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Articles

Bicyclic Compounds as Ring-Constrained Inhibitors of Protein-Tyrosine Kinase p56^{lck} [1]

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A study was undertaken to prepare inhibitors of the lymphocyte protein-tyrosine kinase p56^{lck}. Using the known p56^{lck} inhibitor 3,4-dihydroxy-α-cyanocinnamamide (4) as a lead compound, bicyclic analogues were designed as conformationally constrained mimetics in which the phenyl ring and vinyl side chain of the cinnamamide are locked into a coplanar orientation. Such planarity was rationalized to be an important determinant for binding within a putative flat, cleftlike catalytic cavity. Bicyclic analogues were prepared using the naphthalene, quinoline, isoquinoline, and 2-iminochromene ring systems and examined for their ability to inhibit autophosphorylation of immunopurified p56^{lck}. The most potent analogues were methyl 7,8-dihydroxyisoquinoline-3-carboxylate (12) (IC_{50} = 0.2 μM) and 7,8-dihydroxyisoquinoline-3-carboxamide (13) (IC_{50} = 0.5 μM). Inhibition by 12 was not competitive with respect to ATP. These compounds may represent important new structural motifs for the development of p56^{lck} inhibitors.

Since the initial discovery that the transforming component of the Rous sarcoma virus is a kinase which phosphorylates on tyrosine, protein-tyrosine kinases (PTKs; enzymes which catalyze the transfer of the γ-phosphate at ATP to the 4-hydroxyl of specific tyrosyl residues in protein substrates) have become recognized as critical mediators of both normal and neoplastic cellular signal transduction.[2] The 56-kDa LCK gene product, p56^{lck}, is a member of the src family of nonreceptor (NR) PTKs which is expressed predominantly in T-cells and natural killer (NK) cells. In T-cells, p56^{lck} binds to cell surface glycoproteins CD4 and CD8 and participates in cellular activation and mitogenesis.[3] Recent evidence suggesting an interaction of p56^{lck} with the interleukin-2 (IL-2) receptor β-chain also implies the existence of a role for p56^{lck} in IL-2 mediated signaling.[4] The correlation of p56^{lck} with aberrations in lymphoid proliferative processes[5] highlights the need for p56^{lck} inhibitors. Specific p56^{lck} PTK inhibitors may aid in studies of the role of p56^{lck} in cellular signal transduction affecting cell activation and proliferation. Such inhibitors could also prove to be of potential therapeutic value for certain immune and neoplastic diseases.[6]

Development of rationally designed p56^{lck} inhibitors is hampered by a lack of X-ray structure data for any PTK. However, a recent X-ray determination of the catalytic unit of a cAMP-dependent serine/threonine kinase[7] has important implications for the development of PTK inhibitors due to the high degree of homology that exists between the catalytic domains of PTKs and other protein kinases.[8] PTKs function by the simultaneous binding of both ATP and tyrosyl-containing substrate, and by analogy to the cAMP-dependent kinase, a picture of the catalytic site emerges as a deep planar cleft bounded by extended β-sheets in which the adenosine ring binds to the rear of

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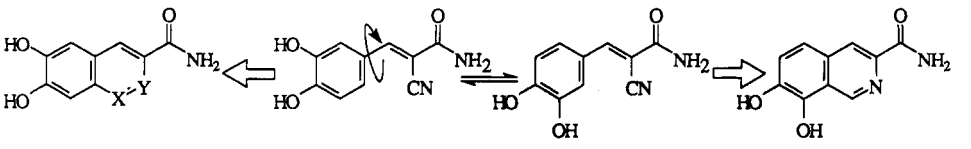


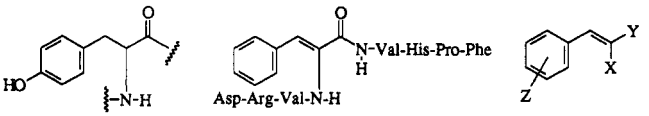
7 X = CH, Y = CH
8 X = N, Y = CH
11 X = CH, Y = N

4 13

Figure 1.

the cleft and the sugar triphosphate chain extends toward the cleft opening where the peptide substrate binds. The the tyrosyl ring would most probably extend into a similar cleft where the actual transfer of phosphate would occur.

Inhibitors could conceptually interact at the catalytic site in modes which are competitive with respect to either ATP, peptide substrate, or both. Additional types of interaction have also recently been described.[9] An important structural motif in the design of PTK inhibitors which are competitive with respect to peptide substrate is the "styryl pharmacophore". Since the first demonstration by Wong in 1984 that replacement of a tyrosyl residue with a dehydrophenylalanine transformed an angiotensin II analogue from a PTK substrate to an inhibitor **2**,[10] a large number of inhibitors of general



1 Tyrosyl Residue

2

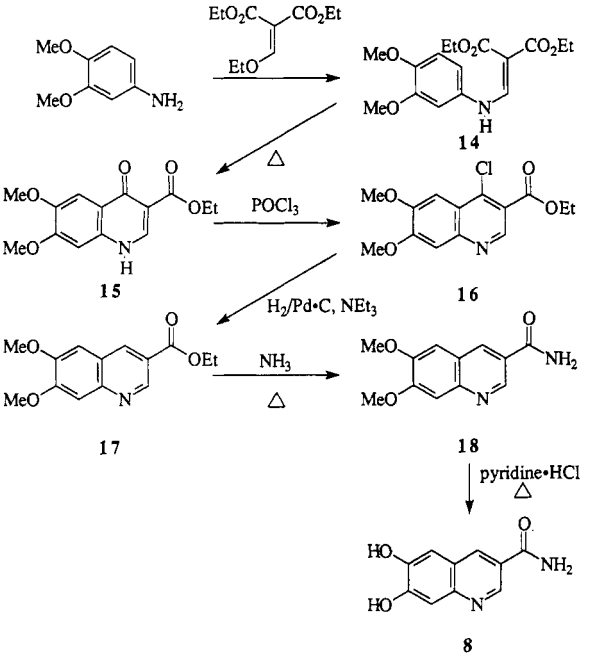
3
4; X = CN
Y = $CONH_2$
Z = 3,4 -OH

structure **3** have been prepared which are competitive with respect to peptide substrate.[6] The structural similarities of styryl-based inhibitors to tyrosyl residues **1** has led to the hypothesis that these agents function at the catalytic site as peptidomimetics.[11] The α-cyanocinnamamides (**3**; X = CN, Y = $CONH_2$) represent one important family of styryl-based PTK inhibitors,[12] and we have previously shown that the 3,4-dihydroxy analogue **4** has good inhibitory activity against $p56^{lck}$.[13] Energy minimization of **4** has shown that the aryl ring and vinyl side chain exhibit global minima in near planar arrangements.[14] Such extended planarity is a consistent requirement for interaction within a flat, cleft-like catalytic cavity.[6] Mimetics of **4** which eliminate phenyl ring rotation can be envisioned by effectively constraining the "vinyl side chain" and aryl ring to the same plane (Figure 1). Locking the phenyl ring into an angle which approximates that required for binding to the enzyme could endow such compounds with increased affinity. Herein is reported work which we have undertaken to develop more potent and selective $p56^{lck}$ inhibitors based on bicyclic analogues designed as conformationally constrained mimics of lead compound **4**.

Synthesis

Naphthyl-, Quinolyl-, and Isoquinolyl-Based Compounds. The 6,7-dihydroxy bicyclics were made following methods reported for the preparation of similar compounds. In the naphthyl series, amidation of 6,7-dimethoxynaphthalene-3-carboxylic acid (**5**)[15] produced **6**, which after BBr_3-mediated demethylation, provided the first target, 6,7-dihydroxynaphthalene-3-carboxamide (**7**). Synthesis of 6,7-dihydroxyquinoline-3-carboxamide (**8**)

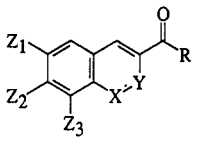
Scheme I



was achieved starting from 4-aminoveratrole as outlined in Scheme I.[16] Isolation and purification of the intermediate anilinopropenate **14** was necessary for the success of the thermal cyclization to the dimethoxyoxoquinoline **15**. Demethylation of **18** could not be realized using the general BBr_3 method, but was accomplished most satisfactorily with the use of pyridine·HCl.

The 6,7-dihydroxyisoquinoline system was accessed from methyl 6,7-dimethoxyisoquinoline-3-carboxylate (**9**) which was synthesized from 3,4-dimethoxyphenylalanine.[17] Conversion to the carboxamide **10** and demethylation with pyridine·HCl provided **11** as the last target in the 6,7-dihydroxy series. The isomeric 7,8-dihydroxyisoquinoline-3-carboxamide target **13** was synthesized from methyl 7,8-dihydroxyisoquinoline-3-carboxylate (**12**) which was prepared by our recently reported procedure for the synthesis of hydroxylated isoquinolines.[18] An important feature of this method is the use of a phenolic starting material which eliminates the need of an extra demethylation step.

Synthesis of methyl 5,6-dihydroxy-2-naphthalenecarboxylate (**23**), which represents the naphthalene analogue of 7,8-dihydroxyisoquinoline (**12**), started from 6-bromo-1,2-naphthoquinone (**19**) (prepared[19] by oxidation of commercially available 6-bromo-2-naphthol using potassium nitrosodisulfonate). Reduction of **19** to the diol **20** using sodium hyposulfite followed by methylation (dimethyl sulfate/aqueous NaOH) as previously described[20]) yielded 1,2-dimethoxy-6-bromonaphthalene (**21**). Lithiation of **21** then reaction with carbon dioxide provided carboxylic acid **22**, which was treated with BBr_3 and



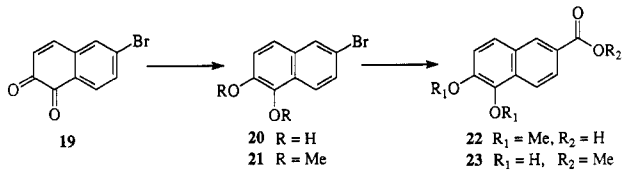
	R	X	Y	Z_1	Z_2	Z_3
5	OH	CH	CH	OMe	OMe	H
6	NH_2	CH	CH	OMe	OMe	H
7	NH_2	CH	CH	OH	OH	H
8	NH_2	N	CH	OH	OH	H
9	OMe	CH	N	OMe	OMe	H
10	NH_2	CH	N	OMe	OMe	H
11	NH_2	CH	N	OH	OH	H
12	OMe	CH	N	H	OH	OH
13	NH_2	CH	N	H	OH	OH
22	OH	CH	CH	H	OMe	OMe
23	OMe	CH	CH	H	OH	OH

quenched with MeOH to give methyl 5,6-dihydroxynaphthalene-2-carboxylate (**23**) (Scheme II).

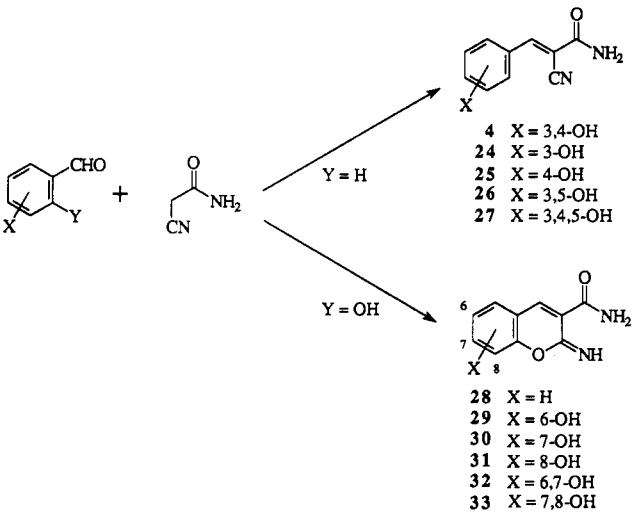
Hydroxylated α-Cyanocinnamamides. Base-catalyzed reaction of aryl aldehydes with active methylene compounds in general and α-cyanoacetamides in particular[21] yields the corresponding α,β-unsaturated condensation products (Scheme III). The α-cyanocinnamamido products are usually obtained as high melting crystalline solids which fall out of solution upon treatment of the aldehyde and α-cyanoacetamide in EtOH with a catalytic quantity of piperidine. Although the stereochemistry of addition is frequently assumed to be trans, direct supportive spectroscopic evidence of such a trans orientation is not usually obtained since the α-cyanocinnamamido products contain a single vinylic hydrogen that does not allow facile NMR assignment based on vicinal proton–proton coupling. A recent study of the Knoevenagel condensation of cyanoacetates with aryl aldehydes confirmed that the phenyl ring is trans to the carboxyl group based on ^{13}C NMR long-range selective proton decoupling experiments.[22] The hydroxylated α-cyanocinnamamides **4, 24–27** prepared as part of this study are also assumed to be in the trans geometry. The observation of a single vinylic signal in the ^1H NMR spectrum supports the existence of only one geometric isomer for each compound. Furthermore, the ability of 2-hydroxylated α-cyanocinnamamides to cyclize to the iminochromenes (see Scheme III) provides confirming evidence for a trans phenyl–carboxamide orientation.

3-Carbamoyl-2-iminochromenes. Aryl aldehydes containing a hydroxyl group in the 2-position undergo initial condensation to the α-cyanocinnamamides upon treatment with α-cyanoacetamide in the presence of base. An additional intramolecular attack of the aryl 2-hydroxyl on the α-cyano group subsequently results in the formation of bicyclic 3-carbamoyl-2-iminochromenes (Scheme III).[23] Evidence for the iminochromene rather than the isomeric open-chain α-cyanocinnamamide includes the absence of an IR nitrile band (2215–2220 cm^{-1}) and the presence of a characteristic imine proton in the NMR (7.6 ppm, DMSO). Iminochromenes **28–33** were accordingly formed by the reaction of α-cyanoacetamide with the appropriately substituted 2-hydroxybenzaldehydes, respectively. In previous publications we incorrectly assigned an open-chain 2,5-dihydroxy-α-cyanocinnamamide structure[13] to 3-carbamoyl-6-hydroxy-2-iminochromene (**29**) based on a prior report.[12b] Others may have also have erred similarly

Scheme II



Scheme III



in assigning open-chain structures to what in reality are iminochromenes.[24]

Results and Discussion

Many NR PTKs are expressed in postmitotic, fully differentiated cells. The fact that several of these are found selectively in hematopoietic cells suggests that such proteins may be involved in specialized cellular processes which are important for the specific function of these cells. Accordingly, $p56^{lck}$ is found almost exclusively in T-lymphocytes, NK cells, some T-cell leukemia lines, EBV-transformed B lymphocytes, and human colon and lung cancer cell lines. In T-cells it has been shown to be involved in early biochemical events associated with T-cell receptor (TCR) mediated cell activation and stimulation.[25] Additionally, IL-2 plays a central role in the growth and function of T-cells, and it has recently been demonstrated that the IL-2/IL-2 receptor interaction provokes a rapid increase in tyrosine phosphorylation of several proteins,[26] perhaps through the action of $p56^{lck}$. In light of the importance of $p56^{lck}$ for mitogenic signal transduction, it is not surprising that high PTK activity has been detected in several leukemias and lymphomas. Additionally, the location of the LCK gene at the site of relatively frequent chromosomal abnormalities in human lymphomas suggests that alterations in $p56^{lck}$ expression may contribute to neoplastic transformation. It therefore becomes of interest to prepare PTK inhibitors which have a high specificity and affinity for $p56^{lck}$ in order to study the mechanism(s) by which the enzyme is involved in cellular activation and proliferation. Such agents could also potentially be of utility as anticancer therapeutics.

To design compounds as potent inhibitors of $p56^{lck}$ we took 3,4-dihydroxy-α-cyanocinnamamide (**4**) as a lead structure which we had previously shown exhibits good activity in this system.[13] Without an X-ray structure of the $p56^{lck}$ catalytic domain, our approach toward the design of analogues of **4** was based on the topology of the catalytic

cavity of the highly homologous c-AMP dependent protein kinase, which has been shown to be a deep cleft.[7] When considered with the fact that energy minimization of 4 provides stable conformations in which the phenyl ring is close to being coplanar with the vinyl side,[14] it becomes probable that the active, inhibitory conformation of 4 is also highly planar. Extended planarity is a feature shared by other PTK inhibitors,[6] which is consistent with their interaction at a planar cleft-like catalytic cavity.

Applying this concept to 4, we designed bicyclic analogues in which the phenyl ring is conformationally constrained to an angle which makes it coplanar with the "vinyl side chain" (Figure 1). In constructing these bicyclic mimetics, note was taken of the fact that the α-cyano group of parent 4 cannot be incorporated directly into the ring system. Since the role of vinyl substituents and the α-cyano group in particular have not been clearly defined for PTK inhibition, three 6,7-dihydroxylated ring systems were examined: naphthyl (7), quinolyl (8), and isoquinolyl (11) (Figure 1). Preliminary examination of these analogues in β-type platelet-derived growth factor (PDGF) receptor preparations indicated that inhibitory potency increased as one went from naphthyl (7) ($IC_{50} > 100\ \mu M$) to quinolyl (8) ($IC_{50} = 5\ \mu M$) to isoquinolyl (11) ($IC_{50} = 0.5\ \mu M$).[1a] On the basis of these initial findings, which identified 6,7-dihydroxyisoquinoline-3-carboxamide (11) as the most potent compound, an additional isomeric 7,8-dihydroxyisoquinoline analogue (13) was prepared. This compound represented a rotationally constrained mimetic of 4 having the phenyl ring rotated 180° relative to the conformation mimicked by the 6,7-dihydroxyisoquinoline 11 (Figure 1). It had been previously demonstrated in epidermal growth factor receptor (EGFR) PTK preparations using constrained analogues of dihydroxylated benzylidene malononitriles (3; X, Y = CN)[27] that a rotamer equivalent to our 7,8-dihydroxy-substituted compound exhibited an approximately 10-fold decrease in IC_{50} value relative to the alternate 180° rotamer.

Surprisingly, when the 6,7-dihydroxylated bicyclics 7, 8, and 11 were examined in p56[lck], the order of potency was reversed relative to that observed in PDGF, with the naphthyl (7), quinolyl (8), and isoquinolyl (11) analogues exhibiting IC_{50} values of 26, 610, and 1900 μM, respectively. Even more unexpected was the dramatic 3000-fold increase in potencies observed for the 7,8-dihydroxyisoquinolines 12 ($IC_{50} = 0.2\ \mu M$) and 13 ($IC_{50} = 0.5\ \mu M$) relative to the 6,7-dihydroxy isomer 11. The extremely large difference in potency between 11 and 13 may indicate that they are binding in entirely different orientations relative to each other. That the hydroxyl substitution pattern may be a critical factor in binding to p56[lck] is further substantiated through the activity shown by the open-chain hydroxycinnamamides 24–27. Although as a whole the members of this set combined the hydroxyl patterns exhibited by rotamers of the parent 3,4-dihydroxy analogue 4 (Figure 1), none of the compounds exhibited significant activity.

The iminochromenes 28–33 may also be viewed as conformationally constrained mimetics of styryl-based PTK inhibitors 3. These compounds add significantly to the present study in that they provide additional evidence that in bicyclic inhibitors, substituents occupying positions equivalent to the vinylic "X group" of open-chain compounds of type 3, have a large impact on inhibitory activity, as evidenced by the 1000-fold loss of potency of the 7,8-dihydroxyiminochromene 33 ($IC_{50} = 750\ \mu M$) relative to the 7,8-dihydroxyisoquinoline 13. Although both the 6,7-dihydroxyiminochromene 32 and its 7,8-dihydroxy isomer 33 had very little activity in p56[lck], they both possessed IC_{50} values in the low micromolar range in PDGF preparations (unpublished results). These latter results indicate that substituents at the pseudovinylic position can also greatly affect interkinase specificity.

The 6,7-dihydroxy- and 5,6-dihydroxynaphthalene analogues (7 and 23, respectively) have moderate inhibitory activity. In contrast to the analogous isoquinoline-based compounds 11 and 12, which exhibited a strong dependence of potency on the pattern of hydroxyl substitution, the naphthalene-2-carboxylate nucleus appears to be insensitive to the pattern of hydroxyl substitution as evidenced by the near equal activity of both 7 and 23. The only previous use of the naphthalene nucleus in PTK inhibitors was in the construction of phosphonate-containing compounds designed as "bisubstrate" analogues.[28] The present findings support the value of naphthalene-2-carboxylate as a pharmacophore for the development of PTK inhibitors.

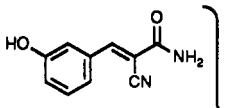
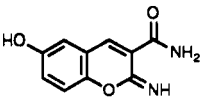
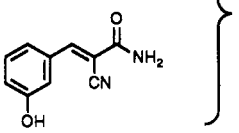
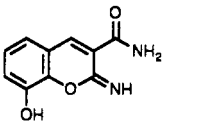
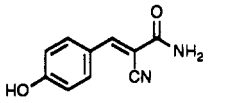
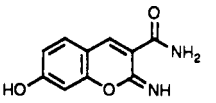
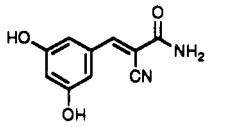
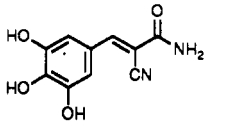
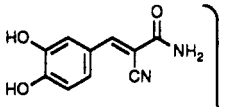
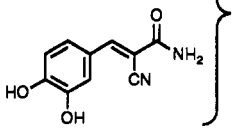
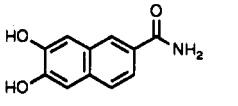
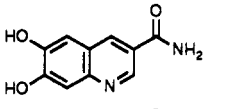
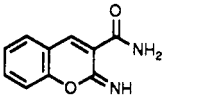
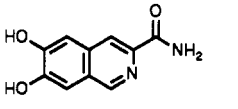
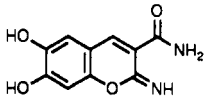
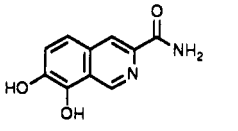
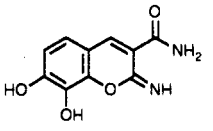
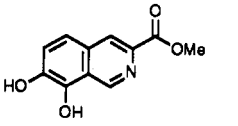
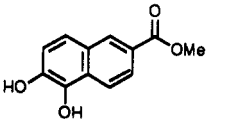
The 7,8-dihydroxyisoquinolines 12 and 13 are among the most potent inhibitors of p56[lck] reported to date[29] and potentially represent an important new structural motif in the design of PTK inhibitors. Since the ultimate value of PTK inhibitors either as pharmacological tools or as anticancer agents will be related to their efficacy in whole cell or animal systems, a significant advantage which both the naphthalene and isoquinoline nuclei could have relative to the cinnamamido (3, Y = $CONH_2$) or benzylidene malononitrile (3, X,Y = CN) inhibitors is the greatly reduced potential for cytotoxicity due to nucleophilic 1,4-Michael-type alkylation.[30] While the bicyclic analogues presented here were designed as conformationally constrained mimetics of styryl-based inhibitors which are competitive with respect to tyrosyl-containing substrate, conceptually their planar nature may allow them to interact at the ATP binding domain, which is situated more deeply within the catalytic cleft. PTK inhibitors which act in a manner competitive with respect to ATP would be expected to function less effectively in an intracellular environment where ATP concentrations are elevated. However, when two analogues (7 and 12) were examined under varying concentrations of ATP, it was found that inhibition of both autophosphorylation and phosphorylation of exogenous substrate (rabbit muscle enolase) was not competitive with respect to ATP, thereby further adding to the potential utility of this class of PTK inhibitors. Specific inhibitors of PTKs may be useful for understanding the mechanisms of signal transduction and cell transformation, and could represent a new type of antitumor therapeutic. Work is presently in progress to utilize the results of this study for the preparation of clinically useful PTK inhibitors.

Experimental Section

Cells. HPB-ALL human T-cell leukemia (CD4+, CD3+) cells were grown in RPMI 1640 (Gibco) supplemented with glutamine and 10% fetal bovine serum (HyClone). HPB-ALL cells express lck, fyn, and yes PTKs.

Biochemical Assay. Immune complex protein-tyrosine kinase assays were conducted as described.[31] Briefly, CD4 was immunoprecipitated from equivalent amounts of cellular protein by a combination of mAB CD4 and RAM and *Staphylococcus aureus* protein A. This regimen was shown to recover effectively CD4-associated p56[lck]. Evaluation of potential inhibitors was conducted by their addition at the concentrations 0, 0.1, 1, 10,

Table I. Inhibition of p56lck Autophosphorylation

no.		IC_{50} (μM)	no.		IC_{50} (μM)
24		1500	**29**		100
24			**31**		100
25		1000	**30**		>2000
26		500			
27		2000			
4		22			
4					
7		26			
8		610	**28**		1500
11		1900	**32**		>2000
13		0.5	**33**		750
12		0.2			
23		25			

50, 100, 250, 500, and 1000 μM with a final dilution of DMSO 1:1000. At this level DMSO was not found to inhibit protein-tyrosine kinase activity. Assays were run in the presence of 0.5 μM cold ATP. Kinetic analysis performed on compounds 7 and 12 using varying concentrations of ATP showed that these inhibitors did not compete with ATP in either autophosphorylation or phosphorylation of the exogenous substrate, rabbit muscle enolase. In vitro kinase assays were resolved by 8% SDS-PAGE and the appropriate bands quantified by cutting from the gel and counting in a β-counter or liquid scintillation counter. IC_{50} values were calculated from the percentage of inhibition (based upon ^{32}P cpm) versus the concentration of inhibitor.

Synthesis. Petroleum ether was of the boiling range 35–60 °C and removal of solvents was performed by rotary evaporation under reduced pressure. Silica gel filtration was carried out using TLC grade silica gel (5–25 μm Aldrich). Melting points were determined on a Mel Temp II melting point apparatus and are uncorrected. Elemental analyses were obtained from Atlantic Microlab Inc., Norcross, GA and are within 0.4% of theoretical values unless otherwise indicated. Fast atom bombardment mass

spectra (FABMS) were acquired with a VG Analytical 7070E mass spectrometer under the control of a VG 2035 data system. ^{1}H NMR data were obtained either on Varian XL-200 (200 MHz) or Bruker AC250 (250 MHz) instruments.

6,7-Dimethoxy-2-naphthalenecarboxamide (6). A suspension of 6,7-dimethoxy-2-naphthalenecarboxylic acid (**5**)[15] in $SOCl_2$ (10 mL, 137 mmol) was refluxed (2 h). Excess $SOCl_2$ was removed by evaporation and the resulting dark solid dried under vacuum, dissolved in ammonia-saturated $CHCl_3$ (15 mL), and stirred at room temperature (1 h). The reaction mixture was taken to dryness and the resulting solid mixed with H_2O, collected by filtration, and recrystallized from CH_3CN yielding **6** (850 mg, 85%): mp 178–182 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.87 (s, 6 H, 2 OCH_3), 7.33 (s, 2 H, ArH and NH), 7.75 (s, 2 H), 8.00 (s, 1 H, NH), 8.30 (s, 1 H); FABMS m/z 232 (M + H). Anal. ($C_{13}H_{13}NO_3 \cdot H_2O$) C, H, N.

6,7-Dihydroxy-2-naphthalenecarboxamide (7). To a stirred solution of naphthalenecarboxamide **6** (200 mg, 0.81 mmol) in anhydrous CH_2Cl_2 (25 mL) was added a solution of BBr_3 (2.5 mL, 1 M in CH_2Cl_2) dropwise at −78 °C under argon. After 1.5 h the reaction was brought to room temperature and stirring continued (2 h). The mixture was cooled on ice, quenched with MeOH, taken to dryness twice from MeOH, and then purified by silica gel chromatography ($CHCl_3$–MeOH 9:1) to give a dark solid. Crystallization from EtOH–H_2O (9:1) provided **7** as colorless plates (100 mg, 57%): mp 159–161 °C; ^{1}H NMR (DMSO-d_6) δ 7.11 (s, 1 H), 7.15 (s, 1 H), 7.24 (br s, 1 H, NH), 7.57 (d, 1 H, J = 8.5 Hz), 7.63 (dd, 1 H, J = 8.5, 1.6 Hz), 7.91 (br s, 1 H, NH), 8.12 (s, 1 H), 9.70 (br s, 1 H, OH), 9.79 (br s, 1 H, OH); FABMS m/z 204 (M + H). Anal. ($C_{11}H_9NO_3$) C, H, N.

Ethyl 2-Carbethoxy-3-(3,4-dimethoxyanilino)-2-propenoate (14). A solution of 4-aminoveratrole (7.9 g, 50 mmol) and diethyl (ethoxymethylene)malonate (10.9 mL, 54 mmol) in anhydrous CH_3CN (50 mL) was stirred at room temperature under N_2 overnight. The resulting dark solution was taken to dryness, decolorized with activated charcoal (boiling ether; petroleum ether), and then concentrated to yield **14** as colorless plates (11 g, 68%): mp 55–56 °C; ^{1}H NMR ($CDCl_3$) δ 1.2–1.4 (m, 6 H, 2 CH_3), 3.87 (s, 3 H, OCH_3), 3.88 (s, 3 H, OCH_3), 4.2–4.4 (m, 4 H, 2 CH_2), 6.6–6.7 (m, 2 H), 6.85 (d, 1 H, J = 8.5 Hz), 8.43 (d, 1 H, J = 14 Hz, vinylic), 11.03 (d, 1 H, J = 14 Hz, NH); FABMS m/z 324 (M + H). Anal. ($C_{16}H_{21}NO_6$) C, H, N.

Ethyl 1,4-Dihydro-6,7-dimethoxy-4-oxoquinoline-3-carboxylate (15). A solution of ethyl propenate **14** (5.84 g, 18.1 mmol) in diphenyl ether (35 mL) was added under argon over 15 min to refluxing diphenyl ether (90 mL) and the reaction stirred at reflux (2 h). The dark mixture was cooled to room temperature, diluted with petroleum ether (100 mL), and the resulting suspension collected and washed with petroleum ether (100 mL). The filtrate was diluted with petroleum ether (100 mL), cooled on ice, and filtered to yield additional product. The combined solid (4.6 g) was triturated with boiling EtOH and filtered while hot and the filter cake washed with EtOH, yielding crude **15** as a light tan-colored solid (3.00 g). Crystallization from boiling DMF (100 mL) yielded pure **15** as white crystals (2.45 g, 49%): mp 275–279 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.25 (t, 3 H, J = 7.1 Hz, CH_3), 3.82 (s, 3 H, OCH_3), 3.86 (s, 3 H, OCH_3), 4.21 (q, 2 H, J = 7.1 Hz, CH_2), 7.04 (s, 1 H), 7.50 (s, 1 H), 8.43 (s, 1 H), 12.06 (br s, 1 H, NH). Anal. ($C_{14}H_{15}NO_5$) C, H, N.

Ethyl 4-Chloro-6,7-dimethoxyquinoline-3-carboxylate (16). A mixture of oxoquinoline **15** (150 mg, 0.54 mmol) in $POCl_3$ (3.1 mL, 33 mmol) was stirred at reflux under N_2 (2 h). The mixture was cooled to room temperature, poured onto ice (30 g), and adjusted to pH 7 by addition of 2 N NaOH, yielding **16** as an off-white precipitate (150 mg, 94%). A sample was crystallized for analysis (EtOH): mp 160–161 °C; ^{1}H NMR (DMSO-d_6) δ 1.35 (t, 3 H, J = 7.2 Hz, CH_3), 4.00 (s, 6 H, 2 OCH_3), 4.40 (q, 2 H, J = 7.2 Hz, CH_2), 7.48 (s, 1 H), 7.51 (s, 1 H), 8.94 (s, 1 H). Anal. ($C_{14}H_{14}NO_4Cl$) C, H, N.

Ethyl 6,7-Dimethoxyquinoline-3-carboxylate (17). A suspension of chloroquinoline **16** (2.00 g, 7.2 mmol) in MeOH–absolute EtOH (1:1; 100 mL) was shaken with NEt_3 (1.46 g, 15.4 mmol) over 10% Pd–C (200 mg) under H_2 (40 psi) for 24 h. Catalyst was removed, the filtrate taken to dryness, and the resulting residue subjected to an extractive work up ($CHCl_3$–dilute aqueous NH_4OH) and taken to dryness. The resulting yellow solid was mixed with boiling ether (300 mL) and the ether mixture cooled to −78 °C and filtered. The filter cake was washed with cold ether (−78 °C) and dried, yielding **17** as a light yellow solid (1.16 g, 62%): mp 138–140 °C; ^{1}H NMR ($CDCl_3$) δ 1.44 (t, 3 H, J = 7.2 Hz, CH_3), 4.02 (s, 3 H, OCH_3), 4.05 (s, 3 H, OCH_3), 4.45 (q, 2 H, J = 7.2 Hz, CH_2), 7.13 (s, 1 H), 7.46 (s, 1 H), 8.66 (d, 1 H, J = 1.9 Hz), 9.26 (d, 1 H, J = 1.9 Hz); FABMS m/z 262 (M + H). Anal. ($C_{14}H_{15}NO_4 \cdot {}^1/_4H_2O$) C, H, N.

6,7-Dimethoxyquinoline-3-carboxamide (18). A mixture of dimethoxyquinoline **17** (1.32 g, 5.1 mmol) and NH_4Cl (265 mg, 5.0 mmol) was heated in a pressure vessel with NH_3-saturated MeOH at 70 °C (4 days). The mixture was taken to dryness, triturated with H_2O, and filtered, yielding **18** as a light yellow solid (1.1 g, 94%). A sample was recrystallized for analysis: mp 266–268 °C; ^{1}H NMR (DMSO-d_6) δ 3.98 (s, 3 H, OCH_3), 4.04 (s, 3 H, OCH_3), 7.54 (s, 1 H), 7.74 (s, 1 H), 7.94 (br s, 1 H, NH), 8.45 (br s, 1 H, NH), 9.36 (s, 1 H); FABMS m/z 233 (M + H). Anal. ($C_{12}H_{12}N_2O_3$) C, H, N.

6,7-Dihydroxyquinoline-3-carboxamide Hydrochloride (8·HCl). A mixture of quinolinecarboxamide **18** (348 mg, 1.5 mmol) and anhydrous pyridine·HCl (3.5 g) was heated from 170 °C to 190 °C over 30 min. Excess pyridine·HCl was then removed by distillation at 140 °C under reduced pressure and the residue cooled on ice and mixed with ice-cold H_2O (25 mL). The resulting yellow solid was collected by filtration and washed with ice-cold H_2O to yield crude **8**·HCl contaminated with a small amount of incompletely demethylated material (263 mg, 64% crude yield). Crystallization from MeOH yielded pure **8**·HCl as light yellow crystals (132 mg, 32%): mp >280 °C dec; ^{1}H NMR (DMSO-d_6) δ 7.57 (s, 1 H), 7.69 (s, 1 H), 7.89 (br s, 1 H, NH), 8.46 (br s, 1 H, NH), 9.23 (s, 2 H); FABMS m/z 205 (M + H). Anal. ($C_{10}H_8N_2O_3 \cdot HCl \cdot CH_3OH \cdot {}^1/_4H_2O$) C, H, N.

6,7-Dimethoxyisoquinoline-3-carboxamide (10). A mixture of methyl 6,7-dimethoxyisoquinoline-3-carboxylate (**9**)[17] (600 mg, 2.41 mmol) and NH_4Cl (13 mg, 0.2 mmol) in methanolic ammonia (30 mL) was stirred in a pressure vessel at 90 °C (20 h). The reaction mixture was cooled on ice and the resulting white suspension collected and washed with cold MeOH (−78 °C) to afford a white solid (418 mg). A second crop was obtained by concentrating and cooling the filtrate to provide a combined yield of **10** as a white crystalline solid (490 mg, 87%); mp 241–242 °C. A sample was recrystallized from MeOH–H_2O for analysis: mp 241–244 °C; ^{1}H NMR (DMSO-d_6) δ 3.96 (s, 6 H, 2OCH_3), 7.58 (s, 2 H, ArH, NH), 7.62 (s, 1 H), 8.14 (br s, 1 H, NH), 8.40 (s, 1 H), 9.10 (s, 1 H); FABMS m/z 233 (M + H). Anal. ($C_{12}H_{12}N_2O_3 \cdot {}^1/_2H_2O$) C, H, N.

6,7-Dihydroxyisoquinoline-3-carboxamide Hydrochloride (11·HCl). A mixture of dimethoxyisoquinoline **10** (232 mg, 1.0 mmol) and pyridine·HCl (6 g) was stirred at 190 °C (30 min). The light brown solution was cooled to 150–160 °C and pyridine·HCl distilled off under reduced pressure. The resulting gray solid was cooled to room temperature and mixed well with ice-cold H_2O and filtered and the filtrate evaporated to a syrup which solidified on cooling. Treatment of the syrup with ether–MeOH (1:1, 20 mL) gave a gummy off-white solid (320 mg), which upon crystallization twice from MeOH yielded **11**·HCl as a white solid (60 mg, 25%): mp >280 °C dec; ^{1}H NMR (DMSO-d_6) δ 7.53 (s, 1 H), 7.78 (s, 1 H), 8.18 (br s, 1 H, NH), 8.69 (br s, 1 H, NH), 8.74 (s, 1 H), 9.35 (s, 1 H), 11.09 (br s, 1 H, OH), 11.91 (br s, 1 H, OH); FABMS m/z 205 (M + H). Anal. ($C_{10}H_8N_2O_3 \cdot HCl \cdot {}^1/_4CH_3OH$) C, H, N.

7,8-Dihydroxyisoquinoline-3-carboxamide Hydrochloride (13·HCl). A solution of methyl 7,8-dihydroxyisoquinoline-3-carboxylate (**12**)[18] (66 mg, 0.3 mmol) in methanolic ammonia (20 mL) was stirred in a sealed reaction vial at 95 °C (24 h). The resulting brown solution was taken to dryness under argon and placed under vacuum, yielding a dark residue which was mixed with 0.2 N HCl (10 mL) and cooled on ice to yield **13**·HCl as a brown solid (49 mg, 68%): mp >270 °C dec; ^{1}H NMR (DMSO-d_6/D_2O) δ 7.76 (d, 1 H, J = 8.7 Hz), 7.79 (d, 1 H, J = 8.7 Hz), 8.76 (s, 1 H), 9.43 (s, 1 H). Anal. ($C_{10}H_8N_2O_3 \cdot HCl \cdot H_2O$) C, H, N.

5,6-Dimethoxy-2-naphthalenecarboxylic Acid (22). To a solution of 1.33 g (5.0 mmol) of 6-bromo-1,2-dimethoxynaphthalene[20] (mp 51–53 °C; lit.[20] oil) in anhydrous ether (20 mL) at −78 °C under argon was added 9.4 mL (15 mmol) of n-butyllithium (1.6 M in hexane) and the reaction then stirred at −15 °C. After

1.5 h the yellow suspension was cooled to −78 °C and solid carbon dioxide added. The resulting thick white suspension was stirred briefly at −78 °C (10 min) and then at 0 °C (10 min) and partitioned between 1 N NaOH (150 mL) and EtOAc (2 × 100 mL). The aqueous phase was acidified with 37% HCl (pH ≤ 2), giving a thick white suspension which was collected by filtration, washed (1 × 50 mL, 1 N HCl; 2 × 50 mL, H_2O) and dried to yield **22** as a white solid (1.0 g, 86%): mp 209–211 °C; ^{1}H NMR (DMSO-d_6) δ 3.90 (s, 3 H, OCH_3), 3.98 (s, 3 H, OCH_3), 7.57 (d, 1 H, J = 9.1 Hz), 7.92 (d, 1 H, J = 9.1 Hz), 7.95 (dd, 1 H, J = 9.0, 1.5 Hz), 8.07 (d, 1 H, J = 9.0 Hz), 8.57 (d, 1 H, J = 1.5 Hz), 12.98 (br s, 1 H, OH). Anal. ($C_{13}H_{12}O_4$) C, H.

Methyl 5,6-Dihydroxy-2-naphthalenecarboxylate (23). To a suspension of **22** (232 mg, 1.0 mmol) in $CHCl_3$ (10 mL) was added 3.0 mL (3 mmol) of BBr_3 (1.0 M in CH_2Cl_2), and the resulting solution was stirred at room temperature under argon overnight. The suspension was quenched with MeOH (5 mL) and taken to dryness twice from MeOH to yield product **23** as a grey solid (222 mg, 100%): mp 184–188 °C; ^{1}H NMR (DMSO-d_6) δ 3.89 (s, 3 H, OCH_3), 7.25 (d, 1 H, J = 8.7 Hz), 7.51 (d, 1 H, J = 8.7 Hz), 7.84 (dd, 1 H, J = 8.8, 1.0 Hz), 8.08 (d, 1 H, J = 8.8 Hz), 8.45 (d, 1 H, J = 1.0 Hz), 9.10 (s, 1 H, OH), 9.80 (s, 1 H, OH). Anal. ($C_{12}H_{10}O_4 \cdot {}^1/_4H_2O$) C, H.

3-Hydroxy-α-cyanocinnamamide (24). General Method A. A solution of 3-hydroxybenzaldehyde (610 mg, 5.0 mmol) and α-cyanoacetamide (462 mg, 5.5 mmol) in absolute EtOH (10 mL) was stirred at reflux with 2 drops piperidine under argon (24 h). The reaction mixture was cooled on ice and the resulting solid collected and washed with absolute EtOH, yielding **24** as tan crystals (602 mg, 64%): mp 220–225 °C; ^{1}H NMR (DMSO-d_6) δ 6.94–7.00 (m, 1 H), 7.30–7.40 (m, 3 H), 7.75 (br s, 1 H, NH), 7.90 (br s, 1 H, NH), 8.05 (s, 1 H, vinylic), 9.25 (s, 1 H, OH); FABMS m/z 279 (M − H + glycerol). Anal. ($C_{10}H_8N_2O_2 \cdot {}^1/_4C_2H_6O$) C, H, N.

4-Hydroxy-α-cyanocinnamamide (25). Compound **25** was prepared from 4-hydroxybenzaldehyde in 66% yield using method A: mp 249.5–251.5 °C (lit. mp 250 °C;[12a] mp 258–259 °C[21]).

3,4-Dihydroxy-α-cyanocinnamamide (4). Compound **4** was prepared from 3,4-dihydroxybenzaldehyde in 60% yield as a yellow solid using method A: mp 234 °C (lit. mp 247 °C;[12a] mp 231 °C[32]).

3,5-Dihydroxy-α-cyanocinnamamide (26). Compound **26** was prepared from 3,5-dihydroxybenzaldehyde (Spectrum Chemical) in 38% yield as a yellow solid using method A: mp 225–228 °C; ^{1}H NMR (DMSO-d_6) δ 6.40 (m, 1 H), 6.78 (m, 2 H), 7.75 (br s, 1 H, NH), 7.85 (br s, 1 H, NH), 7.91 (s, 1 H, vinylic), 9.73 (s, 2 H, 2OH); FABMS m/z 295 (M − H + glycerol). Anal. ($C_{10}H_8N_2O_3 \cdot {}^1/_4H_2O$) C, H, N.

3,4,5-Trihydroxy-α-cyanocinnamamide (27). Compound **27** was prepared from 3,4,5-trihydroxybenzaldehyde in 72% yield using method A: mp >250 °C dec (lit.[32] mp 268 °C); ^{1}H NMR (DMSO-d_6) δ 6.98 (s, 2 H), 7.55 (br s, 1 H, NH), 7.69 (br s, 1 H, NH), 7.81 (s, 1 H, vinylic), 9.31 (br s, 1 H, OH), 9.45 (s, 2 H, 2OH); FABMS m/z 311 (M − H + glycerol). Anal. ($C_{10}H_8N_2O_4$) C, H, N.

3-Carbamoyl-2-iminochromene (28). Compound **28** was prepared from 2-hydroxybenzaldehyde in 64% yield as a cream-colored solid using method A: mp 178–181 °C dec [lit. mp 178 °C;[23c] mp 182 °C[24a] (structure reported as 2-hydroxy-α-cyanocinnamamide)].

3-Carbamoyl-6-hydroxy-2-iminochromene (29). General Method B. A solution of 2,5-dihydroxybenzaldehyde (345 mg, 2.5 mmol) and α-cyanoacetamide (231 mg, 2.75 mmol) in absolute EtOH (10 mL) with 2 drops piperidine was stirred at room temperature under argon (2.5 h). The resulting solid was collected and washed with absolute EtOH, yielding **29** as a green solid (396 mg, 78%): mp 188–190 °C dec (previously reported[12b,13a] as 2,5-dihydroxy-α-cyanocinnamamide, mp 200 °C[12b]); ^{1}H NMR (DMSO-d_6) δ 6.95 (dd, 1 H, J = 2.6, 9.1 Hz), 7.04 (d, 1 H, J = 2.6 Hz), 7.02 (d, 1 H, J = 9.1 Hz), 7.75 (br s, 1 H, =NH), 8.28 (s, 1 H, vinylic), 8.79 (br s, 1 H, NH), 9.55 (br s, 1 H, NH), 9.65 (s, 1 H, OH); FABMS m/z 295 (M − H + glycerol). Anal. ($C_{10}H_8N_2O_3$) C, H, N.

3-Carbamoyl-7-hydroxy-2-iminochromene (30). Compound **30** was prepared from 2,4-dihydroxybenzaldehyde in 93% yield as a tan-colored solid using method A: mp 219–221 °C (lit.[23b] mp 215–217 °C); ^{1}H NMR (DMSO-d_6) δ 4.05–4.15 (m, 1 H, exchangeable), 6.53 (d, 1 H, J = 2.2 Hz), 6.67 (dd, 1 H, J = 2.2, 8.5 Hz), 7.53 (d, 1 H, J = 2.2 Hz), 7.62 (br s, 1 H, =NH), 8.27 (s, 1 H, vinylic), 9.38 (br s, 1 H, NH), 10.6 (br s, 1 H, exchangeable): FABMS m/z 295 (M − H + glycerol). Anal. ($C_{10}H_8N_2O_3$) C, H, N.

3-Carbamoyl-8-hydroxy-2-iminochromene (31). Compound **31** was prepared from 2,3-dihydroxybenzaldehyde in 29% yield as yellow crystals using method B: mp 168–170 °C dec; ^{1}H NMR (DMSO-d_6) δ 7.01–7.16 (m, 3 H), 7.62 (br s, 1 H, =NH), 8.29 (s, 1 H, vinylic), 9.32 (br s, 1 H, NH), 10.09 (br s, 1 H, OH); FABMS m/z 295 (M − H + glycerol). Anal. ($C_{10}H_8N_2O_3 \cdot {}^1/_4C_2H_6O$) C, H, N.

3-Carbamoyl-6,7-dihydroxy-2-iminochromene (32). Compound **32** was prepared from 2,4,5-trihydroxybenzaldehyde (Spectrum Chemical) in 78% yield as light brown crystals using method A: mp > 250 °C dec; ^{1}H NMR (DMSO-d_6/DCl) δ 7.12 (s, 1 H), 7.23 (s, 1 H), 9.02 (s, 1 H, vinylic); FABMS m/z 311 (M − H + glycerol). Anal. ($C_{10}H_8N_2O_4$) C, H, N.

3-Carbamoyl-7,8-dihydroxy-2-iminochromene (33). Compound **33** was prepared from 2,3,4-trihydroxybenzaldehyde in 56% yield as brown crystals using method A: mp > 280 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.40 (br s, 3 H, exchangeable), 6.67 (d, 1 H, J = 8.4 Hz), 7.03 (d, 1 H, J = 8.4 Hz), 7.65 (br s, 1 H, =NH), 8.25 (s, 1 H, vinylic), 9.30 (br s, 1 H, exchangeable); FABMS m/z 311 (M − H + glycerol). Anal. ($C_{10}H_8N_2O_4 \cdot {}^1/_2H_2O$) C, H; N: calcd, 12.22; found, 12.80.

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