Synthesis and Biochemical Evaluation of a Series of Aminoflavones as Potential Inhibitors of Protein-Tyrosine Kinases p56^{lck}, EGFr, and p60$^{v\text{-}src}$

Mark Cushman,*,† Helen Zhu,† Robert L. Geahlen,† and Alan J. Kraker‡

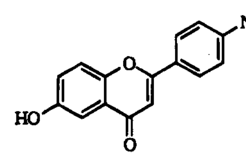
Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907, and Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105

Received May 19, 1994®

A series of nitroflavones, **8a–p**, and their corresponding aminoflavone hydrochloride salts, **10a–p**, was synthesized. The preparation of nitroflavones **8b–i,o,p** began with commercially available *o*-hydroxyacetophenones **2b–f** which were converted to *o*-hydroxynitroacetophenones **3a–h** *via* a variety of nitration methods, followed by condensation with nitrobenzoyl chlorides and cyclization under acidic condition. The nitroflavones **8a,j–n** were prepared by nitration of the corresponding flavones **7a–e**. These new compounds were evaluated for their abilities to inhibit the *in vitro* protein-tyrosine kinase activities of p56^{lck}, EGFr, and p60$^{v\text{-}src}$, and all of the active compounds were amino-substituted flavones. None of the nitroflavones inhibited the enzymes. The most active substance in this series against p56^{lck} was compound **10j**, which had an IC_{50} of 18 μM. When tested versus EGFr, compounds **10a,m** displayed IC_{50}'s of 8.7 and 7.8 μM, respectively. Against p60$^{v\text{-}src}$, **10a,m** showed IC_{50} values of 28.8 and 38.4 μM, respectively.

Eukaryotic cell proliferation is regulated by signaling pathways that are stimulated by the interactions of extracellular ligands with specific cell surface receptors. Recent elucidation of the elements and biochemical mechanisms involved in these signal transduction systems has provided medicinal chemists with innovative strategies for the rational design of new compounds to serve as potential chemotherapeutic agents for the treatment of cancer and immune dysfunction and as molecular probes for deciphering the intricate processes involved in signal transduction.[1,2] Protein-tyrosine kinases (PTK's), which catalyze the transfer of the terminal phosphate of ATP to tyrosine residues on substrate proteins, play key roles in these signal transduction pathways, and in many human malignancies, a specific PTK is activated or overexpressed. Examples include chromosomal translocation of *c-abl* in chronic myelogenous leukemia[3] and Ph^1-positive acute lymphocytic leukemia,[4] amplification of *c-erb-B-2* in human breast cancer,[5] activation of pp60$^{c\text{-}src}$ in colon carcinoma,[6] and overexpression of the epidermal growth factor (EGF) receptor in squamous cell carcinoma.[7,8] This information has aroused a great deal of interest in the development of PTK inhibitors as potential anticancer agents.[9–11]

Prior studies have shown that several naturally occurring flavonoids are inhibitors of protein-tyrosine kinase activity *in vitro*.[12–15] Overall, these flavonoids are competitive inhibitors with respect to ATP and lack selectivity for protein-tyrosine kinases over protein-serine/threonine kinases.[13,14] However, we recently synthesized 4′-amino-6-hydroxyflavone (**1**) and discovered that it is potent and highly selective for the inhibition of the protein-tyrosine kinase p56^{lck} over protein-serine/threonine kinases.[16] Flavone **1** displayed an IC_{50} of 1.2 μM against p56^{lck} and was much less active against protein kinase C and protein kinase A



1

(IC_{50}'s > 300 μM).[17] This raised the question of whether or not aminoflavones in general would be promising PTK inhibitors. In view of the fact that very little has been reported on the preparation and biological properties of aminoflavones, a project was initiated to devise methods for the synthesis of an array of aminoflavones and to evaluate their potencies and specificities as inhibitors of the "nonreceptor" PTK's, p56^{lck} and p60$^{v\text{-}src}$, as well as the "receptor type" PTK, EGFr.

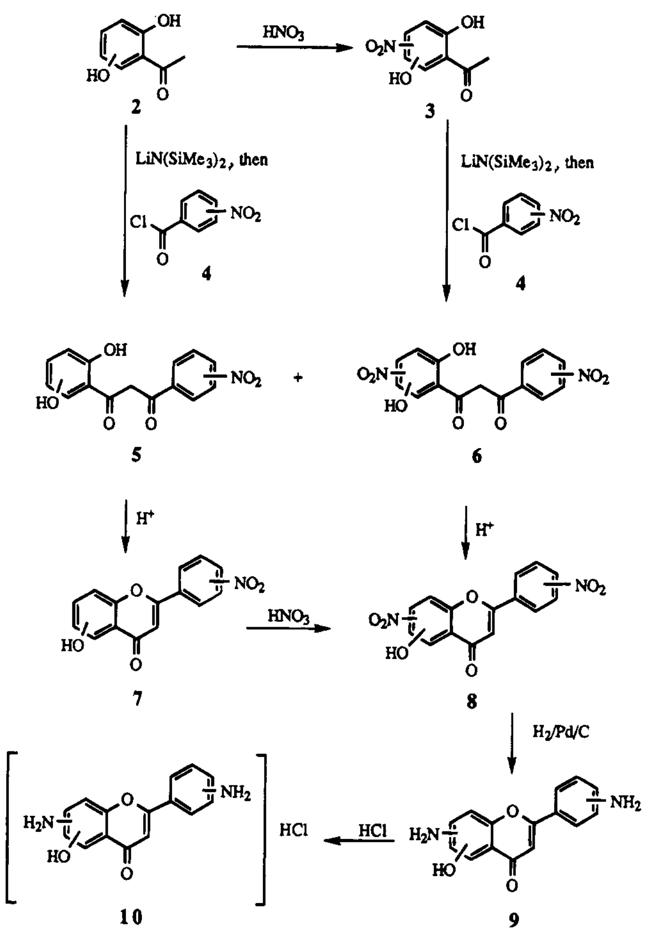
One of the enzymes chosen for study, p56^{lck}, is a lymphoid cell lineage-specific PTK of the *src* family which is overexpressed in several lymphomas.[18–26] In addition, p56^{lck} is associated with both CD4 and CD8 surface glycoproteins in T-lymphocytes, where it exists as a link in the communication of CD4 and CD8 with the T-cell receptor (TCR) ζ chain,[27] and it is also involved as a critical signaling molecule downstream from the interleukin-2 receptor.[28] This evidence indicates that p56^{lck} also plays an important role in immune function.

Two additional enzymes chosen for study were the "receptor type" PTK, EGFr, and the nonreceptor, "*src* type" PTK, p60$^{v\text{-}src}$. The ligand-activated protein-tyrosine phosphorylation of "receptor type" PTK's creates binding sites for enzymes which play critical roles in signal transduction pathways. For example, the binding of EGF or platelet-derived growth factor (PDGF) with their respective receptors results in receptor dimerization and cross-phosphorylation, which creates critical binding sites for proteins that contain SH2 domains, including phosphatidylinositol 3-kinase (PI3K),[1,29–31] phospholipase C-γ (PLC-γ),[32–35] and p21^{ras} GTPase-

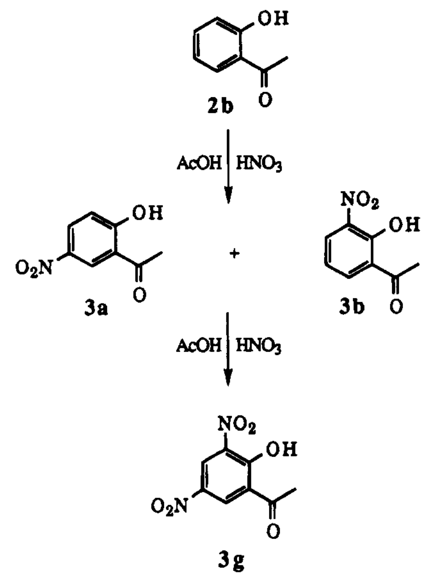
† Purdue University.
‡ Parke-Davis/Warner-Lambert.
® Abstract published in *Advance ACS Abstracts*, September 1, 1994.

0022-2623/94/1837-3353$04.50/0

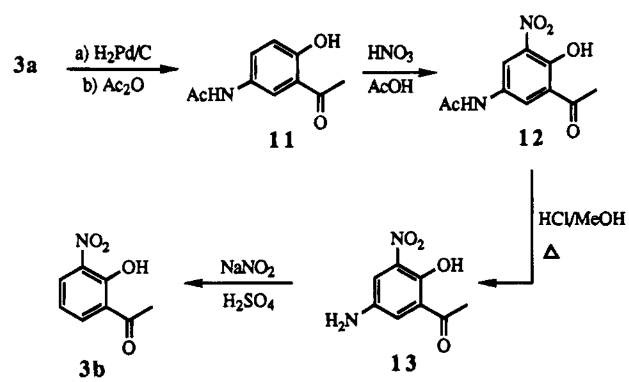
Scheme 1



Scheme 2



Scheme 3



activating protein (GAP).[36,38] Phosphotyrosine-containing sequences in PI3K are also involved in binding to the SH2 domains of cytosolic, nonreceptor, "*src* type" PTK's. For example, the association of PI3K with $p60^{v\text{-}src}$ appears to occur in a reciprocal fashion, in which $p60^{v\text{-}src}$ first phosphorylates the 85 kDa subunit of PI3K and the phosphorylated PI3K then binds to the SH2 domain of $p60^{v\text{-}src}$.[29,39]

Chemistry

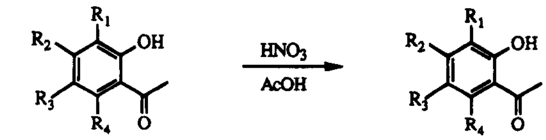
Nitroflavones **8** were prepared from *o*-hydroxyacetophenones **2** and *o*-hydroxynitroacetophenones **3** as shown in Scheme 1. The generation of lithium enolates from the acetyl groups of **2** and **3** was ensured by using 4 equiv of the lithium bis(trimethylsilyl)amide. Treatment of these lithium polyanions with 1 equiv of aroyl chloride **4** afforded the 1,3-diketones **5** and **6** in quantitative yields. The formation of **5** and **6** from the polyanions derived from **2** and **3** and the aroyl chlorides appears to involve direct acylation of the enolate as opposed to *O*-acylation followed by Baker–Venkataraman rearrangement.[40] The 1,3-diketones **5** and **6** cyclized to the corresponding flavones **7** and **8** upon heating in glacial acetic acid containing 0.5% sulfuric acid for 1–1.5 h. The hydroxyflavones **7** were then nitrated by nitric acid to the corresponding nitroflavones **8**. Catalytic hydrogenation of nitroflavones **8** at 40 psi in the presence of 5% palladium on charcoal gave the corresponding aminoflavones **9** which were stablized by conversion to the hydrochloride salts **10**.

Preparation of *o*-Hydroxynitroacetophenones 3. The nitration of **2b** with fuming HNO_3 in acetic acid at 50 °C for 16 h resulted in a mixture of 2-hydroxy-5-nitroacetophenone (**3a**) and 2-hydroxy-3-nitroacetophenone (**3b**), from which **3a** could be isolated by careful fractional crystallization in 36% yield (Scheme 2). The remaing material was then subjected to nitration with fuming nitric acid at 50 °C for 36 h to afford **3g** in 17% yield. Hydrogenation of **3a** gave 5-amino-2-hydroxyacetophenone, which was subsequently treated with acetic anhydride to afford 5-acetamido-2-hydroxyacetophenone, (**11**) (Scheme 3). Nitration of **11** yielded 5-acetamido-2- hydroxy-3-nitroacetophenone (**12**) which was converted to **3b** *via* the diazonium salt derived from **13**.

Friedel–Crafts acetylation of 4-nitroresorcinol may result in a mixture of 2,4-dihydroxy-5-nitroacetophenone (**3c**) and 2,6-dihydroxy-3-nitroacetophenone (**3d**). Therefore, **3c** was obtained by careful nitration of 2,4-dihydroxyacetophenone (**2c**) using a limited reaction time (Scheme 4). In a similar manner, **3d–f** were obtained by nitration of the corresponding hydroxyacetophenones **2d–f**.

2,5-Dihydroxy-3-nitroacetophenone (**3h**) was not obtained from the direct nitration of 2,5-dihydroxyacetophenone (**2a**), since nitration of **2a** gives a mixture of the 4- and 6-nitro compounds, which are difficult to separate. On the other hand, **3h** may be obtained *via* the demethylation of **3e** (Scheme 5). Demethylation using BBr_3 in CH_2Cl_2 or AlI_3 was unsuccessful, and with HBr at 85–90 °C, a mixture of **3h** (70%) and 2,5-dihydroxy-3-nitro-α-bromoacetophenone (**14**; 30%) was

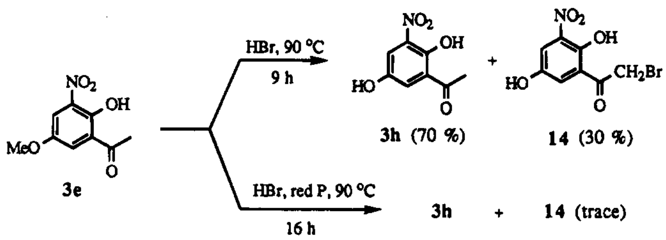
Scheme 4



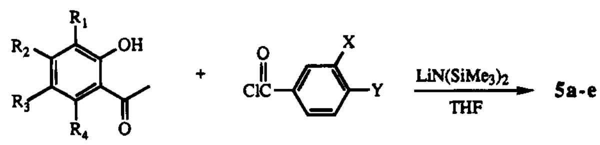
2c-f 3c-f

	R1	R2	R3	R4		R1	R2	R3	R4
2c	H	OH	H	H	3c	H	OH	NO_2	H
2d	H	H	H	OH	3d	NO_2	H	H	OH
2e	H	H	OMe	H	3e	NO_2	H	OMe	H
2f	H	H	H	OMe	3f	NO_2	H	H	OMe

Scheme 5



Scheme 6

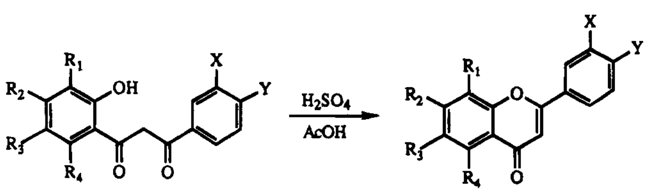


2a-c

4a X = H, Y = NO_2

4b X = NO_2, Y = H

	R1	R2	R3	R4
2a	H	H	OH	H
2b	H	H	H	H
2c	H	OH	H	H



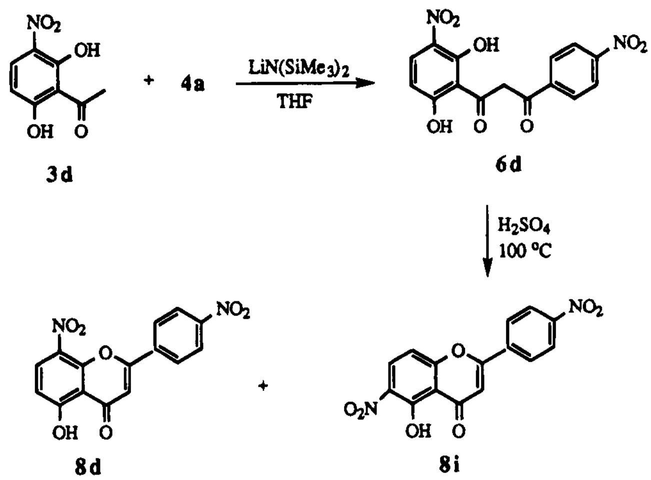
5a-e 7a-e

	R1	R2	R3	R4	X	Y
5a/7a	H	H	OH	H	H	NO_2
5b/7b	H	H	H	H	H	NO_2
5c/7c	H	OH	H	H	H	NO_2
5d/7d	H	H	OH	H	NO_2	H
5e/7e	H	H	H	H	NO_2	H

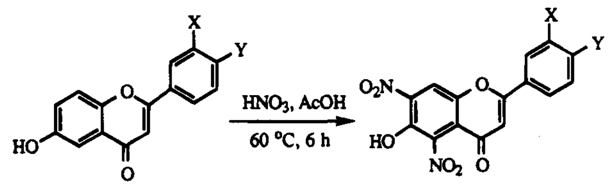
obtained. Fortunately, using HBr and red phosphorus at 85–90 °C for 16 h on **3e** afforded the desired **3h** in high yield (Scheme 5).

Preparation of Flavones 7 and 8. As outlined in Scheme 6, five nitroflavones, **7a–e**, were prepared from *o*-hydroxyacetophenones **2a–c**. Nitroflavones **8b–i,o–p** were prepared using the same methodology as

Scheme 7



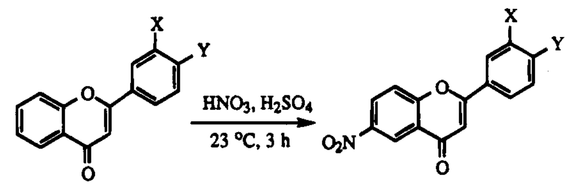
Scheme 8



7a X = H, Y = NO_2 **8j** X = H, Y = NO_2

7d X = NO_2, Y = H **8m** X = NO_2, Y = H

Scheme 9



7b X = H, Y = NO_2 **8a** X = H, Y = NO_2

7e X = NO_2, Y = H **8n** X = NO_2, Y = H

described in Schemes 1 and 6. The conversion of 2,6-dihydroxy-3-nitroacetophenone (**3d**) resulted in 5-hydroxy-4′,8-dinitroflavone (**8d**) (Scheme 7) and 5-hydroxy-4′,6-dinitroflavone (**8i**) in a 1:3 ratio. This ratio may reflect the decreased reactivity of the phenol in **6d** which is hydrogen bonded to the nitro group.[41,42] Compound **8i** is also expected to be stabilized relative to **8d** by hydrogen bonding of the phenol to the nitro group.[41,42] The products **8d,i** were isolated by thin layer chromatography.

Nitration of hydroxyflavones normally proceeds under mild conditions, such as in the conversion of 6-hydroxy-4′-nitroflavone (**7a**) and 6-hydroxy-3′-nitroflavone (**7d**) to 6-hydroxy-4′,5,7-trinitroflavone (**8j**) and 6-hydroxy-3′,5,7-trinitroflavone (**8m**) with HNO_3 in acetic acid at 60 °C (Scheme 8).[43] Nitration of 4′-nitroflavone (**7b**) and 3′-nitroflavone (**7e**) (Scheme 9) using HNO_3 and H_2SO_4 at room temperature afforded the corresponding 4′,6-dinitroflavone (**8a**) and 3′,6-dinitroflavone (**8n**). Nitration of 7-hydroxy-4′-nitroflavone (**7c**) with nitric acid in acetic acid or in pure nitric acid resulted in incomplete conversion to the products at 60 °C. The same procedure worked at 100 °C and provided 7-hydroxy-4′,6,8-

Scheme 10

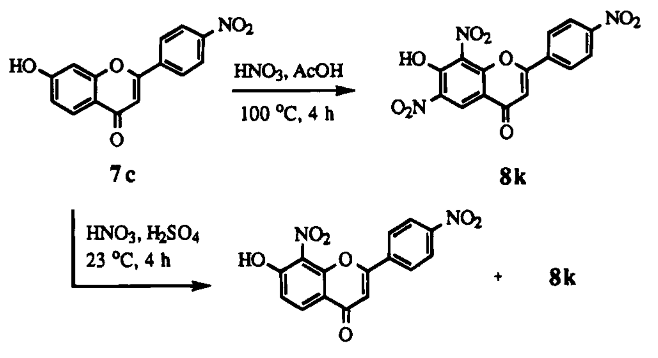
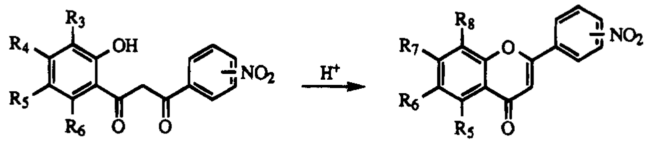


Table 1



flavone	solvent (mL)^a	T (°C)	time (h)
7a	60	95–100	1
7b	70	95–100	1
7c	60	95–100	1
7d	60	95–100	1
7e	80	95–100	1
8b	200	120–125	2
8c	210	120–125	1
8d	60	95–100	1
8e	100	110–115	1.5
8f	100	115–120	1
8g	100	105–110	1
8h	90	105–110	1.5
8o	150	120–125	1.5
8p	160	115–120	1.5

^{a} The diketones (2 g) were dissolved in glacial acetic acid containing 0.5% sulfuric acid.

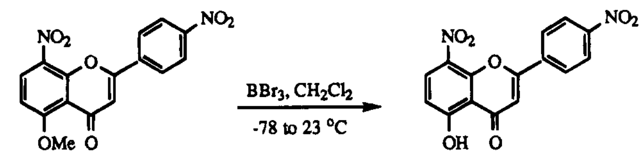
trinitroflavone (**8k**) in 68% yield (Scheme 10). A modified method involved treatment of **7c** with HNO_3 in H_2SO_4 at room temperature and yielded a mixture of **8k** and 7-hydroxy-4′,8-dinitroflavone (**8l**) in a 1:1 ratio as evidenced by ^1H NMR.

As Table 1 indicates, in general, cyclization of 1,3-diaryl β-diketones **6** having electron-withdrawing nitro groups on the phenolic rings requires higher temperatures than were employed during the cyclization of the hydroxy diketones without nitro groups.

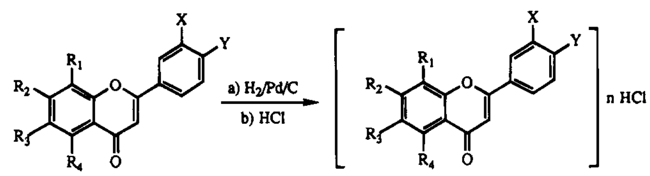
Our attempts to demethylate the methoxyl groups in 6-methoxy-4′,8-dinitroflavone (**8e**) and 5-methoxy-4′,8-dinitroflavone (**8f**) by heating with HBr in acetic acid[44] or HI in acetic anhydride[45] gave only incomplete deprotection or decomposition products. Fortunately, demethylation of **8f** using BBr_3 in CH_2Cl_2 for 3 h afforded the desired 5-hydroxy-4′,8-dinitroflavone (**8d**) in quantitative yield (Scheme 11). However, this method was unsuccessful for removing the methyl group from **8e** in the same manner.

Nitroflavones **8** were catalytically reduced in the presence of 5% Pd/C and hydrogen to afford the corresponding aminoflavones **9**. In general, 4′-aminoflavones **9a–l** were more unstable than 3′-aminoflavones **9m–p**. Compound **9j** was the most unstable and quickly turned to black tar when it was separated from

Scheme 11



Scheme 12



	R_1	R_2	R_3	R_4
8a	H	H	NO_2	H
8b	NO_2	H	H	H
8c	H	OH	NO_2	H
8d	NO_2	H	H	OH
8e	NO_2	H	OMe	H
8f	NO_2	H	H	OMe
8g	NO_2	H	NO_2	H
8h	NO_2	H	OH	H
8i	H	H	NO_2	OH
8j	H	NO_2	OH	NO_2
8k	NO_2	OH	NO_2	H
8l	NO_2	OH	H	H
8m	H	NO_2	OH	NO_2
8n	H	H	NO_2	H
8o	H	OH	NO_2	H
8p	NO_2	H	OMe	H

8a-l: X = H, Y = NO_2; **8m-p**: X = NO_2, Y = H.

	R_1	R_2	R_3	R_4
10a	H	H	NH_2	H
10b	NH_2	H	H	H
10c	H	OH	NH_2	H
10d	NH_2	H	H	OH
10e	NH_2	H	OMe	H
10f	NH_2	H	H	OMe
10g	NH_2	H	NH_2	H
10h	NH_2	H	OH	H
10i	H	H	NH_2	OH
10j	H	NH_2	OH	NH_2
10k	NH_2	OH	NH_2	H
10l	NH_2	OH	H	H
10m	H	NH_2	OH	NH_2
10n	H	H	NH_2	H
10o	H	OH	NH_2	H
10p	NH_2	H	OMe	H

10a-l: X = H, Y = NH_2; **10m-p**: X = NH_2, Y = H.

the solvent. The aminoflavones **9** were stabilized by subsequent conversion to their hydrochloride salts, **10**.

Biological Results and Discussion

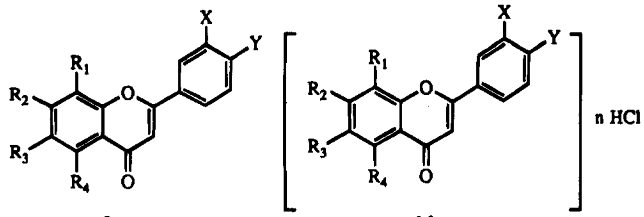
Flavones **8a–p** and **10a–p** (Scheme 12) were evaluated for their abilities to inhibit the *in vitro* protein-tyrosine kinase activities of p56^{lck}, EGFr, and p60$^{v\text{-}src}$. The results of these studies are listed in Table 2.

It is clear that the PTK inhibitory activity associated with the lead compound **1** is not structurally specific. A variety of aminoflavones prepared in the present study display similar activities although there is a high degree of variability in their potencies.

Without exception, the replacement of the amino groups of **10a–p** with nitro groups abolishes the enzyme inhibitory activity. The amino groups therefore evidently play a critical role in allowing recognition by the enzyme.

The relocation of the 4′-amino group to the 3′ position usually resulted in a decrease in activity against p56^{lck}. Examples include the 4′-aminoflavone **10j** (IC_{50} 18 μM) versus the 3′-aminoflavone **10m** (IC_{50} 46 μM), the 4′-aminoflavone **10a** (IC_{50} 103 μM) versus the 3′-aminoflavone **10n** (IC_{50} 200 μM), and the 4′-aminoflavone **10c** (IC_{50} 141 μM) versus the 3′-aminoflavone **10o** (IC_{50} 754 μM). The exception to this general trend was the 4-aminoflavone **10e** (IC_{50} 382 μM) versus the 3′-aminoflavone **10p** (IC_{50} 56 μM).

Table 2. Inhibition of Protein-Tyrosine Kinase Activity of $p56^{lck}$, EGFr, and $p60^{v\text{-}STC}$ by Flavone Derivatives



8 10

flavone	R_1	R_2	R_3	R_4	X	Y	IC_{50} (μM) $p56^{lck}$	EGFr	$p60^{v\text{-}scr}$
8a	H	H	NO_2	H	H	NO_2	>2000	ND	ND
8b	NO_2	H	H	H	H	NO_2	>2000	ND	ND
8c	H	OH	NO_2	H	H	NO_2	>2000	ND	ND
8d	NO_2	H	H	OH	H	NO_2	>2000	ND	ND
8e	NO_2	H	OMe	H	H	NO_2	>2000	ND	ND
8f	NO_2	H	H	OMe	H	NO_2	>2000	ND	ND
8g	NO_2	H	NO_2	H	H	NO_2	>2000	ND	ND
8h	NO_2	H	OH	H	H	NO_2	>2000	ND	ND
8i	H	H	NO_2	OH	H	NO_2	>2000	ND	ND
8j	H	NO_2	OH	NO_2	H	NO_2	>2000	ND	ND
8k	NO_2	OH	NO_2	H	H	NO_2	1565	ND	ND
8l	NO_2	OH	H	H	H	NO_2	1876	ND	ND
10a	H	H	NH_2	H	H	NH_2	103	8.7	28.8
10b	NH_2	H	H	H	H	NH_2	123	>100	>50
10c	H	OH	NH_2	H	H	NH_2	141	14.1	>50
10d	NH_2	H	H	OH	H	NH_2	326	>100	>50
10e	NH_2	H	OMe	H	H	NH_2	382	>100	>50
10f	NH_2	H	H	OMe	H	NH_2	1621	>100	>50
10g	NH_2	H	NH_2	H	H	NH_2	106	>100	>50
10h	NH_2	H	OH	H	H	NH_2	117	>100	>50
10i	H	H	NH_2	OH	H	NH_2	223	>100	>50
10j	H	NH_2	OH	NH_2	H	NH_2	18	>100	>50
10k	NH_2	OH	NH_2	H	H	NH_2	754	>100	>50
10l	NH_2	OH	H	H	H	NH_2	328	>100	>50
10m	H	NH_2	OH	NH_2	NH_2	H	46	7.8	38.4
10n	H	H	NH_2	H	NH_2	H	200	>100	>50
10o	H	OH	NH_2	H	NH32	H	502	>100	>50
10p	NH_2	H	OMe	H	NH_2	H	56	>100	>50

In the two cases investigated, methylation of phenolic hydroxyl groups resulted in a decrease in activity versus $p56^{lck}$. Methylation of the 5-hydroxyl group of **10d** (IC_{50} 326 μM) resulted in the less active methyl ether **10f** (IC_{50} 1621 μM), and methylation of the phenolic hydroxyl group of **10h** (IC_{50} 117 μM) resulted in the less active compound **10e** (IC_{50} 382 μM).

The most active compound in the present series against $p56^{lck}$ proved to be **10j**, which displayed an IC_{50} of 18 μM. The clockwise rotation of the substituents in the 5–7 positions of **10j** to the 6–8 positions resulted in the much less active compound **10k** (IC_{50} 754 μM). Flavone **10m** was the second most potent against $p56^{lck}$, emphasizing the importance of the 5,7-diamino 6-hydroxy substitution patterns.

When tested versus EGFr, compounds **10a,m** displayed IC_{50}'s of 8.7 and 7.8 μM, respectively. Against $p60^{v\text{-}src}$, **10a,m** showed IC_{50} values of 28.8 and 38.4 μM, respectively. The most active inhibitor of $p56^{lck}$ in the present series, compound **10j**, proved to be inactive against EGFr and $p60^{v\text{-}src}$ in the concentration ranges studied. Although the flavones inhibit PTK's by binding to their ATP-binding sites as opposed to their substrate-binding sites, the data in Table 2 nevertheless demonstrate a certain degree of selective enzyme inhibition by certain aminoflavones within the PTK's as a class. In comparing the data in Table 2, it is important to note that the IC_{50} values for $p56^{lck}$ were determined in the presence of 50 μM ATP, while those for EGFr and $p60^{v\text{-}src}$ were determined in the presence of 5 μM ATP. This makes the $p56^{lck}$ IC_{50} values appear higher by a factor of 10 relative to those of the other two enzymes.

The results indicate that certain aminoflavones possess inhibitory activity against $p56^{lck}$, EGFr, and $p60^{v\text{-}src}$. It is not obvious, however, if the amino substituents offer any overall advantage over hydroxyl groups. Both are approximately the same size and can function as hydrogen bond donors as well a hydrogen bond acceptors. It may be noted in this regard that the replacement of the 6-amino of **10g** (IC_{50} 106 μM versus $p56^{lck}$) by a hydroxyl group afforded **10h** (IC_{50} 117 μM versus $p56^{lck}$), which was essentially equipotent.

Experimental Section

The 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-50 spectrometer, ^1H NMR spectra on Chemagnetics A-200 and Varian VXR-500S spectrometers with TMS as an internal standard in $CDCl_3$, CD_3COCD_3-d_6, or DMSO-d_6, IR spectra on a Beckman IR-33 spectrophotometer. Microanalyses were performed at the Purdue Microanalysis Laboratory. All organic solvents were appropriately dried and purified prior to use. Organic reagents were purchased from commercial sources and used without further purification unless stated otherwise.

2-Hydroxy-5-nitroacetophenone (3a) and 2-Hydroxy-3,5-dinitroacetophenone (3g). Nitric acid (fuming, 4 mL) was added to a solution of 2-hydroxyacetophenone (**2b**; 8.17 g, 60 mmol) in glacial acetic acid (70 mL), and the mixture was stirred in a round-bottomed flask equipped with a drying tube ($CaCl_2$) at room temperature for 40 min and then at 45–50 °C for 16 h. Acetic acid was removed at reduced pressure, and water (100 mL) was added. The yellow cloudy solution was neutralized by Na_2CO_3 to pH 6, and then the yellow precipitate was collected and carefully recrystallized from EtOH in several crops to give **3a**, 3.6 g, 33% yield: mp 98–99 °C (lit.[46] mp 99.5 °C); IR (KBr) 3085, 1649, 1579, 1520, 1473, 1432, 1338, 1297, 1250, 1214, 1109, cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 12.9 (s, 1 OH), 8.72 (d, J = 2.7 Hz, 1 H), 8.36 (dd, J = 8.1, 2.7 Hz, 1H), 7.05 (d, J = 8.1 Hz, 1 H), 2.76 (s, 3H).

To the combined remaining crops in glacial acetic acid (60 mL), was added nitric acid (fuming, 2 mL) and the mixture stirred in a round-bottomed flask equipped with a drying tube ($CaCl_2$) at 45–50 °C. After 18 h, more nitric acid (fuming, 2 mL) was added to the reaction and the reaction mixture was stirred for an additional 18 h at 45–50 °C. Acetic acid was removed at reduced pressure, and water (100 mL) was added. The yellow cloudy solution was neutralized by Na_2CO_3 to pH 6, and then the yellow precipitated solid was collected and recrystallized from EtOH to afford **3g**, 2.4 g, 17% yield: mp 123–124 °C (lit.[47] mp 123–124 °C); IR (KBr) 3096, 1655, 1602, 1538, 1461, 1340, 1255, 1185, 1090, cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 14.8 (s, 1 OH), 9.06 (d, J = 2.7 Hz, 1 H), 8.94 (d, J = 2.7 Hz, 1 H), 2.85 (s, 3 H).

5-Acetamido-2-hydroxyacetophenone (11). A solution of **3a** (2.54 g, 14 mmol) in THF (80 mL) was hydrogenated at 40 psi for 2 h in the presence of 5% palladium on charcoal (140 mg). The catalyst was removed by filtration, and acetic anhydride (3 mL) was added and heated at 60 °C for 20 min. Solvent was evaporated at reduced pressure to afford **11**, 2.46 g, 91%: mp 167–168 °C (lit.[48] mp 165 °C); IR (KBr) 3249, 3061, 1655, 1643, 1561, 1485, 1414, 1367, 1291, 1250, 1208, 1015 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 13.6 (s, 1 OH), 8.18 (d, J = 2.5 Hz, 1 H), 7.34 (dd, J = 9, 2.5 Hz, 1 H), 7.20 (br, 1 NH), 6.94 (d, J = 9 Hz, 1 H), 2.64 (s, 3 H), 2.19 (s, 3 H).

5-Acetamido-2-hydroxy-3-nitroacetophenone (12). Nitric acid (d = 1.42, 0.6 mL) in glacial acetic acid (2 mL) was added to a solution of **11** (1.20 g, 6.2 mmol) in glacial acetic acid (14 mL) with stirring at room temperature for 2 h. The solvent was removed at reduced pressure, and the residue was poured into ice−water. The precipitated product was filtered, washed with water, and dried to give **12**, 1.15 g, 71%: mp 174–175 °C (lit.[49] mp 170–171 °C); IR (KBr) 3343, 3096, 3061, 1690, 1655, 1538, 1455, 1367, 1320, 1255, 1103, 1026 cm^{-1}; ^1H NMR [$(CD_3)_2CO$, 200 MHz] δ 13.8 (s, 1 OH), 8.41 (d, J = 3.1 Hz, 1 H), 8.25 (d, J = 3.1 Hz, 1 H), 7.50 (s, 1 NH), 2.61 (s, 6 H).

5-Amino-2-hydroxy-3-nitroacetophenone (13). A mixture of 5-acetamido-2-hydroxy-3-nitroacetophenone (**12**; 1.13 g, 4.74 mmol) in MeOH (12 mL) and H_2O (5 mL) was stirred at 85 °C for 1 h and carefully basified with aqueous $NaHCO_3$. The solution was extracted with ether, and the solvent was removed at reduced pressure to afford **13**, 0.892 g, 96%: mp 129–130 °C (lit.[49] mp 141–142 °C); IR (KBr) 3472, 3378, 3266, 3072, 1649, 1625, 1528, 1355, 1273, 1179, 1090 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 12.6 (s, 1 OH), 7.48 (m, 2 H), 2.65 (s, 3 H), 2.51 (s, 2 NH).

2-Hydroxy-3-nitroacetophenone (3b). $NaNO_2$ (1.0 g) in H_2O (2 mL) was added to a solution of **13** (0.785 g, 4 mmol) in EtOH (14 mL) and H_2SO_4 (0.7 mL) at 0–5 °C (ice−NaCl bath), and the mixture was stirred for 20 min. The mixture was heated at 85 °C for 20 min and then stirred at room temperature for another 1 h. The mixture was poured into water, and the solid was collected to give **3b**, 0.652 g, 90% yield: mp 82–83 °C (recrystallization from EtOH/H_2O) (lit.[46] mp 82–83 °C); IR (KBr) 3085, 1643, 1585, 1520, 1350, 1285, 1250, 1190, 1103 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 13.2 (s, 1 OH), 8.20 (dd, J = 8.1, 1.7 Hz, 1 H), 8.06 (dd, J = 8.1, 1.7 Hz, 1 H), 7.05 (t, J = 8.1 Hz, 1 H), 2.73 (s, 3 H).

2,4-Dihydroxy-5-nitroacetophenone (3c). Nitric acid (d = 1.42, 1.5 mL) in glacial acetic acid (2 mL) was added to a solution of 2,4-dihydroxyacetophenone (**2c**; 2.43 g, 16 mmol) in glacial acetic acid (13 mL) with stirring at room temperature. The temperature was allowed to rise slowly to 30 °C, and the flask was cooled when the reaction became too vigorous. The mixture first turned to a red-orange solution, and then a yellow flocculate precipitate separated out. The reaction mixture was allowed to proceed for another 2 h at room temperature and then poured onto ice (80 g). The precipitated product was filtered, washed with water, and dried to give **3c**, 1.39 g, 44%: mp 145–147 °C (recrystallization from acetic acid) (lit.[50] mp 142 °C); IR (KBr) 3378, 3072, 1665, 1590, 1520, 1444, 1350, 1297, 1250, 1202, 1173 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 12.90 (s, 1 OH), 11.05 (s, 1 OH), 8.64 (s, 1 H), 6.58 (s, 1 H), 2.75 (s, 3 H).

2,6-Dihydroxy-3-nitroacetophenone (3d). Nitric acid (d = 1.42, 1.2 mL) in glacial acetic acid (2 mL) was slowly added to a solution of 2,6-dihydroxyacetophenone (**2d**; 2.43 g, 16 mmol) in glacial acetic acid (13 mL) with stirring on an ice−water bath. Soon the reaction mixture turned to a dark-red solution and was stirred for an additional 40 min at room temperature. The mixture was poured into ice−water (80 g) to afford semisolid product and left for 1 day, and then the resulting solid was filtered to afford **3d**, 3.0 g, 77%: mp 114–115 °C (lit.[51] mp 119 °C); IR (KBr) 3084, 3002, 2650, 1626, 1596, 1461, 1432, 1373, 1297, 1255, 1173, 1103 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 14.5 (s, 1 OH), 12.9 (s, 1 OH), 8.25 (d, J = 9 Hz, 1 H), 6.50 (d, J = 9.0 Hz, 1 H), 2.75 (s, 3 H).

2-Hydroxy-5-methoxy-3-nitroacetophenone (3e). Nitric acid (d = 1.42, 2.6 mL) in glacial acetic acid (5 mL) was added to a solution of 2-hydroxy-5-methoxyacetophenone (**2e**; 6.65 g, 40 mmol) in glacial acetic acid (50 mL) with stirring at room temperature. The mixture first turned to a dark-red solution, and then a yellow flocculate precipitate separated out. The reaction was allowed to proceed for 2 h at room temperature, and the mixture was then poured onto ice (280 g). The precipitated product was filtered, washed with water, and dried to give **3e**, 5.26 g, 83%: mp 111–112 °C (lit.[45] mp 112 °C); IR (KBr) 3072, 2931, 1649, 1585, 1532, 1461, 1426, 1320, 1255, 1167, 1050 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 13.4 (s, 1 OH), 7.64 (q, J = 3.1 Hz, 2 H), 3.76 (s, 3H), 2.65 (s, 3H).

2-Hydroxy-6-methoxy-3-nitroacetophenone (3f). Nitric acid (fuming, 2 mL) was added to a solution of 2-hydroxy-6-methoxyacetophenone (**2f**; 3.32 g, 20 mmol) in glacial acetic acid (20 mL). The mixture was stirred in a round-bottomed flask equipped with a drying tube ($CaCl_2$) at room temperature for 40 min. The reaction mixture was then stirred at 45–50 °C for 16 h. Acetic acid was removed at reduced pressure, and water (80 mL) was added. The precipitated solid was collected and recrystallized from EtOH to give **3f**, 1.27 g, 30% yield (recrystallization from EtOH): mp 98–100 °C (lit.[50] mp 102–103 °C); IR (KBr) 3084, 1620, 1590, 1514, 1444, 1314, 1250, 1109 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 12.63 (s, 1 OH), 8.19 (d, J = 9.4 Hz, 1 H), 6.85 (d, J = 9.4 Hz, 1 H), 3.99 (s, 3 H), 2.62 (s, 3 H).

2,5-Dihydroxy-3-nitroacetophenone (3h). A mixture of 2-hydroxy-5-methoxy-3-nitroacetophenone (**3f**; 1.55 g, 7.34 mmol) and red P (300 mg) in HBr (50 mL, 48% in H_2O) was stirred at 85–90 °C for 16 h under Ar. The mixture was cooled and extracted with three portions of CH_2Cl_2 and then three portions of ether. The combined organic solution was washed by water, and the solvent was moved at reduce pressure to afford **3h**, 1.3 g, 90% yield: mp 136–138 °C (lit.[43] mp 142 °C); ^1H NMR (DMSO-d_6, 200 MHz) δ 8.31 (q, J = 10.9 Hz, 4 H), 8.08 (d, J = 3.1 Hz, 1 H), 7.74 (d, J = 3.1 Hz, 1 H), 7.32 (s, 1 H), 3.90 (s, 3 H).

Preparation of Hydroxynitroflavones 7a–e and 8b–h,o,p. General Procedure. A solution of lithium bis(trimethylsily)amide in THF (1 M, 40 mL, 30 mmol) was added to a well-stirred solution of 2,5-dihydroxyacetophenone (**2a**; 10 mmol) in THF (50 mL) under Ar at −78 °C. The reaction was allowed to proceed for 2 h, and a solution of 4′-nitrobenzoyl chloride (10 mmol) was added slowly. Stirring was continued at −78 °C for 1 h and then at room temperature for 20 h. The reaction mixture was poured into ice−water containing 5% HCl and extracted with two portions of ether. The combined ether layers were dried with $MgSO_4$. Solvents were evaporated, and the residue was dried under vacuum overnight. The residue was mixed with glacial acetic acid (60 mL) and H_2SO_4 (0.3 mL) and then heated at 95–100 °C under Ar for 1 h. Acetic acid was removed at reduced pressure, and about 100 mL of water was added. The precipitated product was filtered, washed with water, and dried. Recrystallization from acetone after treatment with activated charcoal afforded **7a**. Flavones **7b–e** and **8b–h,o,p** were prepared similarly using the modifications detailed in Table 1.

6-Hydroxy-4′-nitroflavone (7a): 60%; mp 326–328 °C (lit.[16] mp 318–320 °C).

4′-Nitroflavone (7b): 74%; mp 244–245 °C (lit.[52] mp 244–246 °C).

7-Hydroxy-4′-nitroflavone (7c): 63%; mp 306–308 °C (lit.[53] mp 308–310 °C).

6-Hydroxy-3′-nitroflavone (7d): 74%; mp 270 °C dec; IR (KBr) 3346, 3074, 1622, 1523, 1469, 1349, 1224 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 8.81 (t, J = 2 Hz, 1 H), 8.53 (d, J = 8 Hz, 1 H), 8.42 (dd, J = 8.2, 2.2 Hz, 1H), 7.88 (t, J = 8.1 Hz, 1 H), 7.75 (d, J = 8.8 Hz, 1 H), 7.32 (m, 2 H), 7.18 (s, 1 H); EIMS m/e (rel intensity) 283 (M^+, 100). Anal. ($C_{15}H_7N_3O_9$) C, H, N.

3′-Nitroflavone (7e): 78%; mp 200–201 °C; IR (KBr) 3074, 1638, 1523, 1469, 1345, 1376, 1344 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 8.84 (t, J = 2.2 Hz, 1 H), 8.56 (m, 1 H), 8.43 (m, 1 H), 8.07 (m, 1 H), 7.88 (m, 3 H), 7.53 (m, 1 H), 7.27 (s, 1 H); EIMS m/e (rel intensity) 267 (M^+, 100). Anal. ($C_{15}H_7N_3O_9$) C, H, N.

4′,6-Dinitroflavone (8a). Nitric acid (d = 1.42, 1.6 mL) was added to a well-stirred mixture of **7b** (1.34 g, 5 mmol) in concentrated sulfuric acid (14 mL) at room temperature. The reaction was allowed to proceed for 3 h, and the mixture was then poured into ice (100 g). The precipitated product was filtered, washed with water, and dried. Recrystallization from acetone/dioxane afforded **8a**, 1.40 g, 90%: mp 260–261 °C; IR (KBr) 3078, 1654, 1638, 1577, 1521, 1460, 1414, 1343, 1064, 1030 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 8.74 (d, J = 2 Hz, 1 H), 8.65 (q, J = 10, 2 Hz, 1 H), 8.43 (s, 4 H), 8.10 (d, J = 10 Hz, 1 H), 7.42 (s, 1 H); EIMS m/e (rel intensity) 312 (M^+, 100). Anal. ($C_{15}H_8N_2O_6$) C, H, N.

4′,8-Dinitroflavone (8b): 70%; mp 268 °C dec (recrystallization from acetone after treatment with activated charcoal); IR (KBr) 3073, 1667, 1612, 1520, 1467, 1345, 1326 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 8.60 (dd, J = 8.0, 1.8 Hz, 1 H), 8.43 (q, $J = 9.4$ Hz, 5 H), 7.71 (t, $J = 8.0$ Hz, 1 H), 7.47 (s, 1 H); EIMS *m/e* (rel intensity) 312 (M^+, 100). Anal. ($C_{15}H_8N_2O_7$) C, H, N.

7-Hydroxy-4′,6-dinitroflavone (8c): 74%; mp 293–295 °C (recrystallization from acetone); IR (KBr) 3067, 1641, 1615, 1574, 1520, 1349, 1256, 1185, 1051 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 8.47 (s, 1 H), 8.38 (s, 4 H), 7.32 (s, 1 H), 7.23 (s, 1 H); EIMS *m/e* (rel intensity) 328 (M^+, 100). Anal. ($C_{15}H_8N_2O_7$) C, H, N.

5-Hydroxy-4′,8-dinitroflavone (8d). Method I (as described in the preparation of hydroxy-4′-nitroflavones **7a–c**). The cyclization resulted in the mixture of **8d** and 5-hydroxy-4′,6-dinitroflavone (**8i**) (ratio of **8d/8i** = 1:3) which was separated by silica gel thin layer chromatography eluting with CH_2Cl_2 to afford the corresponding **8d**, 7%, and **8i**, 23%. **Method II** (demethylation of 5-methoxy-4′,8-dinitroflavone, **8e**). Boron tribromide (8 mL, 1.0 M in CH_2Cl_2, 8 mmol) was added to a well-stirred mixture of 5-methoxy-4′,8-dinitroflavone (**8e**; 0.685 g, 2 mmol) in CH_2Cl_2 (90 mL) at −78 °C for 0.5 h and the reaction allowed to proceed at room temperature for 3 h. The resulting mixture was poured into cold 5% HCl (80 mL) and stirred for 10 min. The mixture was then extracted with one portion of CH_2Cl_2 (40 mL), one portion of 1:1 THF–ether (40 mL), and one portion of ether (40 mL). The organic layers were collected and dried, and the solvent was removed under reduced pressure to afford **8d** in quantitative yield: mp 318 °C dec; IR (KBr) 3084, 2955, 1651, 1608, 1520, 1473, 1426, 1344, 1314, 1114, 1085, 1003 cm^{-1}; ^1H NMR (DMSO-d_6 200 MHz) δ 8.55 (d, $J = 9.4$ Hz, 1 H), 8.48 (s, 4 H), 7.61 (s, 1 H), 6.95 (d, $J = 9.4$ Hz, 1 H); EIMS *m/e* (rel intensity) 328 (M^+, 100). Anal. ($C_{15}H_8N_2O_7$) C, H, N.

6-Methoxy-4′,8-dinitroflavone (8e): 65%; mp 254–256 °C (recrystallization from acetone after treatment with activated charcoal); IR (KBr) 3073, 1649, 1619, 1532, 1467, 1437, 1344, 1320, 1214, 1044, 1020 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 8.31 (q, $J = 10.9$ Hz, 4 H), 8.08 (d, $J = 3.1$ Hz, 1 H), 7.74 (d, $J = 3.1$ Hz, 1 H), 7.32 (s, 1 H), 3.90 (s, 3 H); EIMS *m/e* (rel intensity) 342 (M^+, 89). Anal. ($C_{16}H_{10}N_2O_7$) C, H, N.

5-Methoxy-4′,8-dinitroflavone (8f): 67%; mp 294–296 °C (recrystallization from a mixture of acetone and 1,4-dioxane); IR (KBr) 3096, 3084, 1655, 1596, 1510, 1473, 1214, 1129, 1097, 1020 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.57 (d, $J = 9.6$ Hz, 1 H), 8.40 (dd, $J = 9.2, 5.6$ Hz, 4 H), 7.30 (s, 1 H), 7.20 (d, $J = 9.6$ Hz, 1 H), 4.03 (s, 3 H); EIMS *m/e* (rel intensity) 342 (M^+, 100). Anal. ($C_{16}H_{10}N_2O_7$) C, H, N.

4′,6,8-Trinitroflavone (8g): 72%; mp 273–275 °C (recrystallization from acetone); IR (KBr) 3085, 1661, 1620, 1538, 1520, 1455, 1338, 1297 cm^{-1}; ^1H NMR (acetone-d_6, 200 MHz) δ 9.28 (d, $J = 2.7$ Hz, 1 H), 9.10 (d, $J = 2.7$ Hz, 1 H), 8.46 (q, $J = 9.1$ Hz, 4 H), 7.58 (s, 1 H); FABMS *m/e* (rel intensity) 358 (MH^+, 38). Anal. ($C_{15}H_7N_3O_8$) C, H, N.

6-Hydroxy-4′,8-dinitroflavone (8h): 66%; mp 272 °C dec (recrystallization from acetone after treatment with activated charcoal); IR (KBr) 3344, 3189, 3118, 1697, 1651, 1625, 1518, 1349 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) δ 8.36 (d, $J = 8.9$ Hz, 2 H), 8.27 (d, $J = 8.9$ Hz, 2 H), 8.00 (d, $J = 3$ Hz, 1 H), 7.81 (d, $J = 3$ Hz, 1 H), 7.16 (s, 1 H); EIMS *m/e* (rel intensity) 328 (M^+, 100). Anal. ($C_{15}H_8N_2O_7$) C, H, N.

5-Hydroxy-4′,6-dinitroflavone (8i). The preparation of this compound was described above under the synthesis of **8d**. **8i**: yield 23%; mp 208 °C dec; IR (KBr) 3089, 1649, 1608, 1420, 1344, 1285, 1220, 1120, 1075, 1015 cm^{-1}, ^1H NMR (DMSO-d_6, 200 MHz) δ 8.44 (d, $J = 9.4$ Hz, 1 H), 8.42 (q, $J = 9.6$ Hz, 4 H), 7.56 (s, 1 H), 7.43 (d, $J = 9.4$ Hz, 1 H); EIMS *m/e* (rel intensity) 328 (M^+, 100). Anal. ($C_{15}H_8N_2O_7$) C, H, N.

6-Hydroxy-4′,5,7-trinitroflavone (8j). Nitric acid (d = 1.42, 1.6 mL) in glacial acetic acid (8 mL) was added to **7a** (0.71 g, 2.5 mmol) in glacial acetic acid (5 mL), and the reaction mixture was stirred at 60 °C (oil bath) for 6 h. Acetic acid was removed at reduced pressure, and the mixture was then poured into water (60 mL). The yellow precipitated product was filtered, washed with water, and dried. Recrystallization from acetone afforded **8j**, 0.63 g, 68%: mp 289–291 °C; IR (KBr) 3343, 3072, 1655, 1643, 1572, 1561, 1520, 1461, 1338, 1185, 1114 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 8.73 (s, 1 H), 8.42 (s, 4 H), 7.32 (s, 1 H); EIMS *m/e* (rel intensity) 373 (M^+, 100). Anal. ($C_{15}H_7N_3O_9$) C, H, N.

7-Hydroxy-4′,6,8-trinitroflavone (8k). Nitric acid (d = 1.42, 3 mL) in glacial acetic acid (5 mL) was added to **7c** (1.133 g, 4.0 mmol) in glacial acetic acid (50 mL), and the reaction mixture was stirred at 100–105 °C (oil bath) for 4 h. Acetic acid was removed at reduced pressure, and the mixture was then poured into water (80 mL). The precipitated product was filtered, washed with water, and dried. Recrystallization from acetic acid afforded **8k**, 1.02 g, 68%: mp 256–258 °C; IR (KBr) 3550–3400, 3083, 1635, 1601, 1443, 1420, 1343, 1096 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 8.31 (d, $J = 9.0$ Hz, 2 H), 8.28 (s, 1 H), 8.13 (d, $J = 9.0$ Hz, 2 H), 7.03 (s, 1 H); EIMS *m/e* (rel intensity) 374 (MH^+, 100). Anal. ($C_{15}H_7N_3O_9$) C, H, N.

7-Hydroxy-4′,8-dinitroflavone (8l). Nitric acid (d = 1.41, 0.8 mL) was added to a well-stirred mixture of **7c** (0.80 g, 2.8 mmol) in concentrated sulfuric acid (7 mL) at room temperature. The reaction was allowed to proceed for 4 h, and then the mixture was poured onto ice (100 g). The precipitated product was filtered, washed with water, and dried to give a mixture of 7-hydroxy-4′,8-dinitroflavone and 7-hydroxy-4′,6,8-trinitroflavone (ratio of **8k/8l** = 1:1). Column chromatography on silica gel (CH_2Cl_2, CH_2Cl_2–THF, 8:2, followed by 10% menthol in CH_2Cl_2) afforded the corresponding **8l**, 0.27 g, 27%: mp 289–291 °C; IR (KBr) 3120, 3070, 1648, 1593, 1543, 1468, 1427, 1377, 1309, 1208, 1179, 1079 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 8.42 (d, $J = 10$ Hz, 2 H), 8.17 (d, $J = 10$ Hz, 2 H), 8.12 (d, $J = 9$ Hz, 1 H), 7.26 (s, 1 H), 7.20 (d, $J = 9$ Hz, 1 H); EIMS *m/e* (rel intensity) 328 (M^+, 44). Anal. ($C_{15}H_8N_2O_7$) C, H, N.

6-Hydroxy-3′,5,7-trinitroflavone (8m). The nitration of **7d** was performed as described in the preparation of **8j** above. **8m**: yield 62%; mp 154–156 °C (recrystallization from MeOH); IR (KBr) 3273, 3061, 1655, 1538, 1455, 1343, 1220, 1102 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 8.94 (t, $J = 2$ Hz, 1 H), 8.88 (s, 1 H), 8.61 (dd, $J = 8.1, 1$ Hz, 1 H), 8.46 (dd, $J = 9.7, 2.2$ Hz, 1 H), 7.92 (t, $J = 8.1$ Hz, 1 H), 7.39 (s, 1 H); EIMS *m/e* (rel intensity) 373 (M^+, 41). Anal. ($C_{15}H_7N_3O_9$) C, H, N.

3′,6-Dinitroflavone (8n). The preparation of **7e** was performed as described in the preparation of **8a** above. **8e**: yield 91%; mp 246–248 °C (recrystallization from THF/EtOH); IR (KBr) 3084, 1667, 1614, 1526, 1455, 1344, 1266, 1138, 1036 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 8.93 (t, $J = 1.9$ Hz, 1 H), 8.72 (dd, $J = 14.5, 2.6$ Hz, 1 H), 8.63 (m, 2 H), 8.47 (ddd, $J = 8.1, 2.2, 0.9$ Hz, 1 H), 8.17 (d, $J = 9.1$ Hz, 1 H), 7.90 (t, $J = 8.1$ Hz, 1 H), 7.46 (s, 1 H); EIMS *m/e* (rel intensity) 312 (M^+, 100). Anal. ($C_{15}H_8N_2O_6$) C, H, N.

7-Hydroxy-3′,6-dinitroflavone (8o): 70%; mp 294–296 °C (recrystallization from acetone/THF); IR (KBr) 3260, 3061, 1673, 1632, 1532, 1455, 1355, 1308, 1179, 1109, 1050 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 8.82 (s, 1 H), 8.54 (d, $J = 7.8$ Hz, 1 H), 8.47 (s, 1 H), 8.42 (d, $J = 7.8$ Hz, 1 H), 7.86 (t, $J = 8$ Hz, 1 h), 7.36 (s, 1 H), 7.25 (s, 1 H); EIMS *m/e* (rel intensity) 328 (M^+, 100). Anal. ($C_{15}H_8N_2O_7$) C, H, N.

6-Methoxy-3′,8-dinitroflavone (8p): 76%; mp 279–281 °C (recrystallization from acetone); IR (KBr) 3072, 1661, 1627, 1532, 1479, 1438, 1338, 1108, 1026 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 8.92 (t, $J = 1.8$ Hz, 1 H), 8.57 (dd, $J = 8, 1.6$ Hz, 1 H), 8.47 (dd, $J = 8.1, 2.4$ Hz, 1 H), 8.20 (d, $J = 3$ Hz, 1 H), 7.92 (d, $J = 8$ Hz, 1 H), 7.82 (d, $J = 3$ Hz, 1 H), 7.48 (s, 1 H), 3.98 (s, 3 H); EIMS *m/e* (rel intensity) 342 (M^+, 100). Anal. ($C_{16}H_{10}N_2O_7$) C, H, N.

Catalytic Hydrogenation of Nitroflavones. A solution of nitroflavones **8** (200 mg) in THF (100 mL) was hydrogenated at 40 psi for 20 h in the presence of 5% palladium on charcoal (100 mg). The catalyst was removed by filtration to afford aminoflavones **9**, and then HCl (gas) was bubbled through the mixture while stirring at room temperature for 10 min. Solvent was evaporated at reduced pressure to afford the corresponding aminoflavone hydrochloride salts **10** in 96–100% yields.

4′,6-Diaminoflavone (9a): mp 232 °C dec; IR (KBr) 3331, 3202, 2932, 1608, 1570, 1508, 1473, 1367, 1303, 1244, 1120 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 7.71 (d, $J = 8.7$ Hz, 2 H), 7.39 (d, $J = 8.8$ Hz, 1 H), 7.05 (d, $J = 2.8$ Hz, 1 H), 6.99 (dd, $J = 8.8, 2.8$ Hz, 1 H), 6.63 (d, $J = 8.7$ Hz, 2 H), 6.55 (s, 1

H), 5.93 (s, 2 NH), 5.40 (s, 2 NH); EIMS *m/e* (rel intensity) 252 (M^+, 100). Anal. ($C_{15}H_{12}N_2O_2$·2HCl) C, H, N.

4',8-Diaminoflavone Hydrochloride (10b): mp 178 °C dec; IR (KBr) 3390, 3049, 2930–2700, 2599, 1637, 1608, 1490, 1432, 1372, 1032 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.04 (d, J = 8.4 Hz, 2 H), 7.36 (br, 1 H), 7.26 (br, 1 H), 7.22 (t, J = 7.6 Hz, 1 H), 6.90 (d, J = 8.4 Hz, 2 H), 6.66 (s, 1 H), 1.34 (s, 4 NH); FABMS *m/e* (rel intensity) 253 (MH^+ − 2HCl, 100). Anal. ($C_{15}H_{12}N_2O_2$·2HCl C, H, N.

7-Hydroxy-4',6-diaminoflavone Hydrochloride (10c): mp 224 °C dec; IR (KBr) 3402, 3061, 2970–2880, 2579, 1637, 1508, 1477, 1367, 1296, 1250, 1185 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 7.98 (s, 1 H), 7.86 (d, J = 8.9 Hz, 2 H), 7.37 (s, 1 H), 6.90 (d, J = 8.9 Hz, 2 H), 6.72 (s, 1 H), 1.35 (s, 4 NH); FABMS *m/e* (rel intensity) 269 (MH^+ − 2HCl, 100); HRMS calcd for $C_{15}H_{12}N_2O_3 + H^+$ 269.0926, found 269.0933.

5-Hydroxy-4',8-diaminoflavone Hydrochloride (10d): mp 202 °C dec; IR (KBr) 3405, 2858, 2578, 1658, 1626, 1577, 1485, 1426, 1367, 1297, 1232, 1185, 1003 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 7.87 (d, J = 8.8 Hz, 2 H), 7.76 (d, J = 9 Hz, 1 H), 7.24 (d, J = 9 Hz, 1 H), 6.89 (s, 1 H), 6.72 (d, J = 8.8 Hz, 2 H), 1.38 (s, 4 NH); FABMS *m/e* (rel intensity) 269 (MH^+ − 2HCl, 100); HRMS calcd for $C_{15}H_{12}N_2O_3 + H^+$ 269.0926, found 269.0925.

6-Methoxy-4',8-diaminoflavone Hydrochloride (10e): mp 180 °C dec; IR (KBr) 3477–3320, 2932, 2850, 2568, 1628, 1585, 1508, 1485, 1373, 1220, 1132, 1056, 1026 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 8.05 (d, J = 8.6 Hz, 2 H), 6.95 (d, J = 8.6 Hz, 2 H), 6.78 (s, 1 H), 6.71 (d, J = 1.7 Hz, 2 H), 3.77 (s, 3 H), 1.35 (s, 4 NH); FABMS *m/e* (rel intensity) 283 (MH^+ − 2HCl, 100). Anal. ($C_{16}H_{14}N_2O_3$·2HCl·0.4H_2O) C, H, N.

5-Methoxy-4',8-diaminoflavone Hydrochloride (10f): mp 185 °C dec; IR (KBr) 3402, 3202, 2850, 2759, 1632, 1585, 1508, 1485, 1385, 1261, 1185, 1103, 1032 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 7.93 (d, J = 8.8 Hz, 2 H), 7.77 (d, J = 9.0 Hz, 1 H), 7.02 (d, J = 9.0 Hz, 1 H), 6.77 (d, J = 8.8 Hz, 2 H), 6.67 (s, 1 H), 3.86 (s, 3 H), 1.33 (s, 2 NH), 1.22 (s, 4 NH); FABMS *m/e* (rel intensity) 283 (MH^+ − 2HCl, 53). Anal. ($C_{16}H_{14}N_2O_3$·2HCl) C, H, N.

4',6,8-Triaminoflavone Hydrochloride (10g): mp 225 °C dec; IR (KBr) 3437, 3332, 3202, 2850, 2571, 1614, 1585, 1502, 1385 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 7.94 (d, J = 8.6 Hz, 2 H), 7.03 (d, J = 2.4 Hz, 1 H), 6.92 (d, J = 2.4 Hz, 1 H), 6.72 (s, 1 H), 6.69 (d, J = 8.6 Hz, 2 H), 1.35 (s, 6 NH); FABMS *m/e* (rel intensity) 268 (MH^+ − 2HCl, 100); HRMS calcd for $C_{15}H_{13}N_3O_2 + H^+$ 268.1086, found 268,1083.

6-Hydroxy-4',8-diaminoflavone Hydrochloride (10h): mp 202 °C dec; IR (KBr) 3349–3343, 2920, 1632, 1602, 1508, 1484, 1379, 1126 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 7.96 (d, J = 8.6 Hz, 2 H), 6.68 (d, J = 8.6 Hz, 2 H), 6.66 (s, 1 H), 6.64 (d, J = 2.7 Hz, 1 H), 6.60 (d, J = 2.7 Hz, 1 H), 1.33 (s, 4 NH); FABMS *m/e* (rel intensity) 269 (MH^+ − 2HCl, 63); HRMS calcd for $C_{15}H_{12}N_2O_3 + H^+$ 269.0926, found 269.0890.

5-Hydroxy-4',6-diaminoflavone Hydrochloride (10i): mp 192 °C dec; IR (KBr) 3355, 2849, 2579, 1655, 1608, 1508, 1455, 1402, 1291, 1238, 1191, 1139, 1020 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 7.98 (d, J = 9 Hz, 2 H), 7.64 (d, J = 8.7 Hz, 1 H), 6.89 (s, 1 H), 6.82 (d, J = 8.7 Hz, 1 H), 6.69 (d, J = 9 Hz, 2 H), 4.10–4.85 (br, 4 NH); FABMS *m/e* (rel intensity) 269 (MH^+ − 2HCl, 100); HRMS calcd for $C_{15}H_{12}N_2O_3 + H^+$ 269.0926, found 269.0890.

6-Hydroxy-4',5,7-triaminoflavone Hydrochloride (10j): mp 220 °C dec; IR (KBr) 3402, 3320, 3214, 3047–2800, 1655, 1608, 1497, 1379, 1297, 1244, 1114 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 7.92 (d, J = 8.5 Hz, 2 H), 7.04 (d, J = 8.5 Hz, 2 H), 6.85 (s, 1 H), 6.56 (s, 1 H), 1.35 (s, 6 NH); FABMS *m/e* (rel intensity) 284 (MH^+ − 3HCl, 41); HRMS calcd for $C_{15}H_{13}N_3O_3 + H^+$ 284.1035, found 284.1030.

7-Hydroxy-4',6,8-triaminoflavone Hydrochloride (10k): mp 230 °C dec; IR (KBr) 3343, 3202, 3061, 2960–2830, 1655, 1602, 1508, 1473, 1391, 1244, 1179, cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 8.12 (d, J = 8.7 Hz, 2 H), 7.32 (s, 1 H), 7.05 (d, J = 8.7 Hz, 2 H), 6.75 (s, 1 H), 2.06 (s, 6 NH); FABMS *m/e* (rel intensity) 284 (MH^+ − 3HCl, 61); HRMS calcd for $C_{15}H_{13}N_3O_3 + H^+$ 284.1035, found 284.1029.

7-Hydroxy-4',8-diaminoflavone Hydrochloride (10l): mp 176 °C dec; IR (KBr) 3367, 3100–2800, 2591, 1690, 1602, 1514, 1420, 1391, 1291, 1250, 1173, 1114 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 8.03 (d, J = 8.5 Hz, 2 H), 7.61 (d, J = 8.7 Hz, 1 H), 7.04 (d, J = 8.7 Hz, 1 H), 6.85 (d, J = 8.5 Hz, 2 H), 6.56 (s, 1 H), 1.35 (s, 4 NH); FABMS *m/e* (rel intensity) 269 (MH^+ − 2HCl, 28); HRMS calcd for $C_{15}H_{12}N_2O_3 + H^+$ 269.0926, found 269.0928.

6-Hydroxy-3',5,7-triaminoflavone (9m): mp 158 °C dec; IR (KBr) 3331, 3190, 2850, 2579, 1637, 1578, 1455, 1379, 1267 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 7.43 (d, J = 8.8 Hz, 1 H), 7.18–7.04 (m, 3 H, 2 NH), 6.73 (m, 1 H), 6.63 (s, 1 H), 5.51 (s, 2 NH), 5.39 (s, 2 NH); FABMS *m/e* (rel intensity) 284 (MH^+ − 3HCl, 100); HRMS calcd for $C_{15}H_{13}N_3O_3 + H^+$ 284.1035, found 284.1036.

3',6-Diaminoflavone Hydrochloride (10n): mp 214 °C dec; IR (KBr) 3331, 3214, 2838, 2556, 1627, 1567, 1485, 1461, 1367 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 7.94 (d, J = 8.6 Hz, 2 H), 7.03 (d, J = 2.4 Hz, 1 H), 6.92 (d, J = 2.4 Hz, 1 H), 6.72 (s, 1 H), 6.69 (d, J = 8.6 Hz, 2 H), 1.35 (s, 6 NH); FABMS *m/e* (rel intensity) 253 (MH^+ − 2HCl, 50); HRMS calcd for $C_{15}H_{12}N_2O_2 + H^+$ 253.0977, found 253.0972.

7-Hydroxy-3',6-diaminoflavone Hydrochloride (10o): mp 218 °C dec; IR (KBr) 3367, 3343, 3202, 2908, 2579, 1627, 1579, 1485, 1367, 1261 cm^{-1}; ^1H NMR (DMSO-d_6 500 MHz) δ 7.80 (s, 1 H), 7.69 (m, 1 H, 1 OH), 7.46 (t, J = 8 Hz, 1 H), 7.25 (s, 1 H), 7.23 (d, J = 9 Hz, 1 H), 6.86 (s, 1 H), 6.83 (s, 1 H), 1.33 (s, 4 NH); FABMS *m/e* (rel intensity) 269 (MH^+ − 2HCl, 80); HRMS calcd for $C_{15}H_{12}N_2O_3 + H^+$ 269.0926, found 269.0918.

6-Methoxy-3',8-diaminoflavone Hydrochloride (10p): mp 180 °C dec; IR (KBr) 3343, 2850, 2945, 2873, 2570, 1602, 1485, 1379 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 7.90–7.84 (br, 1 H), 7.47 (t, J = 7.6 Hz, 1 H), 7.24–7.19 (br, 1 H), 6.87 (s, 2 H), 6.65 (m, 2 H), 3.77 (s, 3 H), 1.35 (s, 4 NH); FABMS *m/e* (rel intensity) 283 (MH^+ − 2HCl, 100); HRMS calcd for $C_{16}H_{14}N_2O_3 + H^+$ 283.1083, found 283.1077.

Enzyme Inhibition Studies. The assays for inhibition of p56^{lc} *were* performed as previously described.[54] *In vitro* assays of p56^{lck} 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[55] 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, heparin-agarose, and butyl-agarose.[16] Analogs were screened for inhibition of p56^{lck} at seven concentrations ranging from 0.8 to 2000 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.

p60$^{v\text{-}src}$ kinase was purified from baculovirus-infected insect cell lysates using an antipeptide monoclonal antibody directed against the N-terminal 2–17 amino acids. The antibody was covalently linked to 0.65 μm latex beads in a suspension of insect cell lysis buffer comprised of 150 mM NaCl, 50 mM Tris (pH 7.5), 1 mM DTT, 1% NP-40, 2 mM EGTA, 1 mM sodium vanadate, 1 mM PMSF, and 1 μg/mL each of leupeptin, pepstatin, and aprotinin. Insect cell lysate was incubated with these beads for 3–4 h at 4 °C with rotation. At the end of the lysate incubation, the beads were rinsed three times in lysis buffer, resuspended in lysis buffer containing 10% glycerol and frozen. These latex beads were thawed, rinsed three times in assay buffer which was comprised of 40 mM Tris (pH 7.5) and 5 mM $MgCl_2$, and resuspended in the same buffer. In a Millipore 96-well plate with a 0.65 μm poly(vinylidene fluoride) membrane bottom were added the reaction components: 10 μL of v-src beads, 10 μL of 2.5 mg/mL poly(GluTyr)substrate, 5 μM ATP containing 0.2 μCi of labeled [^{32}P]ATP, and 5 μL of DMSO containing inhibitors or as a solvent control and buffer to make the final volume 125 μL. The reaction was started at room temperature by addition of the ATP and quenched 10 min later by the addition of 125 μL of 30% TCA and 0.1 M sodium pyrophosphate for 5 min on ice. The plate was then filtered, and the wells were washed with two 250 μL aliquots of 15% TCA and 0.1 M pyrophosphate. The filters were punched and counted in a liquid scintillation counter and the data examined for inhibitory activity in comparison to a known inhibitor.

Epidermal growth factor receptor was prepared from human A431 carcinoma cell shed membrane vesicles as previously described.[56] The reactions were carried out in 96-well plates with a 0.65 μm pore size poly(vinylidene fluoride) membrane bottom. The tyrosine kinase activity was assessed by solubilizing the partially purified vesicles in a mixture of 4% Triton X-100 detergent and 10% glycerol. Total vesicle preparation protein (10 μg) was added to a final volume of assay buffer of 125 μL comprised of 20 mM HEPES buffer (pH 7.4), 15 mM $MgCl_2$, 4 mM $MnCl_2$, 0.02% bovine serum albumin, 5 μM ATP containing 0.2 μCi of 0.5–3 Ci/mmol ^{32}P-labeled ATP, 25 μg of random copolymer of glutamate, alanine, and tyrosine in a ratio of 6:3:1, 250 ng of epidermal growth factor, and appropriate solvent controls or inhibitors. The reaction was allowed to progress for 10 min at room temperature and stopped by the addition of 125 μL of cold 30% trichloroacetic acid containing 0.1 M sodium pyrophosphate for 5 min on ice. The precipitate (comprised of acid-insoluble proteins and copolymer) was washed in the 96-well filter plate with the membrane bottom with two 250 μL portions of 15% trichloroacetic acid containing 0.1 M sodium pyrophosphate. Incorporated label was assessed by scintillation counting the filters (membrane bottoms of the wells) in an aqueous fluor. Typically, 80% of the incorporated, precipitated ^{32}P was due to the presence of copolymer substrate. Autophosphorylation controls were done in each experimental assay.

Acknowledgment. This investigation was made possible by Contract NO1-CM-17512, awarded by the National Cancer Institute, DHHS. We are grateful to Charles W. Moore, Michelle Spencer, and Nuphavan Koonchanok for technical support for the kinase assays.

References

(1) Cantley, L. C.; Auger, K. R.; Carpenter, C.; Duckworth, B.; Graziani, A.; Kapeller, R.; Soltoff, S. Oncogenes and Signal Transduction. *Cell* **1991**, *64*, 281–302.
(2) Aaronson, S. A. Growth Factors and Cancer. *Science* **1991**, *254*, 1146–1153.
(3) Konopka, J. B.; Watanabe, S.; Witte, O. N. An Alteration of the Human *c-abl* Protein in K562 Leukemia Cells Unmasks Associated Tyrosine Kinase Activity. *Cell* **1984**, *37*, 1035–1042.
(4) Clark, S. S.; McLaughlin, J.; Timmons, M.; Pendergast, A. M.; Ben-Neriah, Y.; Dow, L. W.; Crist, W.; Rovera, G.; Smith, S. D.; Witte, O. N. Expression of a Distinctive *BCR-ABL* Oncogene in Ph^1-Positive Acute Lymphocytic Leukemia (ALL). *Science* **1988**, *239*, 775–777.
(5) Slamon, D. J.; Clark, G. M.; Wong, S. G.; Levin, W. J.; Ullrich, A.; McGuire, W. L. Human Breast Cancer: Correlation of Relapse and Survival with Amplification of the HER-2/*neu* Oncogene. *Science* **1987**, *235*, 177–182.
(6) Bolen, J. B.; Veillette, A.; Schwartz, A. M.; DeSeau, V.; Rosen, N. Activation of pp60$^{c\text{-}src}$ Protein Kinase Activity in Human Colon Carcinoma. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 2251–2225.
(7) Cowley, G.; Smith, J. A.; Gusterson, B.; Hendler, F.; Ozane, B. The Amount of EGF Receptor is Elevated on Squamous Cell Carcinomas. In *Cancer Cells*; Levine, A. J., Vande Woude, G. F., Topp, W. C., Watson, J. D., Eds.; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY, 1984; Vol. 1, pp 5–10.
(8) Yamamoto, T.; Kamata, N.; Kawano, H.; Shimizu, S.; Kuroki, T.; Toyoshima, K.; Rikimaru, K.; Nomura, N.; Ishizaki, R.; Pastan, I.; Gamou, S.; Shimizu, N. High Incidences of Amplification of the Epidermal Growth Factor Receptor Gene in Human Squamous Carcinoma Cell Lines. *Cancer Res.* **1986**, *46*, 414–416.
(9) Chang, C.-J.; Geahlen, R. L. Protein-Tyrosine Kinase Inhibition: Mechanism-Based Discovery of Antitumor Agents. *J. Nat. Prod.* **1992**, *55*, 1529–1560.
(10) Burke, T. R., Jr. Protein-Tyrosine Kinase Inhibitors. *Drugs Future* **1992**, *17*, 119–131.
(11) Dobrusin, E. M.; Fry, D. W. Protein-Tyrosine Kinases and Cancer. *Annu. Rep. Med. Chem.* **1992**, *27*, 169–178.
(12) Geahlen, R. L.; Koonchanok, N. M.; McLaughlin, J. L.; Pratt, D. E. Inhibition of Protein-Tyrosine Kinase Activity by Flavanoids and Related Compounds. *J. Nat. Prod.* **1989**, *52*, 982–986.
(13) Graziani, Y.; Erikson, E.; Erikson, R. L. The Effect of Quercetin on the Phosphorylation Activity of the Rous Sarcoma Virus Transforming Gene Product *In Vitro* and *In Vivo*. *Eur. J. Biochem.* **1983**, *135*, 583.
(14) Hagiwara, M.; Inoue, S.; Tanaka, T.; Nunoki, K.; Ito, M.; Hidaka, H. Differential Effects of Flavonoids as Inhibitors of Tyrosine Protein Kinases and Serine/Threonine Protein Kinases. *Biochem. Pharmacol.* **1988**, *37*, 2987–2992.
(15) Akiyama, T.; Ishida, J.; Nakagawa, S.; Ogawara, H.; Watanabe, S.; Itoh, N.; Shibuya, M.; Fakami, Y. Genistein, a Specific Inhibitor of Tyrosine-specific Protein Kinases. *J. Biol. Chem.* **1987**, *262*, 5592–5595.
(16) Cushman, M.; Nagarathnam, D.; Burg, D. L.; Geahlen, R. L. Synthesis and Protein-Tyrosine Kinase Inhibitory Activities of Flavonoid Analogues. *J. Med. Chem.* **1991**, *34*, 798–806.
(17) Oberly, L.; Buttner, G. R. Correlation of Antiviral State of the Cell with Superoxide Dismutase Levels. *Cancer Res.* **1979**, *39*, 1141.
(18) Garvin, A. M.; Pawar, S.; Marth, J. D.; Perlmutter, R. J. Structure of the Murine *lck* Gene and Its Rearrangement in a Murine Lymphoma Cell Line. *Mol. Cell Biol.* **1988**, *8*, 3058–3064.
(19) Marth, J. D.; Cooper, J. A.; King, C. S.; Ziegler, S. F.; Tinker, D. A.; Overell, R. W.; Krebs, E. G.; Perlmutter, R. M. Neoplastic Transformation Induced by an Activated Lymphocyte-Specific Protein Tyrosine Kinase (pp56^{lck}). *Mol. Cell Biol.* **1988**, *8*, 540–550.
(20) Koga, Y.; Kimura, N.; Minowada, J.; Mak, T. W. Expression of the Human T-Cell-specific Tyrosine Kinase YT16(*lck*) Message in Leukemic T-Cell Lines. *Cancer Res.* **1988**, *48*, 856–859.
(21) Marth, J. D.; Peet, R.; Krebs, E. G.; Perlmutter, R. M. A Lymphocyte-Specific Protein Tyrosine Kinase Gene is Rearranged and Overexpressed in the Murine T Cell Lymphoma LSTRA. *Cell* **1985**, *43*, 393–404.
(22) Adler, H. R.; Reynolds, P. J.; Kelley, C. M.; Sefton, B. M. Transcriptional Activation of *lck* by Retrovirus Promoter Insertion between Two Lymphoid-Specific Promoters. *J. Virol.* **1988**, *62*, 4113–4122.
(23) Casnellie, J. E.; Harrison, M. L.; Hellstrom, K. E.; Krebs, E. G. A Lymphoma Cell Line Expressing Elevated Levels of Tyrosine Protein Kinase Activity. *J. Biol. Chem.* **1983**, *258*, 10738–10742.
(24) Voronova, A. F.; Sefton, B. M. Expression of a New Tyrosine Protein Kinase Is Stimulated by Retrovirus Promoter Insertion. *Nature* **1986**, *319*, 682–685.
(25) Cheung, R. K.; Dosch, H.-M. The Tyrosine Kinase *lck* Is Critically Involved in the Growth Transformation of Human B Lymphocytes. *J. Biol. Chem.* **1991**, *266*, 8667–8670.
(26) Abraham, K. M.; Levin, S. D.; Marth, J. D.; Forbush, K. A.; Perlmutter, R. M. Thymic Tumorigeneses Induced by Overexpression of p56^{lck}. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 3977–3981.
(27) Bolen, J. B.; Veillette, A. A Function for the *lck* Protooncogene. *Trends Biochem. Sci.* **1989**, *14*, 404–407.
(28) Hatakeyama, M.; Kono, T.; Kobayashi, N.; Kawahara, A.; Levin, S. D.; Perlmutter, R. M.; Taniguchi, T. Interaction of the IL-2 Receptor with the *src*-Family Kinase p56^{lck}. Identification of Novel Intermolecular Association. *Science* **1991**, *252*, 1523–1528.
(29) Kaplan, D. R.; Whitman, M.; Schaffhausen, B.; Pellas, D. C.; White, H.; Cantley, L.; Roberts, T. M. Common Elements in Growth Factor Stimulation and Oncogenic Transformation: 85 kDa Phosphoprotein and Phosphatidylinositol Kinase Activity. *Cell* **1987**, *50*, 1021–1029.
(30) Escobedo, J. A.; Kaplan, D. R.; Kavanaugh, W. M.; Turck, C. W.; Williams, L. T. A Phosphatidylinositol-3 Kinase Binds to Platelet-Derived Growth Factor Receptors through a Specific Receptor Sequence Containing Phosphotyrosine. *Mol. Cell Biol.* **1991**, *11*, 1125–1132.
(31) Coughlin, S. R.; Escobedo, J. A.; Williams, L. T. Role of Phosphatidylinositol Kinase in PDGF Signal Transduction. *Science* **1989**, *243*, 1191–1194.
(32) Meisenhelder, J.; Suh, P.-G.; Rhee, S. G.; Hunter, T. Phospholipase C-γ Is a Substrate for the PDGF and EGF Receptor Protein-tyrosine Kinases *In Vivo* and *In Vitro*. *Cell* **1989**, *57*, 1109–1122.
(33) Kumjian, D. A.; Wahl, M. I.; Rhee, S. G.; Daniel, T. O. Platelet-derived Growth Factor (PDGF) Binding Promotes the Physical Association of PDGF Receptor with Phospholipase-C. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 8232–8236.
(34) Morrison, D. K.; Kaplan, D. R.; Rhee, S. G.; Williams, L. T. Platelet-derived Growth Factor (PDGF)-Dependent Association of Phospholipase C-γ with the PDGF Receptor Signaling Complex. *Mol. Cell Biol.* **1990**, *10*, 2359–2366.
(35) Wahl, M. I.; Olashaw, N. E.; Nishibe, S.; Rhee, S. G.; Pleger, W. J.; Carpenter, G. Platelet-derived Growth Factor Induces Rapid and Sustained Tyrosine Phosphorylation of Phospholipase C-γ in Quiescent Balbc/3T3 Cells. *Mol. Cell Biol.* **1989**, *9*, 2934–2943.

(36) Kaplan, D. R.; Morrison, D. K.; Wong, G.; McCormick, F.; Williams, L. T. PDGF β-Receptor Stimulates Tyrosine Phosphorylation of GAP and Association of GAP with a Signaling Complex. *Cell* **1990**, *61*, 125–133.

(37) Kazlauskas, A.; Ellis, C.; Pawson, T.; Cooper, J. A. Binding of GAP to Activated PDGF Receptors. *Science* **1990**, *247*, 1578–1581.

(38) Molloy, C. J.; Bottaro, D. P.; Fleming, M. S.; Gibbs, J. B.; Aaronson, S. A. PDGF Induction of Tyrosine Phosphorylation of the GTPase Activating Protein. *Nature* **1989**, *342*, 711–714.

(39) O'Brien, M. C.; Fukui, Y.; Hanafusa, H. Activation of the Proto-oncogene p60$^{c\text{-}src}$ by Point Mutations in the SH2 Domain. *Mol. Cell Biol.* **1990**, *10*, 2855–2862.

(40) Cushman, M.; Nagarathnam, D. A Method for the Facile Synthesis of Ring-A Hydroxylated Flavones. *Tetrahedron Lett.* **1990**, *31*, 6497–6500.

(41) Seshadri, S.; Trivedi, P. L. Stabilities of Nitrohydroxy Chalcones and Flavanones. Role of Hydrogen Bonding. *J. Org. Chem.* **1957**, *22*, 1633–1636.

(42) Crawford, M.; Rasburn, J. W. The Stability of Coumarinic Acids. Chelation of the Hydroxyl Group. *J. Chem. Soc.* **1956**, 2155–2160.

(43) Barker, G.; Ellis, G. P. Benzopyrones. Part I. 6-Amino and 6-Hydroxy-2-substituted Chromones. *J. Chem. Soc.* **1970**, 2230–2233.

(44) Doxsee, K. M.; Feigel, M.; Stewart, K. D.; Canary, J. W.; Knobler, C. B.; Cram, D. J. Host-Guest Complexation. 42. Preorganization Strongly Enhances the Tendency of Hemispherands To Form Hemisperaplexes. *J. Am. Chem. Soc.* **1987**, *109*, 3098–3107.

(45) Gowan, J. E.; MacGiolla Riogh, S. P.; MacMahon, G. J.; O'Cleirigh, S.; Philbin,, E. M.; Wheeler, T. S. A Synthesis of 6,8-Dihydroxyflavone. *Tetrahedron* **1958**, *2*, 116–121.

(46) Allan, D.; Loudon, J. D. A Novel Synthesis of Some Quinoline Derivatives. *J. Chem. Soc.* **1949**, 821–825.

(47) Joshi, S. S.; Singh, H. Nitrohydroxy Aromatic Ketones. I. Nitrohydroxyacetophenones. *J. Am. Chem. Soc.* **1954**, *76*, 4993–4994.

(48) Mathieson, D. W.; Newberry, G. Contributions to the Chemistry of Synthetic Antimalarials. Part VIII. Aromatic Carbinolamines. *J. Chem. Soc.* **1949**, 1133–1137.

(49) Kasahara, A. Studies of Flavanones. XXVII. Syntheses and Resolution of 5-Aminoflavanones. *Nippon Kagaku Zasshi* **1958**, *79*, 335–338.

(50) Naik, R. M.; Thakor, V. W. Chromones. Part III. Kostanecki-Robinson Acylation of Some Ortho-Hydroxyacetophenones. *Proc. Indian Acad. Sci.* **1953**, *37a*, 774–782.

(51) Naik, R. M.; Thakor, V. W.; Shah, R. C. γ-Substitution of the Resorcinol Nucleus. Part. XI. Friedel and Crafts Acylation of 4-Nitroresorcinol. *Proc. Indian Acad. Sci.* **1953**, *37a*, 765–773.

(52) Fernandes, P. S.; Coutinho, L. Synthesis of Some New Heterocycles from 4′-Aminoflavone. *J. Indian Chem. Soc.* **1983**, *60*, 864–866.

(53) Gowan, J. E.; Wheeler, T. S. Further Experiments on the Mechanism of the Baker-Venkataramin Transformation. *J. Chem. Soc.* **1950**, 1925–1928.

(54) Cushman, M.; Nagarathnam, D.; Geahlen, R. L. Synthesis and Evaluation of Hydroxylated Flavones and Related Compounds as Potential Inhibitors of the Protein-tyrosine Kinase p56^{lck}. *J. Nat. Prod.* **1991**, *54*, 1345–1352.

(55) Zioncheck, T. F.; Harrison, M. L.; Geahlen, R. L. Purification and Characterization of a Protein-Tyrosine Kinase from Bovine Thymus. *J. Biol. Chem.* **1986**, *261*, 15637–15643.

(56) Cohen, S.; Ushiro, H.; Stoscheck, C.; Chinkers, M. A Native 170,000 Dalton Epidermal Growth Factor Receptor-kinase Complex from Shed Plasma Membrane Vesicles. *J. Biol. Chem.* **1982**, *257*, 1523–1531.