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Synthesis and Evaluation of New Protein-Tyrosine Kinase Inhibitors. Part 1. Pyridine-Containing Stilbenes and Amides.

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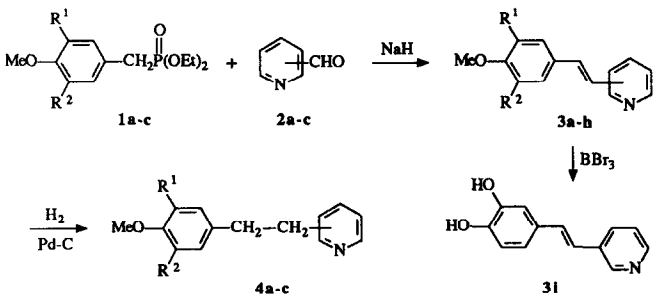
Abstract: A series of pyridine-containing stilbene and amide derivatives based on the structure of piceatannol, a naturally occurring protein-tyrosine kinase inhibitor, has been prepared and tested for inhibition of p56^{lck}. The most potent of these compounds is a competitive inhibitor of p56^{lck} with respect to ATP.

The identification of protein-tyrosine kinases as the products of viral and cellular oncogenes and as receptors for polypeptide growth factors clearly established the link between the phosphorylation of proteins on tyrosine and the stimulation of cell proliferation.[1-3] In many human cancers, these kinases are either overexpressed or inappropriately expressed and are thought to contribute to the transformed phenotype.[4-9] Such observations have generated considerable interest in the design and synthesis of specific inhibitors of these enzymes. Compounds based on the structures of erbstatin[10-13] and of naturally occurring flavones and isoflavones[14-18] have supplied the bulk of new inhibitors.

Our recent efforts in the evaluation of natural products have identified piceatannol (E-3,4,3',5'-tetrahydroxystilbene) as a protein-tyrosine kinase inhibitor.[19] As part of an ongoing study to develop synthetic inhibitors based on this structural lead, we have synthesized a series of pyridine-containing stilbene and amide derivatives as described in Schemes I and II.[20-23] These synthetic compounds were tested for inhibition of angiotensin I phosphorylation catalyzed by the protein-tyrosine kinase p56^{lck}, which was partially purified from bovine thymus as described.[24] IC_{50} values of these compounds for the inhibition of p56^{lck} are summarized in Table I.

Scheme I



Scheme II

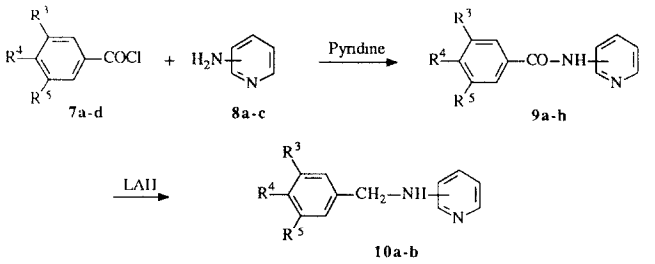


Table I. Physical Characteristics and Protein-Tyrosine Kinase Inhibitory Data of Compounds **3a-l**, **4a-c**, **5a-c**, **9a-h** and **10a-b**.

compd	Ar	Ar'	mp (°C)	lit mp (°C)/ M. Formula	PTKI IC_{50} (μM)
	Stilbenes (E-isomers, Ar-CH=CH-Ar')				
3a	3,4,5-trimethoxyphenyl	4-pyridyl	246-247[a]	247-248[a20]	2285
3b	3,4,5-trimethoxyphenyl	3-pyridyl	105-107	106-108[20]	516
3c	3,4,5-trimethoxyphenyl	2-pyridyl	oil	$C_{16}H_{17}NO_3$**	2175
3d	3,4-dimethoxyphenyl	4-pyridyl	126-128	127-128[25]	622
3e	3,4-dimethoxyphenyl	3-pyridyl	75-7	75-7[21]	178
3f	3,4-dimethoxyphenyl	2-pyridyl	195-200 °C(0.2 mm)[26]		829
3g	4-methoxyphenyl	4-pyridyl	133-134	131.5-132.5[23]	>3787
3h	4-methoxyphenyl	3-pyridyl	101-103	102-103[21]	>3787
3i	3,4-dihydroxyphenyl	3-pyridyl	221-222	220-222[21]	638
3j	2-pyridyl	2-pyridyl	118-119[b]	-	1229
3k	4-pyridyl	4-pyridyl	150-153[b]	-	307
3l	4-pyridyl	2-pyridyl	110-112[b]	-	615
	Dihydrostilbenes (Ar-CH_2-CH_2-Ar')				
4a	3,4,5-trimethoxyphenyl	3-pyridyl	143-144[a]	143-145[a21]	>2927
4b	3,4-dimethoxyphenyl	3-pyridyl	142-144[a]	143-144[a21]	1315
4c	4-pyridyl	4-pyridyl	110-112[b]	-	2909
	Quarternary salts				
5a	methyl iodide salt of **3b**		191-193	190-192[21]	>1936
5b	methyl iodide salt of **3e**		194-196	194-196[21]	>2088
5c	ethyl bromoacetate salt of **3b**		188-190 (dec)	$C_{20}H_{24}BrNO_5$**	>1825
	Amides (ArCONHAr')				
9a	3,4,5-trimethoxyphenyl	4-pyridyl	167-168	166-168.5[22]	>2775
9b	3,4,5-trimethoxyphenyl	3-pyridyl	159-160	158-160[22]	999
9c	3,4,5-trimethoxyphenyl	2-pyridyl	56-57	55-57[22]	260
9d	3,5-dimethoxyphenyl	4-pyridyl	191-192	$C_{14}H_{14}N_2O_3$**	3097
9e	3,5-dimethoxyphenyl	3-pyridyl	77-79	77-79[22]	619
9f	3,5-dimethoxyphenyl	2-pyridyl	130-132	$C_{14}H_{14}N_2O_3$**	205
9g	4-pyridyl	4-pyridyl	182-183	$C_{11}H_9N_3O$**	2323
9h	3-pyridyl	4-pyridyl	192-193	$C_{11}H_9N_3O$**	2137
	Amines (ArCH_2NHAr')				
10a	3,4,5-trimethoxyphenyl	2-pyridyl	oil	$C_{15}H_{18}N_2O_3$**	>2916
10b	3,5-dimethoxyphenyl	2-pyridyl	oil	$C_{14}H_{16}N_2O_2$**	>3275

a) mp of picrate; b) purchased from Aldrich. ** All new compounds gave satisfactory spectral and microanalytical data.

Results and Discussion

Among the two major groups of compounds prepared, viz pyridylstilbenes **3a-l** and benzamide derivatives **9a-h**, compounds **3e**, **3k**, **9c** and **9f** showed significant inhibitory activity (IC_{50} < 310 μM). 1-(3,4-Dimethoxyphenyl)-2-(3-pyridyl)ethene (**3e**) was the most potent of the compounds tested (IC_{50} = 178 μM). The addition or removal of a methoxy group from **3e** resulted in compounds with reduced potency (e. g. **3b**, **3h**). Similarly, quarternization of pyridine nitrogen (**5a-c**) as well as hydrogenation of the olefinic bond (dihydrostilbenes **4a-c**) resulted in considerable loss of inhibitory activity. Substitution of the methoxylated phenyl ring of compounds **3a**, **3d** and **3g** with a 4-pyridyl group increased inhibitory potency (IC_{50} for **3k** = 307 μM). 3,5-Dimethoxy-*N*-(4-pyridyl)benzamide (**9f**) was the most potent of the benzamides derivatives. Reduction of the amide bonds (e. g. **10a-b**) completely eliminated inhibitory activity.

Since most of the reported protein-tyrosine kinase inhibitors contain hydroxyl groups, the dihydroxy compound derived from the most potent dimethoxystilbene **3e** was prepared. The resulting 1-(3,4-dihydroxyphenyl)-2-(3-pyridyl)ethene (**3i**), however, was found to be less potent than **3e**. Additional efforts are underway to prepare several analogs having combined features of both piceatannol and pyridylstilbenes.

The most potent of these compounds (**3e**) was investigated in detail to determine its mode of interaction with the tyrosine kinase. A kinetic evaluation of the mechanism of inhibition revealed that **3e** was a competitive inhibitor of $p56^{lck}$ with respect to ATP (Fig. 1A). This is in contrast to piceatannol, which inhibits by competing with the tyrosine-containing peptide or protein substrate for binding to the enzyme. The inhibition of $p56^{lck}$ was uncompetitive with respect to the peptide substrate angiotensin I (Fig. 1B). This inhibition pattern is consistent with an ordered reaction mechanism in which the peptide binds first to the enzyme. Such a mechanism has been proposed also for the phosphorylation of peptide substrates by the epidermal growth factor receptor tyrosine kinase.[27] Other inhibitors, primarily the naturally occurring

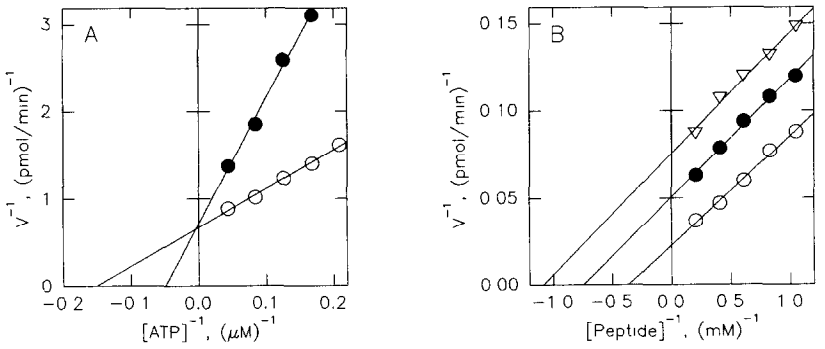


Fig. 1. Lineweaver-Burk plots showing inhibition of $p56^{lck}$ by compound **3e**. A, Effect of increasing concentrations of [γ-^{32}P]ATP on the inhibition of $p56^{lck}$ by **3e** {0 (o) or 40 (•) μg/ml}. B, Effect of increasing concentrations of angiotensin on the inhibition of $p56^{lck}$ by **3e** {0 (o), 40 (•) or 100 (▽) μg/ml}. Assay conditions are as described previously.[24]

flavonoids, have been shown to compete with ATP for binding to tyrosine kinases. These inhibitors, however, are generally mixed-type inhibitors with respect to peptide substrates.[14,17]

These observations provide new information regarding the structural features that influence the interaction of compounds with the ATP-binding site on tyrosine kinases. Such observations are currently being exploited in an effort to develop new and more potent inhibitors.

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