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# STRUCTURAL INFLUENCES OF STYRYL-BASED INHIBITORS ON EPIDERMAL GROWTH FACTOR RECEPTOR AND p56$^{lck}$ TYROSINE-SPECIFIC PROTEIN KINASES.

Terrence R. Burke, Jr.*[1] Zhen-Hong Li,[2,3] Joseph B. Bolen[3] and Victor E. Marquez[1]

[1]Laboratory of Medicinal Chemistry, Developmental Therapeutics Program, Division of Cancer Treatment, Building 37, Rm 5C06, and [2]Medicine Branch and [3]Laboratory of Tumor Virus Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892

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**Summary:** A structure activity study was conducted on two important members of the styryl class of tyrosine-specific protein kinase inhibitors to examine relative roles which the aryl rings and vinyl side chains play in their inhibitory activity. The ability of four analogs (**1a-d**) to inhibit autophosphorylation of epidermal growth factor receptor (EGFR) and p56$^{lck}$ tyrosine kinases was examined, with results showing that both the pattern of aromatic hydroxylation and the type of side chain functionality can greatly influence both selectivity and potency.

Tyrosine-specific protein kinases (TPKs) constitute an important class of phosphoryl transfer enzymes which serve vital regulatory functions in cellular growth and differentiation.[1] In view of the fact that several TPKs are the products of proto-oncogenes, inhibitors of these kinases offer a potential approach toward the treatment of various proliferative disorders. Since the isolation of erbstatin **1a** from a culture of *Streptomyces*[2] and the demonstration of its potent inhibitory properties in the epidermal growth factor receptor (EGFR) TPK ($IC_{50}$ = 3 μM)[3] a large number of inhibitors bearing general structure **1** have been reported.[4-16] These styrene-containing compounds are generally competitive with respect to substrate and can be envisioned to function at the catalytic site as peptidomimetics of tyrosine-containing peptides.[17,18] Studies which have shown the importance of aromatic hydroxyl groups for inhibitory activity in this class of compounds[15] support the hypothesis that the aryl ring mimics features of the 4-hydroxyphenyl ring of tyrosine itself while the vinylic side chain provides recognition features normally present in the tyrosyl amide bonds. At the present time a lack of three dimensional structural information of TPKs either free or bound with inhibitors necessitates reliance on structure-activity studies to probe the nature of these interactions.

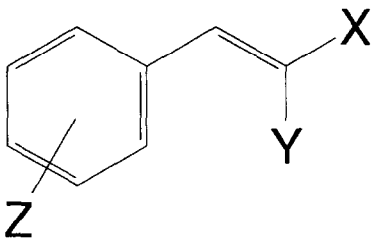


Figure 1.General Structure of styryl-based TPK inhibitors.

Structurally, styryl-based inhibitors can be differentiated according to aryl (Z, Figure 1) and vinyl substitution (X and Y, Figure 1). Two important nuclei which have emerged within this class of inhibitors are the formamido-containing compounds, such as erbstatin **1a**,[4] and the α-cyanocinnamamido-based analogs typified by **1b**.[8,12,14,15] Since it is known that TPK inhibitors can exhibit selectivity with respect to various families of TPKs,[16,18] a limited study was undertaken to examine how these aryl and side chain moieties contribute to the TPK inhibitory profile of **1a** and **1b**. Both compounds were accordingly dissected into their respective aryl and side chain portions and two new analogs **1c** and **1d** were prepared from the exchange of side chains between the two (Figure 2). Derivative **1c** incorporates the aryl ring of erbstatin **1a** with the side chain of **1b**, while analog **1d** combines the 3,4-dihydroxyphenyl ring of **1b** with the formamido side chain of erbstatin (Figure 2). Synthesis of the formamido analogs was performed as previously described[19] (**1a** mp 148 °C; lit.[20] mp 149-151 °C and **1d** mp 196-198 °C; lit.[21] mp 185-187 °C) and preparation of the α-cyanocinnamamido-based analogs was according to the method of Gazit et al.[15] (**1b** mp 234 dec.; lit.[22] 231°C and **1c** mp 188-190 °C; lit.[8] mp 200 °C).

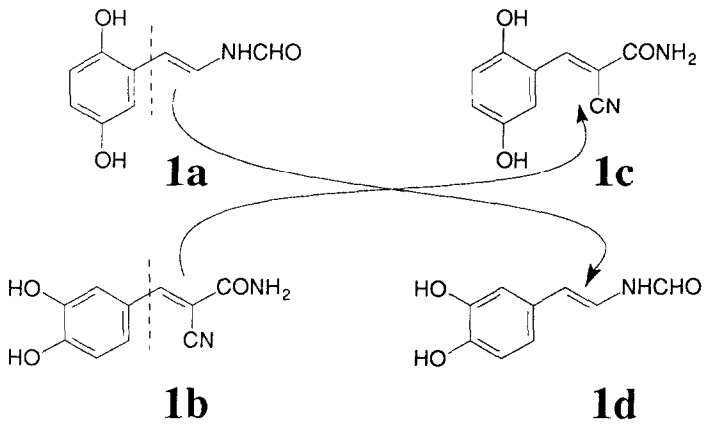


Figure 2. Rearrangement of vinyl side chains.

The TPK inhibitory activity of these analogs was then compared using immunopercipitated EGFR and $p56^{lck}$ preparations as previously described[23] (Figure 3). The data indicate a high degree of structural specificity between EGFR and $p56^{lck}$ for these analogs. In EGFR assays, compounds **1b** and **1d**, bearing the 3,4-dihydroxyphenyl ring, were poor inhibitors ( $IC_{50}$ values > 1000 μM), while inhibitors **1a** and **1c**, bearing the 2,5-dihydroxyphenyl ring, were better inhibitors. In the latter group the formamido side chain-containing **1a** exhibited both a slightly better $IC_{50}$ value (5 μM) (reported[3] $IC_{50}$ = 3 μM) and a greater level of maximal inhibition (90%) than analog **1c**, which bears the α-cyanocinnamamido structure ($IC_{50}$ = 10 μM with 60% maximal inhibition). The poor inhibitory properties of the 3,4-dihydroxyphenyl analogs **1b** and **1d** were not consistent with previously reported values against EGFR (**1b** $IC_{50}$ = 4 μM[15]; **1d** $IC_{50}$ = 7 μM[4]). However, previous estimates of inhibition evaluated EGFR kinase activity following addition of EGF, while in the present case, inhibitory activity was measured using basal EGFR kinase activity. An interesting feature exhibited by these compounds is that maximal inhibition varies greatly (Figure 3). While this has previously been observed with (+/-)-phenazocine in the same EGFR assay system,[24] the reasons for this behavior are not clear.

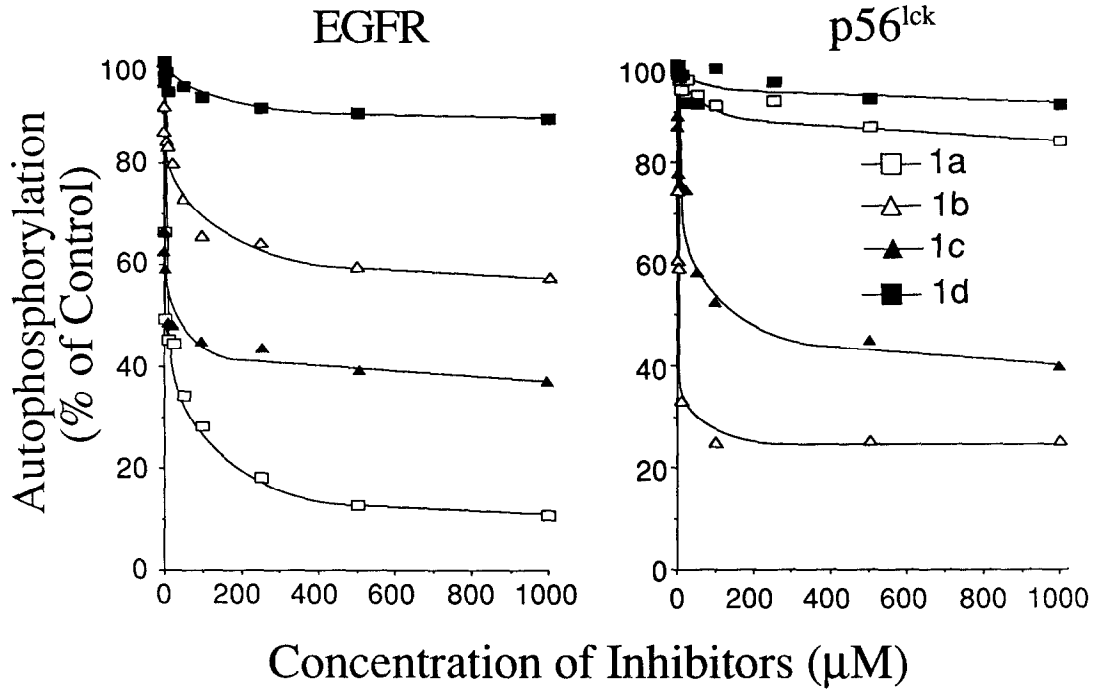


Figure 3. Plot of autophosphorylation of EGFR and $p56^{lck}$ against inhitor concentration.

In the $p56^{lck}$ assays compounds **1a** and **1d**, bearing the formamido side chain were essentially inactive regardless of ring substitution, while the α-cyanocinnamamide-based analogs showed significant activity (**1b** $IC_{50}$ = 7 μM; **1c** $IC_{50}$ = 100 μM). A variation in maximal inhibition was also observed, similar to that seen in the EGFR system.

This is the first example of a comparison of inhibitory potencies between EGFR and $p56^{lck}$ for the styryl class of compounds. These findings are consistent with previous reports that certain inhibitors can exhibit marked differences in potency among select TPKs.[8,15,18] While previous work has shown that TPK inhibitory potency increases as hydroxyls are added to the aryl ring,[15] the present study indicates that the hydroxyl substitution pattern is also of great importance. Results of this study show that the 2,5-dihydroxyphenyl ring confers specificity for EGFR relative to $p56^{lck}$. Additionally, while the formamido side chain exhibited good activity in EGFR, it was inactive in $p56^{lck}$ regardless of ring substitution.This study, while limited in scope, supports previous findings that significant changes in both potency and specificity can be achieved by selective modification of either ring or side chain portions of this very important class of inhibitors. Further studies are in progress to more fully exploit this behavior in the development of inhibitors selective for specific TPKs.

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