosine phosphorylation and cell transformation.[1] As receptors for polypeptide growth factors and as protooncogene products, it is clear that protein-tyrosine kinases play important roles in the regulation of normal cell growth and differentiation.[2-6] There is now substantial evidence accumulating to suggest that the inappropriate or elevated expression of these enzymes may also contribute to the transformed state of many human malignancies.[7-12] For this reason, there is considerable interest in the discovery and development of specific inhibitors of protein-tyrosine kinases as chemotherapeutic agents.

The search for such inhibitors has uncovered several classes of natural products with inhibitory activity. These include such compounds as herbimycin A,[13] adriamycin,[14] and erbstatin[15] from *Streptomyces* fermentation broths, genistein from *Pseudomonas* fermentation broths,[16] and piceatannol[17] from higher plants. The identification of naturally occurring inhibitors provides important information as to the types of small molecules that can interact with and inhibit tyrosine kinases and provides a basis for the design of additional synthetic inhibitors. Several analogues structurally related to erbstatin, for example, have been prepared that show enhanced inhibitory activity.[18-22]

We have initiated a similar approach for the discovery and development of new protein-tyrosine kinase inhibitors. We[23] and others[24-26] have previously shown that several

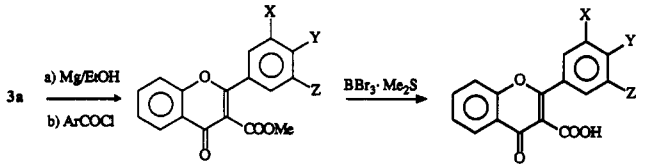
Scheme I



	R1	R2	R3	R4
2a	H	H	H	H
2b	H	OMe	H	H
2c	H	H	OMe	H
2d	H	OBn	H	OBn
2e	OH	OH	H	H

	R1	R2	R3	R4	R5
3a	H	H	H	H	Me
3b	H	OMe	H	H	Me
3c	H	H	OMe	H	Me
3d	H	OBn	H	OBn	Me
3e	H	H	H	H	Et
3f	H	OBn	H	OBn	Et
3g	OH	OH	H	H	Me

Scheme II



	X	Y	Z
4a/9a	OMe	OMe	OMe
4b/9b	OMe	H	OMe
4c/9c	OMe	OMe	H
4d/9d	H	OMe	H
4e/9e	H	Br	H

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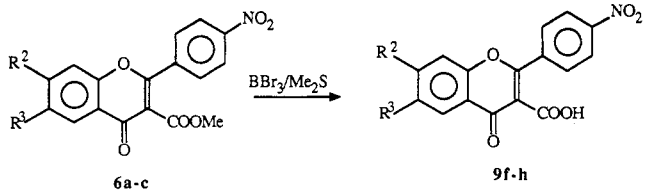
Scheme III



	R^2	R^3
6a/9f	H	H
6b/9g	OMe	H
6c/9h	H	OMe

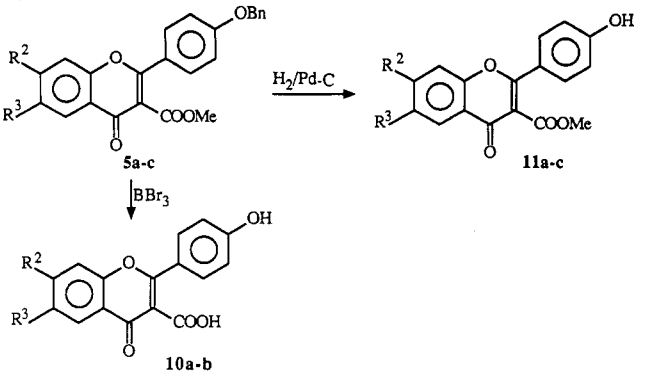
naturally occurring flavonoids are inhibitors of protein-tyrosine kinase activity in vitro. These flavonoids are, in general, competitive inhibitors with respect to ATP and lack selectivity for protein-tyrosine kinases over protein-serine/threonine kinases.[24,25] We have used these compounds as structural models for the design of new, more potent, and more selective inhibitors. In this paper we present the synthesis of new flavonoid analogues and describe the ability of these compounds to serve as inhibitors of the protein-tyrosine kinase $p56^{lck}$.

Chemistry

Methyl 3-(2-hydroxyphenyl)-3-oxopropanoate (**3a**) is a potential intermediate for the synthesis of 3-(methoxycarbonyl)-2-aryl-4*H*-benzopyran-4-ones,[27] including **4a–e**, and the synthesis of **3a** and methyl 3-(3,4-dimethoxy-2-hydroxyphenyl)-3-oxopropanoate were reported from our laboratory in 38–40% yields.[28,29] A general and improved experimental procedure for the synthesis of this class of compounds in optimized yields (85–96%) has now been devised as described below. Scheme I outlines the synthesis of methyl 3-(2-hydroxyaryl)-3-oxopropanoates **3a–d** by the reaction of lithium bis(trimethylsilyl)amide with 2′-hydroxyacetophenones **2a–d** and subsequent treatment with dimethyl carbonate. The progress of these reactions was closely monitored by working up aliquots of the reaction mixtures and monitoring by ^{1}H NMR for complete disappearance of the 2′-hydroxyacetophenones. The reaction was also extended for the preparation of ethyl 3-(2-hydroxyaryl)-3-oxopropanoates **3e–f**. The same procedure worked very well for the condensation of 2′,3′,4′-trihydroxyacetophenone (**2e**), provided 5 equiv of lithium bis(trimethylsilyl)amide were employed to give methyl (2,3,4-trihydroxyphenyl)-3-oxopropanoate (**3g**). ^{1}H NMR of compounds **3a–e** in $CDCl_3$ and **3g** in DMSO-d_6 showed that all these products exist exclusively in the keto tautomeric form.

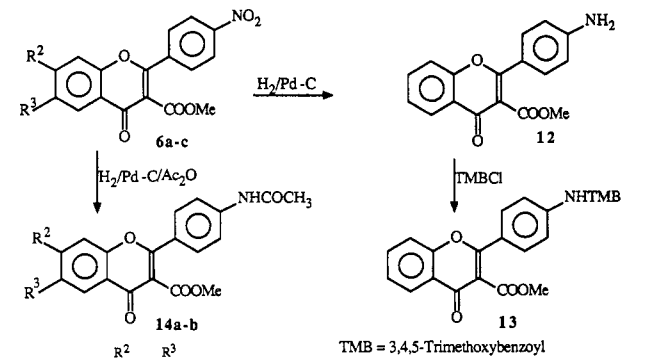
Compound **3a** was reacted with magnesium and ethanol followed by reaction with acid chlorides to provide 2-aryl-3-(methoxycarbonyl)-4*H*-benzopyran-4-ones **4a–e** according to Scheme II. By a similar methodology, alkyl 3-(2-hydroxyaryl)-3-oxopropanoates **3a–c** were reacted with 4-(benzyloxy)benzoyl chloride to get the 4′-benzyloxy-substituted flavones **5a–c**. Likewise, reaction of 4-nitrobenzoyl chloride with **3a–d** afforded 3-(methoxycarbonyl)-2-(4-nitrophenyl)-4*H*-benzopyran-4-ones **6a–d** in 68–74%.

Scheme IV



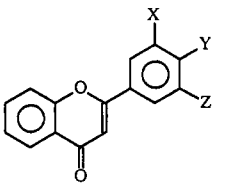
a: R^2=R^3=H; b: R^2=OMe, R^3=H; c: R^2=H, R^3=OMe

Scheme V



	R^2	R^3
6a	H	H
6b/14a	OMe	H
6c/14b	H	OMe

Our attempts to demethylate the methoxyl groups in 3-(methoxycarbonyl)-2-(3,4,5-trimethoxyphenyl)-4*H*-benzopyran-4-one (**4a**) by heating with pyridine hydrochloride in quinoline for 2.5 h resulted in the isolation of 3′,4′,5′-trimethoxyflavone (**7a**) and 3′,5′-dimethoxy-4′-hydroxyflavone (**7b**). Reaction of compound **4d** with 48% hydrobromic acid gave 4′-hydroxyflavone (**8**). As the methoxycarbonyl group was lost in both these reactions, we reacted compound **4a** with dimethyl sulfide–tribromoborane in CH_2Cl_2 and this reaction gave the hydrolysis product **9a**. Similar results were obtained with **4b–e** (Scheme II) and **6a–c** (Scheme III) to give carboxylic acids **9b–h**. All our attempts to synthesize hydroxyl-substituted flavones from the methoxyl compounds by treatment with a large excess of dimethyl sulfide–tribromoborane for extended reaction times were not successful. However, a similar reaction of 4′-(benzyloxy)-flavones **5a,b** with tribromoborane gave 2-(4-hydroxyphenyl)-4-oxo-4*H*-benzopyran-3-carboxylic acids **10a,b**, with concomitant debenzylation and hydrolysis. Catalytic hydrogenation of 4′-benzyloxyflavones **5a–c** at 40 psi in the presence of 5% palladium on charcoal gave 2-(4-



	X	Y	Z
7a	OMe	OMe	OMe
7b	OMe	OH	OMe
7c	H	OH	H

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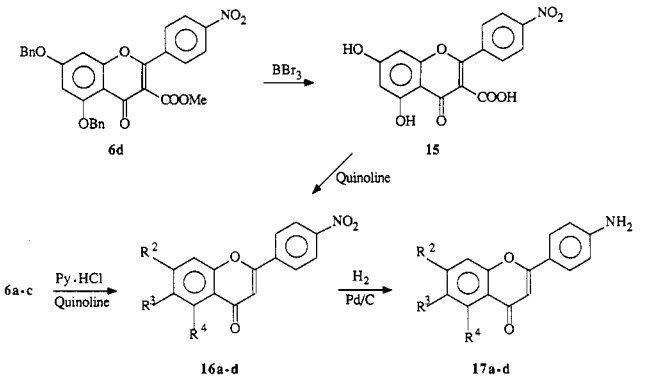
Scheme VI



hydroxyphenyl)-3-(methoxycarbonyl)-4*H*-benzopyran-4-ones **11a–c** in 88–92% yields (Scheme IV).

Scheme V describes the catalytic reduction of nitroflavone **6a** to the amino compound **12**. Our efforts to prepare an analytical sample of this product by chromatography and recrystallizations showed that this compound was unstable and hence it was converted to 3,4,5-trimethoxybenzamide derivative **13**, by reacting with 3,4,5-trimethoxybenzoyl chloride in the presence of pyridine. Catalytic hydrogenation of nitro compounds **6b,c** in acetic anhydride provided acetamidoflavones **14a,b**.

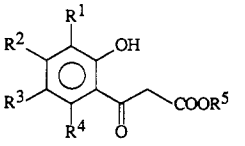
5,7-Bis(benzyloxy)-3-(methoxycarbonyl)-2-(4-nitrophenyl)-4*H*-benzopyran-4-one **6d** on reaction with tribromoborane in CH_2Cl_2 gave 5,7-dihydroxy-2-(4-nitrophenyl)-4-oxo-4*H*-benzopyran-3-carboxylic acid (**15**), which was subsequently decarboxylated by heating in quinoline as shown in Scheme VI to provide 5,7-dihydroxy-4′-nitroflavone (**16d**).

3-(Methoxycarbonyl)-2-(4-nitrophenyl)-4*H*-benzopyran-4-ones **6a–c** when heated with pyridine hydrochloride and quinoline provided 4′-nitroflavones **16a–c**. Hydrogenation of these nitro compounds **16a–d** gave 4′-aminoflavones **17a–d** (Scheme VI).

Biology

Flavonoid analogues and synthetic intermediates were tested for their ability to inhibit the in vitro protein-tyrosine kinase activity of p56^{lck}. The product of the *lck* gene, p56^{lck} is a 56-kDA enzyme that exhibits considerable sequence similarity to pp60^{src} and other members of the *src* family of tyrosine kinases.[30,31] The expression of p56^{lck} is normally restricted to lymphoid cells, primarily those of the T cell lineage,[30,31] where it is thought to play a role in mediating signal transduction via the CD4 receptor during lymphocyte activation.[32,33] An elevated expression of p56^{lck} due to retroviral promoter insertion has been found in two independent murine T cell lymphomas, suggesting a role for the kinase in the malignant transformation of these cells. The *lck* gene is also expressed in many human T cell leukemias[34] and is localized to a site of frequent chromosomal aberations seen in non-Hodgkins lymphomas and neuroblastomas.[35] Interestingly, *lck*

Table I. Inhibition of Protein-Tyrosine Kinase Activity of p56^{lck} by Alkyl 3-(2-Hydroxyaryl)-3-oxopropanoates **3a–e**



no.	R^1	R^2	R^3	R^4	R^5	IC_{50}, μM
3a	H	H	H	H	Me	>2000
3b	H	OMe	H	H	Me	1160
3c	H	H	OMe	H	Me	>2000
3d	H	OBn	H	OBn	Me	>2000
3e	H	H	H	H	Et	>2000
3f	H	OBn	H	OBn	Et	>2000
3g	OH	OH	H	H	Me	>2000

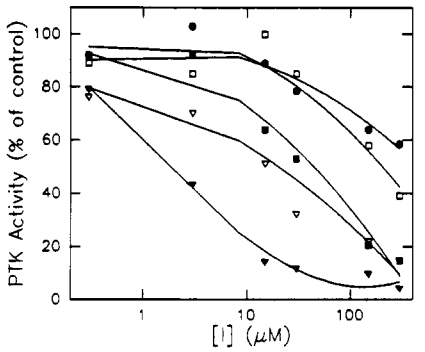
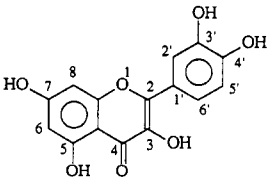


Figure 1. Inhibition of p56^{lck} by derivatives of 4′-aminoflavone. The activity of p56^{lck} was determined in the presence of increasing concentrations of **17a** (●), **17b** (□), **17c** (▼), or **17d** (▽). The effect of increasing concentrations of **1** (■) on kinase activity is shown for comparison.

transcripts are observed in many human colon carcinomas and in occasional small-cell lung cancers, which are both tissues that do not normally express p56^{lck}.[36] This makes the enzyme an interesting target for antitumor drug design. p56^{lck} was isolated from bovine thymus and assayed in vitro by monitoring the extent of phosphorylation of a synthetic tyrosine-containing peptide substrate (angiotensin I).

Results and Discussion

Several naturally occurring flavonoids were shown previously to be inhibitors of protein-tyrosine kinase activity in vitro.[23-26,37] Polyphenols related in structure to quercetin (3,5,7,3′,4′-pentahydroxyflavone, **1**) were the most



Quercetin (1)

potent inhibitors. Quercetin, which has been shown to inhibit a wide range of protein kinases, was also an inhibitor of p56^{lck} (IC_{50} = 13 μM, Table II and Figure 1). One of the major goals of this project was to develop synthetic approaches to the preparation of flavonoid analogues that inhibited tyrosine kinase activity with po-

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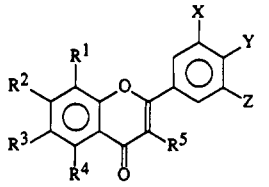
Table II. Inhibition of Protein-Tyrosine Kinase Activity of p56^{lck} by Flavone Derivatives



no.	R^1	R^2	R^3	R^4	R^5	X	Y	Z	IC_{50}, μM
4a	H	H	H	H	COOMe	OMe	OMe	OMe	>2000
4b	H	H	H	H	COOMe	OMe	H	OMe	>2000
4c	H	H	H	H	COOMe	OMe	OMe	H	>2000
4d	H	H	H	H	COOMe	H	OMe	H	>2000
4e	H	H	H	H	COOMe	H	Br	H	>2000
5a	H	H	H	H	COOMe	H	OBn	H	>2000
5b	H	OMe	H	H	COOMe	H	OBn	H	>2000
5c	H	H	OMe	H	COOMe	H	OBn	H	>2000
6a	H	H	H	H	COOMe	H	NO_2	H	>2000
6b	H	OMe	H	H	COOMe	H	NO_2	H	>2000
6c	H	H	OMe	H	COOMe	H	NO_2	H	>2000
6d	H	OBn	H	OBn	COOMe	H	NO_2	H	>2000
7a	H	H	H	H	H	OMe	OMe	OMe	>2000
7b	H	H	H	H	H	OMe	OH	OMe	26
8	H	H	H	H	H	H	OH	H	504
9a	H	H	H	H	COOH	OMe	OMe	OMe	>2000
9b	H	H	H	H	COOH	OMe	H	OMe	>2000
9c	H	H	H	H	COOH	OMe	OMe	H	>2000
9d	H	H	H	H	COOH	H	OMe	H	>2000
9e	H	H	H	H	COOH	H	Br	H	>2000
9f	H	H	H	H	COOH	H	NO_2	H	>2000
9g	H	OMe	H	H	COOH	H	NO_2	H	>2000
9h	H	H	OMe	H	COOH	H	NO_2	H	>2000
10a	H	H	H	H	COOH	H	OH	H	1595
10b	H	OMe	H	H	COOH	H	OH	H	1032
11a	H	H	H	H	COOMe	H	OH	H	439
11b	H	OMe	H	H	COOMe	H	OH	H	>2000
11c	H	H	OMe	H	COOMe	H	OH	H	>2000
12	H	H	H	H	COOMe	H	NH_2	H	810
13	H	H	H	H	COOMe	H	NHTMB[a]	H	>2000
14a	H	OMe	H	H	COOMe	H	NHAc	H	>2000
14b	H	H	OMe	H	COOMe	H	NHAc	H	>2000
15	H	OH	H	OH	COOH	H	NO_2	H	>2000
16a	H	H	H	H	H	H	NO_2	H	>2000
16b	H	OH	H	H	H	H	NO_2	H	>2000
16c	H	H	OH	H	H	H	NO_2	H	>2000
16d	H	OH	H	OH	H	H	NO_2	H	>2000
17a	H	H	H	H	H	H	NH_2	H	210
17b	H	OH	H	H	H	H	NH_2	H	138
17c	H	H	OH	H	H	H	NH_2	H	1.2
17d	H	OH	H	OH	H	H	NH_2	H	7.4
1	H	OH	H	OH	OH	OH	OH	H	13

[a] TMB = 3,4,5-trimethoxybenzoyl.

tencies greater than those of these naturally occurring flavonoids. To this end, reaction conditions were sought that would allow the synthesis of compounds bearing a wide range of substituents. These flavonoid analogues and synthetic intermediates were tested for inhibitory activity against p56^{lck} in vitro. The relative potencies of the test compounds are shown in Tables I and II and, for selected compounds, in Figure 1.

One of the methyl 3-(2-hydroxyaryl)-3-oxopropanoate intermediates, compound **3b**, showed weak inhibitory activity. However, other ethyl or methyl 3-(2-hydroxyaryl)-3-oxopropanoates were inactive (compounds **3a**, **3c–j**). Compound **3g** was prepared on the basis of the observation that the presence of three adjacent phenolic hydroxyl groups enhanced the inhibitory potency of the structurally related erbstatin analogues.[18,21,22] However, **3g** was inactive as an inhibitor of p56^{lck}.

Analogues bearing a methoxycarbonyl group at position 3 and methoxyl substituents on the phenyl ring (**4a–d**) were inactive as kinase inhibitors. This is consistent with previous observations that methoxyl-substituted flavonoids either lacked[23] or had low inhibitory activity[26,37] when tested against p40 and EGF receptor, respectively. Product **7a**, which lacked the methoxycarbonyl function, was also inactive. However, compound **7b**, which has a 4′-hydroxyl group instead of a methoxyl group, showed surprising inhibitory activity. The presence of the 3′- and 5′-methoxyl substituents appears to be important for inhibitory activity since other analogues with a 4′-hydroxyl group that were lacking these methoxyl substituents (eg. **10a,b** and **11a–c**) were either weak inhibitors or were inactive. Efforts are currently underway to prepare additional analogues of this general structural type.

A comparison of 3-methoxycarbonyl derivatives bearing different groups at the 4′-position (**4e**, **5a–c**, **6a–d**, **11a–c**, **12**, **13**, **14a,b**) indicated that hydroxyl- and amino-substituted compounds were active while bromo-, nitro-, benzyloxy-, or acetamido-substituted flavones were inactive. Similar results were obtained with the 3-carboxylic acids **9a–h**, **10a,b**, and **15**. The identification of inhibitory activity in 4′-amino-substituted compounds prompted an extensive examination of related compounds. A detailed

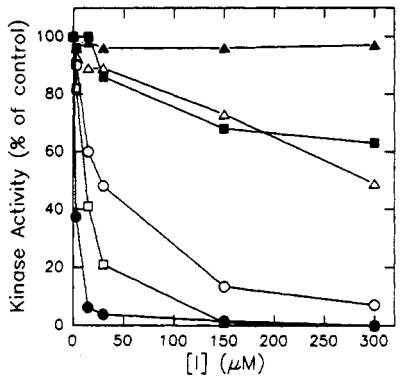


Figure 2. Inhibition of protein kinase activity by quercetin (1) and **17c**. Increasing concentrations of 1 (open symbols) or **17c** (closed symbols) were examined for their ability to inhibit the activity of $p56^{lck}$ (○, ●), protein kinase C (□, ■), or protein kinase A (△, ▲).

characterization of the 4′-aminoflavones showed that the absence of the 3-methoxycarbonyl group (compare **12** to **17a**) and the presence of additional hydroxyl groups (compounds **17b–d**) led to substantial increases in inhibitory activity. The most potent inhibitor, compound **17c** (Figure 1), was considerably more active than quercetin as an inhibitor of $p56^{lck}$.

The influence of hydroxyl substituents on the activity of the 4′-aminoflavones was similar to that seen previously for naturally occurring flavonoids.[23,25,37] In this series, flavonoids with hydroxyl groups at both positions 5 and 7 were more active as inhibitors than were compounds with only one hydroxyl group at position 7. This same pattern was seen for the 4′-aminoflavones (compare **17d** and **17b**). The influence of a hydroxyl group at position 6, which is found in the most potent 4′-aminoflavone inhibitor (**17c**), has not previously been tested since commonly occurring natural flavonoids are not modified at this position.

An analysis of the kinetics of the inhibition of $p56^{lck}$ indicated that **17c** was a competitive inhibitor with respect to ATP (data not shown). This pattern of inhibition is consistent with that observed previously for other flavonoid inhibitors.[23-26] Unlike flavonoids such as quercetin (1), however, **17c** exhibited considerable selectivity for the inhibition of the protein-tyrosine over protein-serine/threonine kinases. As shown in Figure 2, while **17c** was a more potent inhibitor of $p56^{lck}$ than quercetin, it was much less active for the inhibition of either protein kinase C or protein kinase A.

In summary, the data presented here indicate that the presence of a 4′-amino and a 6-hydroxyl group are important structural determinants that promote high affinity interactions of flavone derivatives with $p56^{lck}$ and decrease interactions with protein-serine/threonine kinases. These results indicate that it is possible to design flavonoid analogues that act as selective inhibitors of protein-tyrosine kinases.

Experimental Section

Melting points were determined in capillary tubes on a Mel-Temp apparatus and are uncorrected. Spectra were obtained as follows: EI and CI mass spectra on a Finnegan 4000 spectrometer, FAB mass spectra on a Kratos MS-50 spectrometer, high-resolution mass spectra on a Kratos MS-25 spectrometer, ^1H NMR spectra on a Chemagnetics A-200 or Nicolet QE-300 or Varian VXR-500S spectrometers with TMS as an internal standard in $CDCl_3$ or DMSO-d_6, ^{13}C NMR spectra on a Varian VXR-500S spectrometer, IR spectra on a Beckman IR-33 spectrophotometer. 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. 2′-Hydroxyacetophenones **2a–c** and **2e** and solutions of lithium bis(trimethylsilyl)amide in THF and dimethyl sulfide–tribromoborane in CH_2Cl_2 were obtained from commercial sources. Compound **2d** was prepared by reacting 2′,4′,6′-trihydroxyacetophenone with benzyl chloride.[38]

Preparation of Alkyl 3-(2-Hydroxyaryl)-3-oxopropanoates 3a–e. General Procedure. A well-stirred solution of lithium bis(trimethylsilyl)amide in THF (1 M, 75 mL, 75 mmol) was cooled to −78 °C under a dry argon atmosphere and a solution of 2′-hydroxyacetophenone **2a–d** (25 mmol) in dry THF (100 mL) was added in 20 min. The resultant orange solution was stirred at −78 °C for 1 h and at −10 °C for 2 h. It was cooled again to −78 °C, a solution of dimethyl carbonate or diethyl carbonate (27 mmol) in THF (10 mL) was added rapidly and the reaction mixture was allowed to warm to 25 °C over a period of 3–4 h. The mixture was then stirred at room temperature for 4 h and poured into a mixture of concentrated HCl (15 mL) and ice (250 g). The mixture was extracted with $CHCl_3$ (3 × 75 mL) and the combined $CHCl_3$ extracts were washed with water (2 × 100 mL). Solvents were evaporated from the dried (Na_2SO_4) extracts and the residue was crystallized from appropriate solvents. An analytical sample of compound **3e** was prepared by preparative thin-layer chromatography on silica gel with $CHCl_3$–hexane (1:1) as eluent.

Methyl 3-(2-hydroxyphenyl)-3-oxopropanoate (3a): 4.46 g; 92%; mp 56 °C (lit.[28] mp 56–57 °C).

Methyl 3-(2-hydroxy-4-methoxyphenyl)-3-oxopropanoate (3b): 5.26 g; 94%; mp 51–52 °C (ether–hexane); IR (KBr) 3420, 1730, 1620, 1560, 1480, 1425, 1345, 1285, 1255, 1205, 1195, 1140, 1130 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 12.33 (s, 1 H, exchanges with D_2O), 7.56 (d, J = 9 Hz, 1 H), 6.46 (m, 2 H), 3.95 (s, 2 H), 3.85 (s, 3 H), 3.77 (s, 3 H); CIMS (isobutane) m/e 225 (MH^+, 100). Anal. ($C_{11}H_{12}O_5$) C, H.

Methyl 3-(2-hydroxy-5-methoxyphenyl)-3-oxopropanoate (3c): 5.38 g; 96%; mp 53–54 °C (ether–hexane); IR (KBr) 3140, 3040, 1745, 1650, 1620, 1590, 1495, 1440, 1360, 1310, 1290, 1260, 1180, 1150, 1040, 1000 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 11.46 (s, 1 H, exchanges with D_2O), 7.14 (dd, 1 H), 7.09 (d, J = 3 Hz, 1 H), 6.95 (d, J = 9 Hz, 1 H), 4.00 (s, 2 H), 3.80 (s, 3 H), 3.78 (s, 3 H); CIMS (isobutane) m/e 225 (MH^+, 100). Anal. ($C_{11}H_{12}O_5$) C, H.

Methyl 3-[4,6-bis(benzyloxy)-2-hydroxyphenyl]-3-oxopropanoate (3d): 8.95 g; 88%; mp 87–88 °C ($CHCl_3$–hexane); IR (KBr) 3460–3420, 3080, 3020, 1725, 1620, 1585, 1455, 1425, 1385, 1345, 1290, 1270, 1210, 1180, 1100 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 10.05 (s, 1 H, exchanges with D_2O), 7.42–7.35 (m, 10 H), 6.18 (s, 1 H), 6.06 (s, 1 H), 5.06 (s, 2 H), 5.05 (s, 2 H), 3.92 (s, 2 H), 3.57 (s, 3 H); CIMS (isobutane) m/e 407 (MH^+, 100). Anal. ($C_{24}H_{22}O_6$) C, H.

Ethyl 3-(2-hydroxyphenyl)-3-oxopropanoate (3e): 4.94 g; 95%; oil; IR (neat) 3420, 1730, 1635, 1585, 1480, 1440, 1360, 1330, 1300, 1250, 1180, 1140, 1020 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 11.86 (s, 1 H, exchanges with D_2O), 7.67 (d, J = 8 Hz, 1 H), 7.51 (t, J = 8 Hz, 1 H), 7.01 (d, J = 8 Hz, 1 H), 6.93 (t, J = 8 Hz, 1 H), 4.23 (q, J = 7 Hz, 2 H), 4.01 (s, 2 H), 1.28 (t, J = 7 Hz, 3 H); CIMS (isobutane) m/e 209 (MH^+, 100). Anal. ($C_{11}H_{12}O_4$) C, H.

Ethyl 3-[4,6-bis(benzyloxy)-2-hydroxyphenyl]-3-oxopropanoate (3f): 9.47 g; 90%; mp 92–93 °C ($CHCl_3$–hexane); IR (KBr) 3460–3420, 2980, 2960, 1745, 1630, 1610, 1590, 1405, 1340, 1220, 1200, 1165, 1100, 1020 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 13.59 (s, 1 H, exchanges with D_2O), 7.40–7.33 (m, 10 H), 6.17 (s, 1 H), 6.05 (s, 1 H), 5.07 (s, 2 H), 5.04 (s, 2 H), 4.05 (q, J = 7 Hz, 2 H), 3.94 (s, 2 H), 1.16 (t, J = 7 Hz, 3 H); CIMS (isobutane) m/e 421 (MH^+, 15). Anal. ($C_{25}H_{24}O_6$) C, H.

Methyl 3-(2,3,4-Trihydroxyphenyl)-3-oxopropanoate (3g). This compound was prepared in the same manner as that described for **3a–f** using 5 equiv of lithium bis(trimethylsilyl)amide: 4.75 g; 84%; mp 153–154 °C (acetone–hexane); IR (KBr) 3460, 3400, 1720, 1650, 1620, 1530, 1460, 1420, 1380, 1320, 1240, 1190, 1140, 1050, 1000 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 12.25 (s, 1 H, exchanges with D_2O), 8.82 (s, 1 H, exchanges with D_2O), 7.63 (s, 1 H, exchanges with D_2O), 7.15 (d, J = 8.9 Hz, 1 H), 6.50 (d, J = 8.9 Hz, 1 H), 3.95 (s, 2 H), 3.75 (s, 3 H); CIMS (isobutane)

(38) Tsukayama, M.; Fujimoto, K.; Horie, T.; Masumura, M.; Nakayama, M. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 136.

m/e 227 (MH^+, 100). Anal. ($C_{10}H_{10}O_6$) C, H.

3-(Methoxycarbonyl)-2-(3,4,5-trimethoxyphenyl)-4*H*-benzopyran-4-one (4a). A mixture containing methyl 3-(2-hydroxyphenyl)-3-oxopropanoate (**3a**) (0.776 g, 4 mmol), magnesium (0.096 g, 4 mg-atom), absolute ethanol (24 mL), and dry CCl_4 (6 drops) was heated at 50–55 °C until all of the magnesium had dissolved (5 h). The solvent was evaporated under reduced pressure, and benzene (50 mL) and 3,4,5-trimethoxybenzoyl chloride (0.92 g, 4 mmol) were added to the residue. This mixture was heated under reflux for 6 h and cooled to room temperature. Aqueous acetic acid (10%, 40 mL) was added and the mixture was extracted with $CHCl_3$ (3 × 50 mL). The combined organic layer was washed with brine (2 × 50 mL) and dried (Na_2SO_4). Solvents were evaporated and the residue was purified by column chromatography (SiO_2, 230–400 mesh, 25 g), using hexane–ethyl acetate as gradient eluent (20:1 to 4:1) to get **4a** (0.965 g, 65%). An analytical sample was prepared by further recrystallization: mp 97–98 °C (ether–hexane); IR (KBr) 2940, 2810, 1730, 1640, 1565, 1500, 1460, 1410, 1370, 1320, 1245, 1120, 1080 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 8.27–7.44 (m, 4 H), 6.99 (s, 2 H), 3.93 (s, 3 H), 3.91 (s, 9 H); CIMS (isobutane) *m/e* 371 (MH^+, 100); HRMS calculated for $C_{20}H_{19}O_7$ (MH^+) 371.1131, found 371.1133.

2-Aryl-3-(methoxycarbonyl)-4*H*-benzopyran-4-ones 4b–e were prepared by using the same procedure as that reported above in 4-mmol scale with the corresponding acid chlorides. The crude products were purified by column chromatography followed by crystallization.

2-(3,5-Dimethoxyphenyl)-3-(methoxycarbonyl)-4*H*-benzopyran-4-one (4b): 0.865 g; 64%; mp 108–109 °C (ether–hexane); IR (KBr) 3000, 2940, 1730, 1635, 1605, 1565, 1460, 1415, 1375, 1275, 1230, 1220, 1200, 1150, 1090, 1060, 1050, 1000 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 8.24–7.30 (m, 4 H), 6.87 (d, *J* = 2 Hz, 2 H), 6.61 (t, 1 H), 3.82 (s, 9 H); CIMS (isobutane) *m/e* 341 (MH^+, 100); HRMS calculated for $C_{19}H_{17}O_6$ (MH^+) 341.1025, found 341.1022.

2-(3,4-Dimethoxyphenyl)-3-(methoxycarbonyl)-4*H*-benzopyran-4-one (4c): 0.92 g; 68%; mp 110–111 °C (ethyl acetate–hexane); IR (KBr) 3000, 2940, 1725, 1620, 1595, 1560, 1510, 1460, 1415, 1370, 1325, 1260, 1230, 1215, 1140, 1090, 1010 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.23 (d, *J* = 8.4 Hz, 1 H), 7.72 (t, 1 H), 7.53 (d, *J* = 8.4 Hz, 1 H), 7.42 (d, 1 H), 7.37 (t, 1 H), 7.28 (s, 1 H), 6.97 (d, 1 H), 3.97 (s, 3 H), 3.95 (s, 3 H), 3.83 (s, 3 H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 173.86, 165.40, 161.63, 155.09, 151.72, 148.59, 134.63, 125.77, 124.77, 123.04, 122.02, 121.31, 118.46, 116.47, 111.57, 110.45, 55.63, 55.54, 52.61; CIMS (isobutane) *m/e* 341 (MH^+, 100); HRMS calcd for $C_{19}H_{17}O_6$ (MH^+) 341.1025, found 341.1024.

3-(Methoxycarbonyl)-2-(4-methoxyphenyl)-4*H*-benzopyran-4-one (4d): 0.89 g; 72%; mp 92–93 °C (ethyl acetate–hexane); IR (KBr) 3000, 2940, 1730, 1630, 1600, 1530, 1470, 1430, 1380, 1270, 1260, 1240, 1190, 1080 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 8.26 (dd, 1 H), 7.73 (m, 3 H), 7.50 (dd, 1 H), 7.43 (t, 1 H), 6.99 (d, *J* = 8.0 Hz, 2 H), 3.89 (s, 3 H), 3.83 (s, 3 H); CIMS (isobutane) *m/e* 311 (MH^+, 100). Anal. ($C_{18}H_{14}O_5$) C, H.

2-(4-Bromophenyl)-3-(methoxycarbonyl)-4*H*-benzopyran-4-one (4e): 1.04 g; 72%; mp 123–124 °C ($CHCl_3$–hexane); IR (KBr) 1735, 1645, 1625, 1575, 1470, 1405, 1385, 1220, 1100, 1090, 1000 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.26 (dd, 1 H), 7.73 (dt, 1 H), 7.64 (q, 4 H), 7.53 (dd, 1 H), 7.46 (dt, 1 H), 3.81 (s, 3 H); CIMS (isobutane) *m/e* 362 (93), 360 (MH^+, 100). Anal. ($C_{17}H_{11}BrO_4$) C, H.

2-[4-(Benzyloxy)phenyl]-3-(methoxycarbonyl)-4*H*-benzopyran-4-one (5a). A mixture of methyl 3-(2-hydroxyaryl)-3-oxopropanoate (**3a**, 1.94 g, 10 mmol), magnesium (0.24 g, 10 mg-atom), absolute ethanol (100 mL), and dry CCl_4 (6 drops) was heated at 50–55 °C until all the magnesium had dissolved (5–6 h). The solvent was removed under reduced pressure, and 4-(benzyloxy)benzoyl chloride (2.50 g, 10 mmol) and benzene (150 mL) were added to the residue. The mixture was heated under reflux for 8 h and then cooled and poured into 10% ice-cold acetic acid (200 mL). The mixture was extracted with $CHCl_3$ (3 × 60 mL), and the combined organic extracts were washed twice with brine (50 mL). Solvents were evaporated from the dried (Na_2SO_4) solution, and the residue was chromatographed on a column of silica gel (230–400 mesh, 80 g). Elution with $CHCl_3$–ethyl acetate (9:1) gave the desired product as a viscous oil, which was crystallized from ethanol (2.55 g, 66%). An analytical sample was prepared by one more recrystallization from ethanol: mp 156–157 °C; IR (KBr) 1730, 1640, 1620, 1605, 1505, 1465, 1390, 1380, 1240, 1180, 1105, 1095, 1000 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 8.25 (d, 1 H), 7.73–7.39 (m, 10 H), 7.08 (d, *J* = 9 Hz, 2 H), 5.14 (s, 2 H), 3.82 (s, 3 H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 173.89, 165.26, 161.78, 161.05, 155.20, 136.29, 134.84, 129.56, 128.41, 127.96, 127.78, 125.92, 124.87, 123.29, 122.08, 118.52, 116.35, 115.30, 69.53, 52.60; CIMS (isobutane) *m/e* 387 (MH^+, 100). Anal. ($C_{24}H_{18}O_5$) C, H.

Preparation of 2-[4-(Benzyloxy)phenyl]-7-methoxy-3-(methoxycarbonyl)-4*H*-benzopyran-4-one (5b) and 2-[4-(Benzyloxy)phenyl]-6-methoxy-3-(methoxycarbonyl)-4*H*-benzopyran-4-one (5c). These compounds were prepared in the same manner as that described for **5a**.

Product **5b**: 2.62 g; 63%; mp 156–157 °C; IR (KBr) 1740, 1635, 1610, 1500, 1440, 1385, 1355, 1250, 1220, 1145, 1090, 1010 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.15 (d, *J* = 9 Hz, 1 H), 7.69 (d, *J* = 9 Hz, 2 H), 7.46–7.35 (m, 5 H), 7.08 (d, *J* = 9 Hz, 2 H), 7.00 (dd, 1 H), 6.90 (d, *J* = 2.3 Hz, 1 H), 5.15 (s, 2 H), 3.93 (s, 3 H), 3.83 (s, 3 H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 173.14, 165.38, 164.22, 161.20, 160.95, 157.07, 136.30, 129.43, 128.41, 127.97, 127.80, 126.30, 123.28, 116.18, 115.76, 115.26, 115.19, 100.91, 69.53, 56.20, 52.57; CIMS (isobutane) *m/e* 417 (MH^+, 100). Anal. ($C_{25}H_{20}O_6$) C, H.

Product **5c**: 2.95 g; 71%; mp 157–158 °C; IR (KBr) 1735, 1640, 1620, 1610, 1585, 1510, 1490, 1435, 1380, 1265, 1245, 1200, 1180, 1105, 1090, 1010 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.71 (d, *J* = 8.8 Hz, 2 H), 7.60 (d, *J* = 3.3 Hz, 1 H), 7.46–7.27 (m, 7 H), 7.07 (d, *J* = 8.8 Hz, 2 H), 5.15 (s, 2 H), 3.91 (s, 3 H), 3.84 (s, 3 H); CIMS (isobutane) *m/e* 417 (MH^+, 100). Anal. ($C_{25}H_{20}O_6$) C, H.

Preparation of 3-(Methoxycarbonyl)-2-(4-nitrophenyl)-4*H*-benzopyran-4-ones 6a–d. These compounds were prepared from the corresponding methyl 3-(2-hydroxyaryl)-3-oxopropanoates **3a–d** and 4-nitrobenzoyl chloride in 10-mmol scale following the procedure reported for **5a**. The crude products were purified by crystallization from methanol.

3-(Methoxycarbonyl)-2-(4-nitrophenyl)-4*H*-benzopyran-4-one (6a): 2.4 g; 74%; mp 153–154 °C; IR (KBr) 1735, 1640, 1610, 1600, 1560, 1510, 1460, 1400, 1380, 1340, 1310, 1220, 1085 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.38 (d, *J* = 8.3 Hz, 2 H), 8.28 (dd, 1 H), 7.94 (d, *J* = 8.3 Hz, 2 H), 7.78 (dt, 1 H), 7.55 (dd, 1 H), 7.52 (dt, 1 H), 3.83 (s, 3 H); CIMS (isobutane) *m/e* 326 (MH^+, 100). Anal. ($C_{17}H_{11}NO_6$) C, H.

7-Methoxy-3-(methoxycarbonyl)-2-(4-nitrophenyl)-4*H*-benzopyran-4-one (6b): 2.42 g; 68%; mp 202–203 °C; IR (KBr) 1740, 1620, 1600, 1510, 1425, 1370, 1340, 1250, 1185, 1130, 1090, 1080 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.36 (d, *J* = 8.4 Hz, 2 H), 8.18 (d, *J* = 9 Hz, 1 H), 7.926 (d, *J* = 8.4 Hz, 2 H), 7.06 (dd, 1 H), 6.94 (d, *J* = 2.3 Hz, 1 H), 3.95 (s, 3 H), 3.81 (s, 3 H); CIMS (isobutane) *m/e* 356 (MH^+, 100). Anal. ($C_{18}H_{13}NO_7$) C, H.

6-Methoxy-3-(methoxycarbonyl)-2-(4-nitrophenyl)-4*H*-benzopyran-4-one (6c): 2.56 g; 72%; mp 192–193 °C ($CHCl_3$–hexane); IR (KBr) 1720, 1630, 1600, 1560, 1470, 1450, 1350, 1330, 1260, 1240, 1185, 1075, 1000 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.37 (d, *J* = 8.4 Hz, 2 H), 7.93 (d, *J* = 8.4 Hz, 2 H), 7.62 (d, *J* = 3.3 Hz, 1 H), 7.49 (d, *J* = 9.2 Hz, 1 H), 7.36 (dd, 1 H), 3.94 (s, 3 H), 3.83 (s, 3 H); CIMS (isobutane) *m/e* 356 (MH^+, 100), 326 (14). Anal. ($C_{18}H_{13}NO_7$) C, H.

5,7-Bis(benzyloxy)-3-(methoxycarbonyl)-2-(4-nitrophenyl)-4*H*-benzopyran-4-one (6d): 3.17 g; 59%; mp 190–191 °C; IR (KBr): 1720, 1650, 1610, 1580, 1520, 1450, 1430, 1380, 1350, 1290, 1175, 1140, 1110, 1080 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.34 (d, *J* = 9 Hz, 2 H), 7.91 (d, *J* = 9 Hz, 2 H), 7.59–7.39 (m, 10 H), 6.61 (d, *J* = 2.3 Hz, 1 H), 6.55 (d, *J* = 2.3 Hz, 1 H), 5.24 (s, 2 H), 5.13 (s, 2 H), 3.81 (s, 3 H); FABMS *m/e* 538 (MH^+, 27), 523 (5), 389 (11), 309 (8), 181 (11), 155 (54). Anal. ($C_{31}H_{23}NO_8$) C, H.

Reaction of 3-(Methoxycarbonyl)-2-(3,4,5-trimethoxyphenyl)-4*H*-benzopyran-4-one (4a) with Pyridine Hydrochloride To Afford 7a and 7b. A mixture of 3-(methoxycarbonyl)-2-(3,4,5-trimethoxyphenyl)-4*H*-benzopyran-4-one (**4a**, 0.095 g, 0.26 mmol), pyridine hydrochloride (2 g), and quinoline (6 drops) was heated at 180–185 °C under nitrogen atmosphere for 2.5 h and cooled to room temperature. Then it was dissolved in water (15 mL), acidified to pH 2 with concentrated HCl, and extracted with ether (4 × 15 mL). The combined organic extracts

were washed with water (2 × 15 mL) and dried (Na_2SO_4) and the solvents were evaporated. The residue was purified by preparative thin-layer chromatography on silica gel with $CHCl_3$–ethyl acetate (1:1) as eluent. Two product **7a** and **7b** were obtained at about R_f 0.66 and 0.33, respectively.

Product **7a**: 18 mg; 23%; mp 175–176 °C (lit.[39] mp 174 °C).

Product **7b**: 44 mg; 57%; mp 224–225 °C; IR (KBr) 3240–3180, 1620, 1600, 1550, 1500, 1450, 1410, 1375, 1200, 1100 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 8.28–7.28 (m, 4 H), 7.20 (s, 2 H), 6.78 (s, 1 H, exchanges with D_2O), 5.96 (s, 1 H), 4.03 (s, 6 H); CIMS (isobutane) *m/e* 299 (MH^+, 28); HRMS calcd for $C_{17}H_{14}O_5$ (M^+) 298.0841, found 298.0843.

4′-Hydroxyflavone (8). A mixture of 3-(methoxycarbonyl)-2-(4-methoxyphenyl)-4*H*-benzopyran-4-one (**4d**, 0.310 g, 1 mmol) and 48% aqueous hydrobromic acid (15 mL) was heated at 110–120 °C under nitrogen atmosphere for 24 h and the excess hydrobromic acid was distilled off under reduced pressure. The residue was crystallized from ethyl acetate–hexane to afford 4′-hydroxyflavone (**8**) as a white, crystalline solid (0.185 g, 78%): mp 270–271 °C (lit.[39] mp 270 °C); CIMS (isobutane) *m/e* 239 (MH^+, 100).

Preparation of 2-Aryl-4-oxo-4*H*-benzopyran-3-carboxylic Acids 9a–h. General Procedure. A solution of dimethyl sulfide–tribromoborane in CH_2Cl_2 (1 M, 4 mL, 4 mmol) was added to a well-stirred solution of 2-aryl-3-(methoxycarbonyl)-4*H*-benzopyran-4-one (**4a–e** or **6a–c**, 2 mmol) in CH_2Cl_2 (25 mL) at −78 °C and the reaction mixture was allowed to warm to room temperature. After 4 h, solvent was removed under reduced pressure and ice was added to the residue. The precipitate was filtered, washed with water and hexane, and dried. The crude product was recrystallized from acetone–hexane.

2-(3,4,5-Trimethoxyphenyl)-4-oxo-4*H*-benzopyran-3-carboxylic acid (9a): 0.676 g; 95%; mp 222–223 °C; IR (KBr) 3240–3180, 2840, 1745, 1620, 1590, 1460, 1380, 1335, 1245, 1130, 1100, 995 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 8.11–7.51 (m, 4 H), 7.17 (s, 2 H), 6.52 (bs, 1 H, exchanges with D_2O), 3.84 (s, 6 H), 3.76 (s, 3 H); CIMS (isobutane) *m/e* 357 (MH^+, 100); HRMS calcd for $C_{19}H_{17}O_7$ (MH^+) 357.0974, found 357.0975.

2-(3,5-Dimethoxyphenyl)-4-oxo-4*H*-benzopyran-3-carboxylic acid (9b): 0.626 g; 96%; mp 230–232 °C; IR (KBr) 3240–3180, 1720, 1610, 1580, 1560, 1500, 1465, 1390, 1215, 1150, 1080 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 8.06 (d, 1 H), 7.86 (t, 1 H), 7.76 (d, 1 H), 7.54 (t, 1 H), 6.96 (s, 2 H), 6.73 (s, 1 H), 6.62 (bs, 1 H, exchanges with D_2O), 3.80 (s, 6 H); CIMS (isobutane) *m/e* 327 (MH^+, 100), 309 (18); HRMS calcd for $C_{18}H_{15}O_6$ (MH^+) 327.0869, found 327.0859.

2-(3,4-Dimethoxyphenyl)-4-oxo-4*H*-benzopyran-3-carboxylic acid (9c): 0.639 g; 98%; mp 246–247 °C; IR (KBr) 3000–2940, 1715, 1620, 1595, 1560, 1550, 1510, 1470, 1390, 1260, 1210, 1150, 1140, 1085, 1000 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 13.49 (bs, 1 H, exchanges with D_2O), 8.09 (d, *J* = 3.1 Hz, 1 H), 7.90–7.77 (m, 2 H), 7.56–7.44 (m, 3 H), 7.17 (d, *J* = 1.6 Hz, 1 H), 3.86 (s, 3 H), 3.84 (s, 3 H); CIMS (isobutane) *m/e* 327 (MH^+, 100); HRMS calcd for $C_{18}H_{15}O_6$ (MH^+) 327.0869, found 327.0872.

2-(4-Methoxyphenyl)-4-oxo-4*H*-benzopyran-3-carboxylic acid (9d): 0.556 g; 94%; mp 195–197 °C; IR (KBr) 3240–3180, 1715, 1610, 1580, 1570, 1500, 1470, 1460, 1395, 1335, 1265, 1210, 1150, 1080 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 8.04 (d, 1 H), 7.78 (m, 3 H), 7.68 (d, 1 H), 7.49 (t, 1 H), 7.47 (d, *J* = 8.4 Hz, 2 H), 6.78 (bs, 1 H, exchanges with D_2O), 3.80 (s, 3 H); CIMS (isobutane) *m/e* 297 (MH^+, 100); HRMS calcd for $C_{17}H_{12}O_5$ (M^+) 296.0685, found 296.0684.

2-(4-Bromophenyl)-4-oxo-4*H*-benzopyran-3-carboxylic acid (9e): 0.678 g; 98%; mp 215–217 °C; IR (KBr) 3000–2960, 1720, 1610, 1590, 1560, 1470, 1450, 1390, 1370, 1210, 1080 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 12.78 (bs, 1 H, exchanges with D_2O), 7.23 (dd, 1 H), 7.02 (dt, 1 H), 6.97 (d, *J* = 8.8 Hz, 2 H), 6.91 (d, *J* = 8.8 Hz, 2 H), 6.88 (d, 1 H), 6.69 (dt, 1 H); CIMS (isobutane) *m/e* 348 (92), 346 (MH^+, 100). Anal. ($C_{16}H_9BrO_4$) C, H.

2-(4-Nitrophenyl)-4-oxo-4*H*-benzopyran-3-carboxylic acid (9f): 1.14 g; 92%; mp 240–241 °C; IR (KBr) 3440–3400, 1740, 1620, 1600, 1580, 1515, 1480, 1460, 1450, 1380, 1340, 1090 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 12.67 (bs, 1 H, exchanges with D_2O), 8.23 (d, *J* = 8.7 Hz, 2 H), 8.19 (dd, 1 H), 7.81 (d, *J* = 8.7 Hz, 2 H), 7.72 (dt, 1 H), 7.47 (dd, 1 H), 7.45 (dt, 1 H); CIMS (isobutane) *m/e* 312 (MH^+, 100), 294 (45.97). Anal. ($C_{16}H_9NO_6$) C, H.

7-Methoxy-2-(4-nitrophenyl)-4-oxo-4*H*-benzopyran-3-carboxylic acid (9g): 1.12 g; 82%; mp 258–260 °C; IR (KBr) 3440–3400, 1740, 1625, 1590, 1565, 1540, 1460, 1435, 1340, 1270, 1225, 1100, 1090 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 12.94 (bs, 1 H, exchanges with D_2O), 8.42 (d, *J* = 8.8 Hz, 2 H), 8.06 (d, *J* = 8.8 Hz, 2 H), 7.99 (d, *J* = 8.9 Hz, 1 H), 7.28 (d, *J* = 2.2 Hz, 1 H), 7.14 (dd, 1 H), 3.92 (s, 3 H); CIMS (isobutane) *m/e* 342 (MH^+, 100). Anal. ($C_{17}H_{11}NO_7$) C, H.

6-Methoxy-2-(4-nitrophenyl)-4-oxo-4*H*-benzopyran-3-carboxylic acid (9h): 1.18 g; 86%; mp 250–252 °C; IR (KBr) 3460–3440, 1740, 1625, 1590, 1555, 1540, 1510, 1460, 1435, 1340, 1275, 1225, 1100, 990 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.43 (d, *J* = 9.0 Hz, 2 H), 8.06 (d, *J* = 9.0 Hz, 2 H), 8.02 (d, *J* = 8.9 Hz, 1 H), 7.29 (d, *J* = 2.2 Hz, 1 H), 7.14 (dd, 1 H), 3.92 (s, 3 H); FABMS *m/e* 342 (MH^+, 6), 159 (10), 157 (100). Anal. ($C_{17}H_{11}NO_7$) C, H.

2-(4-Hydroxyphenyl)-4-oxo-4*H*-benzopyran-3-carboxylic Acids (10a,b). A 1 M solution of tribromoborane (8 mL, 8 mmol) in CH_2Cl_2 was added to a well-stirred solution of compound **5a** or **5b** (2 mmol) in dry CH_2Cl_2 (50 mL) at 0 °C and the mixture was allowed to warm to room temperature. After 48 h, the solvent was distilled off at reduced pressure and the residue was treated with ice (15 g). The precipitated product was filtered, washed with water, and dried. The crude products were recrystallized from acetonitrile.

2-(4-Hydroxyphenyl)-4-oxo-4*H*-benzopyran-3-carboxylic acid (10a): 0.40 g; 71%; mp 278–280 °C; IR (KBr) 3220–3210, 1700, 1680, 1610, 1560, 1530, 1495, 1485, 1460, 1370, 1285, 1220, 1160, 1090 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 10.38 (bs, 1 H, exchanges with D_2O), 8.06 (dd, 1 H), 7.83 (t, *J* = 7.2 Hz, 1 H), 7.72 (m, 3 H), 7.52 (t, *J* = 7.2 Hz, 1 H), 6.94 (d, *J* = 8.9 Hz, 2 H); FABMS *m/e* 283 (MH^+, 64), 265.03 (29), 234.98 (25), 158.98 (58), 158 (45), 156.98 (100). Anal. ($C_{16}H_{10}O_5$) C, H.

2-(4-Hydroxyphenyl)-7-methoxy-4-oxo-4*H*-benzopyran-3-carboxylic acid (10b): 0.49 g; 79%; mp 320–322 °C; IR (KBr) 3220–3160, 1700, 1605, 1520, 1490, 1465, 1425, 1350, 1260, 1230, 1200, 1155, 1080 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 10.31 (bs, 1 H, exchanges with D_2O), 7.95 (dd, 1 H), 7.70 (d, *J* = 8.6 Hz, 2 H), 7.25 (d, *J* = 2.1 Hz, 1 H), 7.10 (dd, 1 H), 6.93 (d, *J* = 8.6 Hz, 2 H), 3.91 (s, 3 H); FABMS *m/e* 313.06 (MH^+, 9), 309 (8), 287.12 (8), 177.02 (14), 157.02 (10), 155.02 (55), 152 (21). Anal. ($C_{17}H_{12}O_6$) C, H.

2-(4-Hydroxyphenyl)-3-(methoxycarbonyl)-4*H*-benzopyran-4-ones 11a–c. A solution of **5a–c** (5 mmol) in dioxane (80 mL) was hydrogenated at 40 psi of hydrogen in the presence of 50 mg of 5% palladium on charcoal for 20 h. The catalyst was filtered off and the filtrate was concentrated to leave the crude product as an oil. It was crystallized from 95% ethanol to give an analytical sample.

2-(4-Hydroxyphenyl)-3-(methoxycarbonyl)-4*H*-benzopyran-4-one (11a): 1.30 g; 88%; mp 237–238 °C; IR (KBr) 3170–3160, 1720, 1620, 1600, 1580, 1550, 1490, 1430, 1250, 1220, 1170, 1140, 1080 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 10.30 (bs, 1 H, exchanges with D_2O), 8.13 (d, *J* = 8.0 Hz, 1 H), 7.64–7.35 (m, 5 H), 6.87 (d, *J* = 8.5 Hz, 2 H), 3.71 (s, 3 H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 173.89, 165.45, 162.10, 160.90, 155.13, 134.66, 129.68, 125.76, 124.85, 122.07, 121.45, 118.38, 115.90, 115.86, 52.57; CIMS (isobutane) *m/e* 297 (MH^+, 100). Anal. ($C_{17}H_{12}O_5$) C, H.

2-(4-Hydroxyphenyl)-7-methoxy-3-(methoxycarbonyl)-4*H*-benzopyran-4-one (11b): 1.50 g; 92%; mp 178–179 °C; IR (KBr) 3180–3160, 1720, 1630, 1600, 1580, 1550, 1490, 1430, 1250, 1220, 1170, 1140, 1080 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 10.40 (bs, 1 H, exchanges with D_2O), 7.95 (d, *J* = 8.5 Hz, 1 H), 7.59 (d, *J* = 8.8 Hz, 2 H), 7.26 (d, *J* = 2.4 Hz, 1 H), 7.09 (dd, 1 H), 6.95 (d, *J* = 8.8 Hz, 2 H), 3.91 (s, 3 H), 3.72 (s, 3 H); EIMS *m/e* 326 (M^+, 56), 295 (100). Anal. ($C_{18}H_{14}O_6$) C, H.

2-(4-Hydroxyphenyl)-6-methoxy-3-(methoxycarbonyl)-4*H*-benzopyran-4-one (11c): 1.42 g; 87%; mp 270–271 °C; IR (KBr) 3200, 1720, 1620, 1600, 1570, 1545, 1490, 1470, 1450, 1415, 1355, 1260, 1235, 1210, 1160, 1080 cm^{-1}; ^1H NMR (DMSO-d_6, 500

(39) Gaydou, E. M.; Bianchini, J.-P. *Bull. Soc. Chim. Fr.* **1978**, II-44.

MHz) δ 9.81 (bs, 1 H, exchanges with D_2O), 7.59 (d, J = 8.7 Hz, 2 H), 7.53 (d, J = 3 Hz, 1 H), 7.51 (d, J = 9 Hz, 1 H), 7.33 (dd, 1 H), 6.94 (d, J = 8.7 Hz, 2 H), 3.91 (s, 3 H), 3.80 (s, 3 H); EIMS m/e 326 (M^+, 70). Anal. ($C_{18}H_{14}O_6$) C, H.

2-(4-Aminophenyl)-3-(methoxycarbonyl)-4*H*-benzopyran-4-one (12). A solution of 3-(methoxycarbonyl)-2-(4-nitrophenyl)-4*H*-benzopyran-4-one (**6a**, 0.650 g, 2 mmol) in dioxane (80 mL) was hydrogenated at 40 psi in presence of 5% palladium on charcoal (100 mg) for 4 h and the catalyst was filtered off. Dioxane was removed from the filtrate at reduced pressure and the residue was crystallized from 95% ethanol to give **12** as a bright yellow solid (0.496 g, 84%); mp 210–212 °C; IR (KBr) 3460, 3360, 1730, 1650, 1610, 1560, 1510, 1460, 1390, 1320, 1250, 1230, 1180, 1090 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.24 (dd, 1 H), 7.69 (t, 1 H), 7.58 (d, J = 8.4 Hz, 2 H), 7.51 (d, J = 8.3 Hz, 1 H), 7.42 (t, 1 H), 6.73 (d, J = 8.4 Hz, 2 H), 4.10 (bs, 2 H), 3.90 (s, 3 H); CIMS (isobutane) m/e 296 (MH^+, 100).

The 3,4,5-trimethoxybenzamide (**13**) of this aminoflavone was prepared as follows: 3,4,5-trimethoxybenzoyl chloride (0.230 g, 1 mmol) and pyridine (1 mL) were added to a solution of **12** (0.295 g, 1 mmol) in $CHCl_3$ (25 mL), and the mixture was stirred at room temperature for 4 h. This mixture was washed with 10% HCl (15 mL) followed by water (25 mL) and the $CHCl_3$ layer was dried (Na_2SO_4). Evaporation of the solvent gave the crude product which was recrystallized from ethyl acetate–hexane to get pure **13** (0.385 g, 79%); mp 212 °C; IR (KBr) 3400, 1715, 1680, 1650, 1610, 1600, 1520, 1500, 1460, 1380, 1335, 1250, 1215, 1185, 1125, 1080 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.37 (bs, 1 H), 8.18 (dd, 1 H), 7.75–7.68 (m, 5 H), 7.50 (dd, 1 H), 7.45 (dt, 1 H), 7.02 (s, 2 H), 3.95 (s, 3 H), 3.91 (s, 3 H), 3.88 (s, 6 H); CIMS (isobutane) m/e 490 (MH^+, 100); HRMS calcd for $C_{27}H_{24}NO_8$ (MH^+) 490.1502, found 490.1497.

2-(4-Acetamidophenyl)-3-(methoxycarbonyl)-4*H*-benzopyran-4-ones 14a,b. A solution of **6b** or **6c** (3.55 g, 10 mmol) in acetic anhydride (50 mL) was hydrogenated at 40 psi for 6 h in the presence of 5% palladium on charcoal (0.2 g) and the catalyst was filtered off. Excess acetic anhydride was removed by distillation at reduced pressure to leave the product as a white powder. Analytical samples were prepared by recrystallization from ethanol.

2-(4-Acetamidophenyl)-7-methoxy-3-(methoxycarbonyl)-4*H*-benzopyran-4-one (14a): 3.60 g; 98%; mp 232–233 °C; IR (KBr) 3280, 1715, 1675, 1640, 1620, 1590, 1530, 1490, 1425, 1370, 1345, 1310, 1250, 1210, 1165, 1140, 1080, 1000 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.15 (d, J = 8.1 Hz, 1 H), 7.75 (d, J = 8.7 Hz, 2 H), 7.66 (d, J = 8.7 Hz, 2 H), 7.51 (bs, 1 H), 7.01 (dd, 1 H), 6.91 (d, J = 2.2 Hz, 1 H), 3.93 (s, 3 H), 3.81 (s, 3 H), 2.23 (s, 3 H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 173.11, 168.82, 165.26, 164.23, 161.12, 157.07, 142.40, 128.49, 126.31, 125.03, 118.64, 116.33, 115.78, 115.22, 100.89, 56.19, 52.56, 24.15; EIMS m/e 367 (M^+, 100), 336 (25), 294 (25), 150 (15). Anal. ($C_{20}H_{17}NO_6$) C, H.

2-(4-Acetamidophenyl)-6-methoxy-3-(methoxycarbonyl)-4*H*-benzopyran-4-one (14b): 3.60 g; 98%; mp 227–228 °C; IR (KBr) 3460, 1720, 1660, 1620, 1595, 1510, 1465, 1420, 1150 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.69–7.67 (m, 4 H), 7.65 (bs, 1 H), 7.60 (d, J = 3 Hz, 1 H), 7.46 (d, J = 9.0 Hz, 2 H), 7.31 (dd, 1 H), 3.91 (s, 3 H), 3.82 (s, 3 H), 2.22 (s, 3 H); EIMS m/e 367 (M^+, 100). Anal. ($C_{20}H_{17}NO_6$) C, H.

5,7-Dihydroxy-4′-nitroflavone (16d). A 1 M solution of tribromoborane in CH_2Cl_2 (8 mL, 8 mmol) was added to a well-stirred solution of **6d** (0.537 g, 1 mmol) in CH_2Cl_2 (50 mL) at 0 °C and the reaction mixture was allowed to warm to room temperature. After 40 h, the reaction mixture was cooled again to 0 °C, and 2 mL of water was added. Solvents were removed from the reaction mixture under reduced pressure, and the residue was recrystallized from acetonitrile–water. The ^1H NMR analysis showed this product as 5,7-dihydroxy-2-(4-nitrophenyl)-4-oxo-4*H*-benzopyran-3-carboxylic acid (**15**). [^1H NMR (DMSO-d_6, 300 MHz) δ 12.25 (s, 1 H, exchanges with D_2O), 11.16 (s, 1 H, exchanges with D_2O), 8.42 (d, J = 8.3 Hz, 2 H), 8.04 (d, J = 8.3 Hz, 2 H), 6.48 (s, 1 H), 6.23 (s, 1 H).] This product was decarboxylated by heating it in quinoline (1 mL) at 180–185 °C under an argon atmosphere for 10 min and pouring the mixture into ice water (25 mL) containing 5 mL of hydrochloric acid. The precipitated 5,7-dihydroxy-4′-nitroflavone (**16d**) was filtered, washed with water, and dried. Recrystallization from acetone gave an analytical sample (0.15 g, 50%): mp 304–305 °C; IR (KBr) 3380, 1660, 1610, 1590, 1510, 1420, 1335, 1255, 1175, 1150, 1100, 1080, 1010 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 12.67 (bs, 1 H, exchanges with D_2O), 11.03 (bs, 1 H, exchanges with D_2O), 8.36 (d, J = 8.0 Hz, 2 H), 8.34 (d, J = 8.0 Hz, 2 H), 7.18 (s, 1 H), 6.55 (d, J = 1.7 Hz, 1 H), 6.25 (d, J = 1.7 Hz, 1 H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 181.46, 164.61, 161.30, 160.40, 157.25, 148.91, 136.45, 127.63, 123.91, 107.48, 104.04, 99.14, 94.14; EIMS (isobutane) m/e 299 (M^+, 100). Anal. ($C_{15}H_9NO_6$) C, H.

4′-Nitroflavone (16a). A mixture of 3-(methoxycarbonyl)-2-(4-nitrophenyl)-4*H*-benzopyran-4-one (**6a**, 0.65 g, 2 mmol), pyridine hydrochloride (10 g), and quinoline (5 mL) was heated at 180–185 °C under nitrogen atmosphere for 6 h and poured into a mixture of ice (100 g) and hydrochloric acid (20 mL). The precipitated product was filtered, washed with water, and dried. Recrystallization from acetic acid–water afforded pure product (0.385 g, 72%); mp 244–245 °C (lit.[40] mp 244–246 °C).

7-Hydroxy-4′-nitroflavone (16b) and 6-hydroxy-4′-nitroflavone (16c) were prepared in an analogous way.

Product **16b**: 0.37 g; 65%; mp 308–309 °C (lit.[41] mp 308–310 °C); ^1H NMR (DMSO-d_6, 500 MHz) δ 11.05 (bs, 1 H, exchanges with D_2O), 8.35 (AB q, J = 8.1 Hz, 4 H), 7.92 (d, J = 8.66 Hz, 1 H), 7.11 (s, 1 H), 7.03 (d, J = 1.95 Hz, 1 H), 6.95 (dd, 1 H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 176.09, 162.99, 159.34, 157.36, 148.70, 137.15, 127.41, 126.52, 123.90, 116.04, 115.34, 108.85, 102.56; EIMS m/e 283 (M^+, 100), 255 (55), 225 (15), 224 (11), 209 (22), 208 (17), 181 (11), 152 (11), 136 (18), 108 (20).

Product **16c**: 0.33 g; 58%; mp 318–320 °C; IR (KBr) 3420–3400, 1655, 1605, 1580, 1520, 1485, 1350, 1290, 1200, 1060, 1010 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 13.55 (bs, 1 H, exchanges with D_2O), 8.34 (AB q, J = 7.9 Hz, 4 H), 7.50 (d, J = 9.15 Hz, 1 H), 7.07 (m, 2 H), 7.04 (s, 1 H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 176.19, 159.62, 155.02, 149.27, 148.82, 137.26, 127.54, 124.15, 123.97, 123.43, 119.94, 107.99, 107.35; EIMS m/e 283 (M^+, 22), 136 (14). Anal. ($C_{15}H_9NO_5$) C, H.

4′-Aminoflavones 17a–d. A solution of 4′-nitroflavone **16a–d** (1 mmol) in dioxane (20 mL) was hydrogenated at 40 psi for 8 h in the presence of 5% palladium on charcoal and the catalyst was removed by filtration. Solvent was evaporated from the filtrate at room temperature and the residue was crystallized from methanol–water to afford the desired compound.

4′-Aminoflavone (17a): 0.20 g; 84%; mp 233–235 °C (lit.[40] mp 233–235 °C).

4′-Amino-7-hydroxyflavone (17b): 0.22 g; 87%; mp 342–343 °C (lit.[41] mp 338–340 °C); ^1H NMR (DMSO-d_6, 500 MHz) δ 9.94 (bs, 1 H, exchanges with D_2O), 7.84 (d, J = 8.8 Hz, 1 H), 7.65 (d, J = 8.8 Hz, 2 H), 6.85 (d, J = 2.4 Hz, 1 H), 6.81 (dd, 1 H), 6.63 (d, J = 8.8 Hz, 2 H), 6.49 (s, 1 H), 4.93 (bs, 2 H, NH_2, exchanges with D_2O); EIMS m/e 253 (M^+, 16), 224 (17), 208 (18), 195 (14), 117 (11).

4′-Amino-6-hydroxyflavone (17c): 0.23 g; 91%; mp 193–194 °C; IR (KBr) 3320, 3200, 1625, 1590, 1580, 1525, 1465, 1350, 1250, 1180, 1120, 1080, 1060, 1030 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 9.94 (bs, 1 H, exchanges with D_2O), 7.86 (d, J = 8.5 Hz, 2 H), 7.59 (d, J = 8.9 Hz, 1 H), 7.29 (d, J = 2.4 Hz, 1 H), 7.20 (m, 1 H), 6.88 (d, J = 8.5 Hz, 2 H), 6.72 (s, 1 H), 4.93 (bs, 2 H, NH_2, exchanges with D_2O); EIMS m/e 253 (M^+, 90). Anal. ($C_{15}H_{11}NO_3$) C, H.

4′-Amino-5,7-dihydroxyflavone (17d): 0.245 g; 91%; mp 344–346 °C; IR (KBr) 3480, 3390, 3220, 1650, 1610, 1585, 1565, 1500, 1445, 1370, 1245, 1185, 1170, 1115, 1025 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 13.13 (bs, 1 H, exchanges with D_2O), 10.73 (bs, 1 H, exchanges with D_2O), 7.76 (d, J = 8.7 Hz, 2 H), 6.66 (d, J = 8.7 Hz, 2 H), 6.62 (s, 1 H), 6.43 (d, J = 2.1 Hz, 1 H), 6.15 (d, J = 2.1 Hz, 1 H), 6.08 (bs, 2 H, NH_2, exchanges with D_2O); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 181.33, 164.42, 163.71, 161.30, 157.04, 152.79, 128.04, 116.36, 113.35, 103.45, 100.61, 98.54, 93.72; EIMS m/e 269 (M^+, 64), 241 (5), 120 (4). Anal. ($C_{15}H_{11}NO_4$) C, H.

Protein-Tyrosine Kinase Assays. In vitro assays of protein-tyrosine kinase activity were carried out using angiotensin

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I (1.2 mM) and [γ-^{32}P]ATP (50 μM) as described previously for the routine assay of the p40 protein-tyrosine kinase[42] 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. p56^{lck} was partially purified from bovine thymus by sequential chromatography on columns of DEAE-cellulose and heparin-agarose exactly as described for the purification of p40.[42] The first peak of tyrosine kinase activity to elute from the heparin-agarose column, which contains p56^{lck}, was further fractionated by chromatography on butyl-agarose.[42]

The catalytic subunit of cAMP-dependent protein kinase was isolated from bovine heart by method I of Bechtel et al.[43] Kinase activity was measured in 25-μL reactions containing 0.5 mM Leu-Arg-Ala-Ser-Leu-Gly (Kemptide, Sigma Chemical Co.), 180 mM Hepes, pH 7.4, 10 mM $MgCl_2$, 50 μM [γ-^{32}P]ATP, 5 mM *p*-nitrophenyl phosphate, and 8% DMSO. Protein kinase C was kindly provided by Dr. Curtis Ashendel, Purdue University. Activity was measured in 125-μL reactions containing 0.24 mg/mL phosphatidylserine, 1 nM 12-*O*-tetradecanoylphorbol-13-acetate, 4% glycerol, 20 μM [γ-^{32}P]ATP, 5 mM *p*-nitrophenyl phosphate, and 1.6% DMSO.

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Dihydropyrimidine Calcium Channel Blockers. 3.[1] 3-Carbamoyl-4-aryl-1,2,3,4-tetrahydro-6-methyl-5-pyrimidinecarboxylic Acid Esters as Orally Effective Antihypertensive Agents

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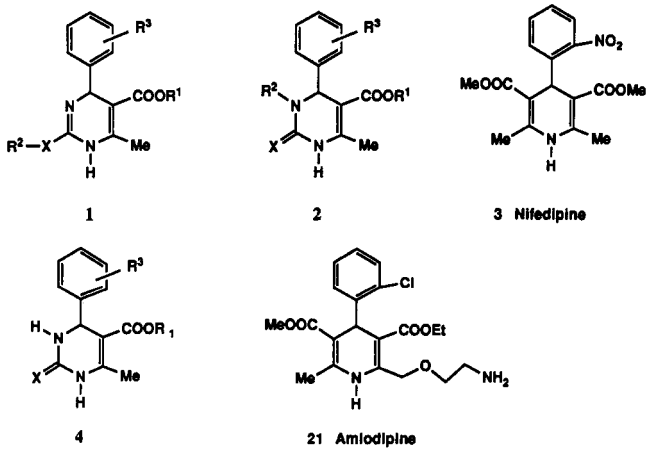
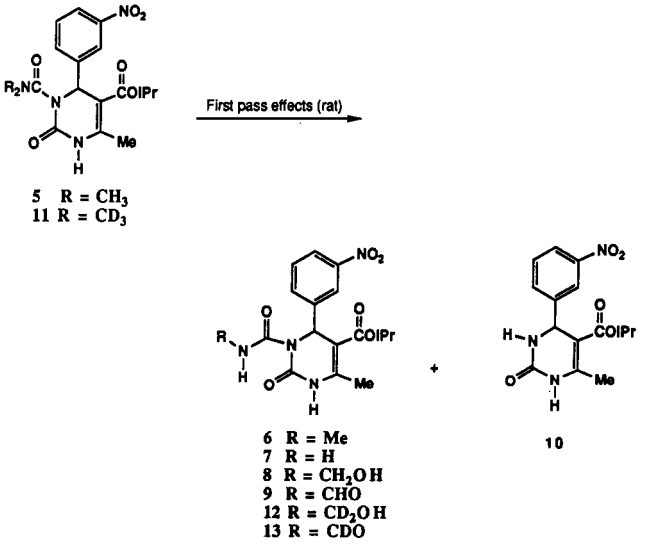
In order to explain the potent antihypertensive activity of the modestly active (IC_{50} = 3.2 μM) dihydropyrimidine calcium channel blocker **5**, we carried out drug metabolism studies in the rat and found **5** is metabolized to compounds **6–10**. Two of the metabolites, **6** (IC_{50} = 16 nM) and **7** (IC_{50} = 12 nM), were found to be responsible for the antihypertensive activity of compound **5**. Potential metabolism of **6** into **7** in vivo precluded our interest in pursuing compounds related to **6**. Structure–activity studies aimed at identifying additional aryl-substituted analogues of **7** led to **17g,j,p** with comparable potential in vivo, though these compounds were less potent than **7** in vitro. To investigate the effects of absolute stereochemistry on potency, we resolved **7** via diastereomeric ureas **19a,b**, prepared from **18** by treatment with (*R*)-α-methylbenzylamine. Our results demonstrate that the active *R*-(−)-enantiomer **20a** of **7** is both more potent and longer acting than nifedipine (**1**) as an antihypertensive agent in the SHR. The in vivo potency and duration of **20a** is comparable to the long-acting dihydropyridine amlodipine. The superior oral antihypertensive activity of **20a** compared to that of previously described carbamates **2** (R^2 = COOEt) could be explained by its improved oral bioavailability, possibly resulting from increased stability of the urea functionality.

Introduction

In previous papers we described the synthesis and structure–activity studies with 1,4-dihydropyrimidines such as **1** and **2**.[1,2] Similar to the structurally analogous dihydropyridines **3**, the dihydropyrimidines possess potent calcium channel blocking activity. However, throughout our early investigations with the dihydropyrimidines, we observed that the potency in vitro was not accompanied by antihypertensive activity when the compounds were orally administered to spontaneously hypertensive rats



Scheme I



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