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SELECTIVE INHIBITION OF THE TYROSINE KINASE PP60^{src} BY ANALOGS OF 5,10-DIHYDROPYRIMIDO[4,5-b]QUINOLIN-4(1H)-ONE

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Abstract: 7,8-Dimethoxy-5,10-dihydropyrimido[4,5-b]quinolin-4(1H)-one (**1b**) has been discovered to be a potent and selective inhibitor of the tyrosine-specific kinase activity associated with pp60*c-src*. Mode of inhibition studies reveal that this agent inhibits in a pure-mixed-noncompetitive mode with respect to nucleotide co-factor.

Introduction:

Since the initial discovery of retroviral tyrosine protein kinases, significant advances have been made in our understanding of the role this class of enzymes plays in transformation and regulation of cell proliferation.[1] Recently, the normal cellular variants, which include a number of growth factor receptors, have been shown to be involved in cellular regulation.[2,3] Clinical evidence now points to inappropriate expression of these normal cellular tyrosine-specific kinases being linked to human malignancies.[4-8] These findings suggest a role for selective inhibitors of tyrosine-specific protein kinases in the treatment of cancer and other hyperproliferative disorders.

Screening and directed synthesis efforts have successfully identified a number of structurally distinct tyrosine-specific kinase inhibitors.[9,10] This report details the discovery and preliminary SAR for a 5,10-dihydropyrimido[4,5-b]quinolin-4(1H)-one-based series (**1**) of tyrosine-specific kinase inhibitors identified as part of a screening program.

Chemistry:

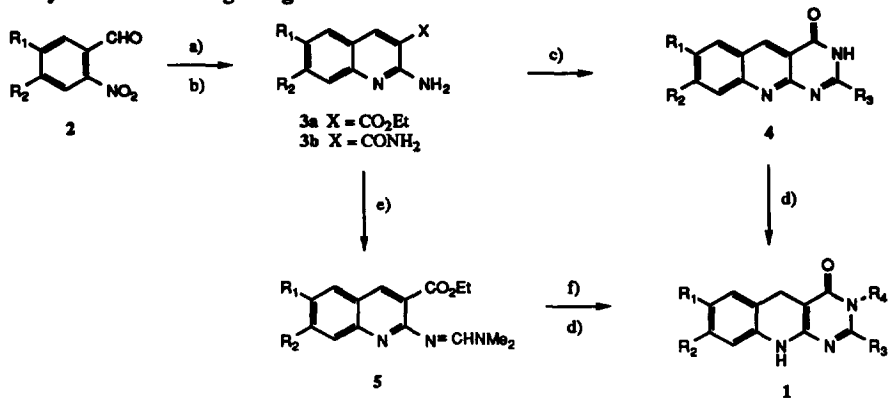
The general synthetic routes utilized to prepare the core 5,10-dihydropyrimido[4,5-b]quinolin-4(1H)-one (**1**) backbone,[11] as well as, C-2 and N-3 substituted variants are shown in the attached Scheme. Condensation of an o-nitrobenzaldehyde (**2**) with ethyl cyanoacetate or cyanoacetamide, followed by reductive cyclization affords the corresponding 2-aminoquinoline intermediate **3**. Reaction of ester **3a** with carboxamides at elevated temperatures (Niementowski reaction), or alternatively, treatment of carboxamides **3b** with an ortho ester in the presence of an acid catalyst provides the fully-aromatized tricyclic core **4**. Reduction to the dihydro-targets **1** is efficiently accomplished by heating **4** with an excess of ammonium formate in formamide. Preparation of the corresponding N-3 functionalized analogs is initiated by condensation of **3a** with dimethylformamide dimethyl acetal. Trapping and cyclization of **5** with the requisite amine affords the fully-aromatized tricyclic, which upon reduction under the standard conditions affords N-3 substituted analogs of **1**. Syntheses of analogs **6**,[12] **7**[13] and **8**[14] have previously been reported.

Results and Discussion:

During the course of a high-throughput screening effort directed towards the identification of inhibitors of the tyrosine-specific kinase activity associated with the cellular oncogene pp60$^{c\text{-}src}$ (c-*src*), a member of the 5,10-dihydropyrimido[4,5-b]quinolin-4-(1H)-one chemical class (**1**) was discovered to potently inhibit this enzyme. The Table details the results of our preliminary SAR follow-up on this series. Inhibitory activities of

these compounds against c-AMP-dependent kinase (PKA), a member of the serine-/threonine-specific kinase family, were also determined to provide a measure of tyrosine kinase selectivity.

Scheme - Syntheses of Analogs 1a-g



a) $(CO_2Et)CH_2CN$ or $(CONH_2)CH_2CN$, piperdine, EtOH, reflux b) zinc or iron dust, AcOH, reflux c) R_3-$CONH_2$, ≥150°C or R_3-$CH(OEt)_3$, PTSA, reflux d) HCO_2NH_4, $HCONH_2$, 180°C e) $(MeO)_2CHNMe_2$, PTSA, toluene, reflux f)R_4-NH_2, EtOH, reflux.

The parent compound (**1a**) in this series inhibits c-*src* with an IC_{50} of 9 μM, while exhibiting no substantial inhibition of PKA at concentrations up to 3 mM.[15] Incorporation of methoxy groups into the 7- and 8-positions (**1b**) produces an 18-fold enhancement in c-*src* inhibitory potency. Compound **1b** possesses a remarkable selectivity (6000-fold) for inhibition of c-*src* over a representative serine-/threonine-specific kinase. Given this profile, we were encouraged to investigate the issue of enzymatic selectivity within the tyrosine-specific kinase family. Evaluation of **1b** against the tyrosine-specific kinase activity associated with the epidermal growth factor receptor (EGFR) revealed a 150-fold selectivity for inhibition of c-*src*.

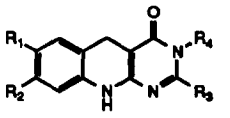
To test whether both of the methoxyl substituents present in **1b** are required for expression of sub-micromolar c-*src* inhibitory activity, the mono-methoxylated derivatives **1c** and **1d** were prepared. As can be seen in theTable, the 8-methoxy substituent (R_2 in Table) is largely responsible for the potency boost observed for **1b** relative to the parental lead **1a**.

Analogs **1e - 1g** were prepared as structure-activity probes of the pyrimidinone ring system. Methylation of the N-3 position (**1e**) produces only a 2-fold reduction in potency against c-*src*, suggesting that the pyrimidinone N-H functionality of **1b** is not interacting in a critical manner with the target enzyme. However, an attempt to enhance the solubility properties within this series by the incorporation of a 2-morpholinoethyl substituent (**1f**) leads to a substantial (200-fold) reduction in potency. It remains to be determined whether this potency loss is driven by unfavorable steric or ionic interactions. Based on the loss (~4-fold) in activity observed for **1g**, introduction of substituents into the 2-position of the pyrimidinone ring system appear to be disfavored.

The enzyme inhibition results obtained for compounds **4b - 8** (Table) reveal some very interesting structure-activity-relationships concerning the tricyclic core structure. The fully aromatized precursor (**4b**) of **1b** was shown to be a 60-fold less potent inhibitor of c-*src*. Blocking of the central ring N-H functionality with a relatively small alkyl substituent (**6**) leads to a >50-fold reduction in tyrosine kinase inhibitory activity,

suggesting that the N-H group may be directly interacting with the enzyme. Elimination of the central methylene linker of **1**, generating either indole-based derivative **7** or uncyclized phenylaminopyrimidinone **8** produces a substantial loss of activity. These results suggest a very specific relationship between the 5,10-dihydropyrimido[4,5-b]quinolin-4(1H)-one core structure and c-*src* inhibitory activity.

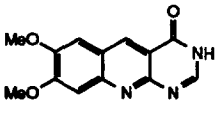
Table - In Vitro Kinase Inhibitory Activities.



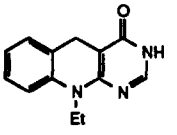
1

#	R_1	R_2	R_3	R_4	c-*Src* IC_{50} (μM)	PKA IC_{50} (μM)	EGFR IC_{50} (μM)
1a	H	H	H	H	9.1	>3000	N.D.
1b	OMe	OMe	H	H	0.5	>3000	75
1c	OMe	H	H	H	3.7	>3000	N.D.
1d	H	OMe	H	H	0.5	>3000	N.D.
1e	OMe	OMe	H	Me	1.2	>3000	160
1f	OMe	OMe	H	$(CH_2)_2$-N-Morpholino	105	>1000	N.D.
1g	H	H	Et	H	38	>3000	N.D.
4b					32	>750	N.D.
6					550	960	N.D.
7					293	N.D.	N.D.
8					1550	>4000	N.D.

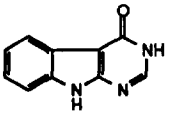
N.D. = Not determined



4b



6



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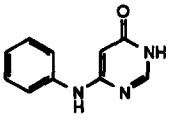

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In order to better understand the nature of c-*src* inhibition elicited by this class of inhibitors, mode of inhibition studies were performed on **1b**.[15] While the effects of **1b** are not altered by varying peptide substrate concentrations, significant effects on V_{max} and K_M are observed with varying concentrations of the ATP cofactor. Modeling using the weighted nonlinear least-squares curve-fitting program Enzyme PC reveals **1b** inhibits c-*src* in a pure-mixed-noncompetitive mode with respect to ATP. This finding suggests that **1b** binds at a site distinct from that of the nucleotide-binding pocket, which may play a role in the selective inhibition observed.

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