Bioorganic & Medicinal Chemistry Letters. Vol 2, No 12, pp. 1771-1774, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00

ARYLAMIDES OF HYDROXYLATED ISOQUINOLINES AS PROTEIN-TYROSINE KINASE INHIBITORS

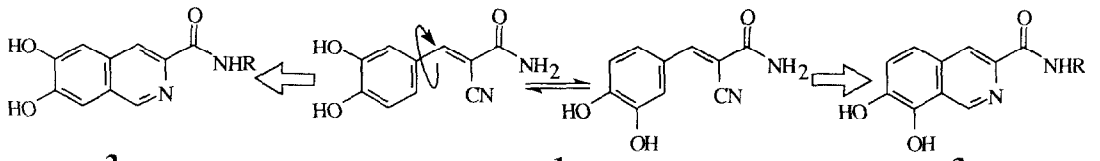
Terrence R. Burke, Jr.,*,† Harry Ford,† Nir Osherov,§ Alexander Levitzki,§
Irena Stefanova,¶ Ivan D. Horak¶ and Victor E. Marquez†

†Laboratory of Medicinal Chemistry, Developmental Therapeutics Program, Division of Cancer Treatment, Bldg. 37, Rm 5C06, and ¶Metabolism Branch, Division of Cancer Biology, Diagnosis and Centers, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, §Department of Biological Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

(*Received* 8 *October* 1992)

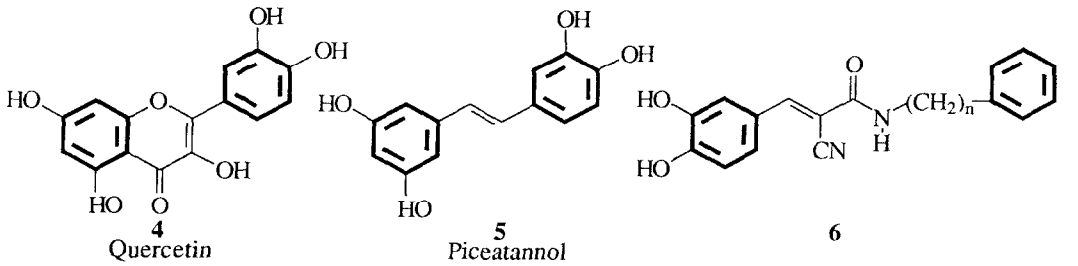
Abstract: Aryl-substituted amides of isomeric 6,7- and 7,8-dihydroxyisoquinoline-3-carboxamides (**2** and **3**) were prepared. Divergent structural requirements were observed for inhibiting $p56^{lck}$ and epidermal growth factor receptor (EGFR) protein-tyrosine kinases (PTKs), with the 7,8-dihydroxy substitution pattern being essential for $p56^{lck}$ activity, and the arylamide being required for EGFR potency. The work presents a useful approach toward the design of inhibitors which can discriminate between different PTKs.

Kinase mediated phosphorylation of hydroxyl-bearing amino acid residues within certain proteins is a key component of the complex mechanism of cellular signal transduction. Tyrosyl residues represent particularly important substrates for such enzymes, with protein-tyrosine kinases (PTKs) playing central roles in the regulation of cellular activation and mitogenesis. The association of aberrantly or overly expressed PTKs with a number of proliferative disorders makes inhibitors of specific PTKs appealing as antineoplastic therapeutics.[1,2] However, the development of PTK inhibitors is hampered by both a lack of information regarding the three dimensional characteristics of any PTK and by a lack of clarity on the mechanisms of action for many known PTK inhibitors. Solution of the X-ray crystal structure of the catalytic domain of the serine/threonine-specific protein kinase A (PKA) provided useful insight into the design of PTK inhibitors due to the high degree of homology of PKA with PTKs at the catalytic domain.[3] Based on the PKA results, a PTK catalytic site would be expected to resemble a deep planar cleft. This is consistent with the ability of a number of PTK inhibitors to assume a high degree of planarity.[1] Predicated on the premise that extended planarity may be an important determinant for interaction at PTK catalytic sites, we have recently prepared a series of planar bicyclic analogues as conformationally constrained mimetics of the broad styryl-based class of PTK inhibitors typified by α-cyano-3,4-dihydroxycinnamamide **1**.[4,5]



2 **1** **3**

As shown above, isomeric 6,7- and 7,8-dihydroxyisoquinoline-3-carboxamides **2** and **3** were designed to represent the two 180° rotational conformers of **1**. Initial testing in the lymphocyte-specific PTK p56^{lck} indicated that the open-chain prototype **1** exhibited an IC_{50} value of 20 μM while the 6,7- and 7,8-dihydroxyisoquinolines **2a** and **3a** (R = H) showed IC_{50} values of 1900 and 0.5 μM respectively.[4] In order to further explore structural features which would enhance PTK selectivity and affinity of the hydroxylated isoquinoline nucleus, note was taken of the frequent occurrence of a second aryl ring in many PTK inhibitors (Figure 1). Aryl 3-carboxamides were therefore prepared of both 6,7-dihydroxyisoquinoline (**2**) and 7,8-dihydroxyisoquinoline (**3**), and these analogues tested for inhibitory potency in p56^{lck} and epidermal growth factor receptor (EGFR) PTK preparations (Table 1).



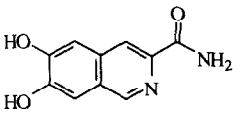
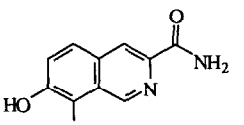
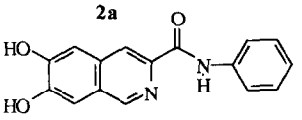
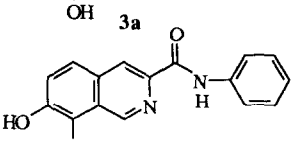
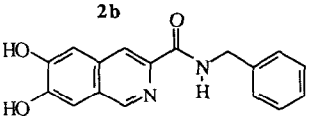
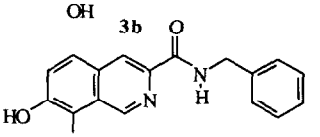
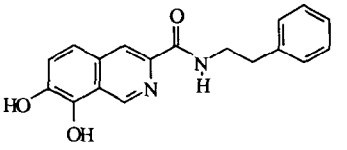
4 Quercetin **5** Piceatannol **6**

Figure 1. Examples of PTK inhibitors containing two aryl rings. Quercetin **4**[6] and Piceatannol **5**[7] are natural products, while arylamide **6**[8] is a synthetic derivative of cinnamamide **1**.

Two significant SAR conclusions can be drawn from this study. First, it is evident that the 7,8 - dihydroxyl substitution (**3a** - **d**) is a critical determinant for inhibition of p56^{lck}. All 6,7-dihydroxy isomers (**2a** - **c**) were essentially inactive against this PTK regardless of amide substitution. Equally interesting is the apparent insensitivity of p56^{lck} inhibition to the type of amide substitution. An alternative finding is indicated with EGFR PTK, where activity is independent of hydroxyl substitution. In this PTK the major structural requirement for activity is the presence of an aryl-substituted amide. While primary amides **2a** and **3a** had low activity, all aryl amides had good potency, with little difference between either 6,7-dihydroxylated (**2b** - **c**) or 7,8-dihydroxylated (**3b** - **d**) isomers.

The divergent and independent SAR criteria for activity against the $p56^{lck}$ and EGFR PTKs has important implications for the design of kinase-specific PTK inhibitors, and has in the present case resulted in the development of two inhibitors which have the ability to discriminate between $p56^{lck}$ / EGFR PTK; **3a** being selective for $p56^{lck}$ and **2c** being selective for EGFR. More importantly, this work highlights the value of using conformationally inflexible pharmacophores for the elaboration of highly specific inhibitors. This latter fact is consistent with a decreased ability of such analogues to conform to structural variations between different enzymes. These principles are presently being applied toward the development of new PTK-specific inhibitors.

Table 1. Inhibition of protein-tyrosine kinases[a]

	IC_{50} (μM)			IC_{50} (μM)	
	EGFR	$p56^{lck}$		EGFR	$p56^{lck}$
 **2a**	>100	1900	 **3a**	>100	0.5
 **2b**	5.6	600	 **3b**	7.7	1
 **2c**	3.1	>1000	 **3c**	1.4	1
			 **3d**	1.4	40

[a] Immunopurified enzyme preparations were utilized as previously described for EGFR PTK[9] and $p56^{lck}$ PTK.[6] Compounds were prepared as described.[10]

Acknowledgements. Appreciation is expressed to Dr. James Kelley and Ms. Pamela Russ of the LMC for providing mass spectral analyses.

References and Notes

1. Burke, T. R., Jr. *Drugs of the Future* **1992**, *17*, 119.
2. Dobrusin, E. M.; Fry, D. W. *Annual Reports in Medicinal Chemistry* **1992**, *27*, 169.
3. Knighton, D. R.; Zheng, J. H.; Ten Eyck, L. F.; Ashford, V. A.; Xuong, N. H.; Taylor, S. S.: Sowadski, J. M. *Science* **1991**, *253*, 407.
4. Burke, T. R., Jr.; Lim, B.; Russ, P.; Marquez, V. E.; Li, Z.-h.; Bolen, J. B.; Stefanova, I.; Horak, I. *J. Med. Chem.* (in review).
5. Gazit, A.; Yaish, P.; Gilon, C.; Levitzki, A. *J Med. Chem* **1989**, *32*, 2344.
6. Horak, I. D.; Popovic, M.; Horak, E. M.; Lucas, P., J.; Gress, R. E.; June, C. H.; Bolen, J. B. *Nature* **1990**, *348*, 557.
7. Geahlen, R. L.; McLaughlin, J. L. *Biochem. and Biophys. Res. Comm.* **1989**, *165*, 241.
8. Gazit, A.; Osherov, N.; Posner, I.; Yaish, P.; Poradosu, E.; Gilon, C.; Levitzki, A. *J. Med. Chem.* **1991**, *34*, 1896.
9. Levitzki, A.; Gazit, A.; Osherov, N.; Posner, I.; Gilon, C. *Methods Enzymol.* **1991**, *201*, 347.
10. Compounds **2a** and **3a** have previously been reported.[4] Compounds **2c**, **3c** and **3d** were prepared from the known[11] methyl 6,7-dihydroxyisoquinoline-3-carboxylate (for **2c**) and methyl 7,8-dihydroxyisoquinoline-3-carboxylate (for **3c** and **3d**) by heating at 100° C with the appropriate amine (benzyl amine for **2c** and **3c** and phenethyl amine for **3d**) as a methanolic solution in a sealed reaction vial for 1 - 3 days. Treatment of the reaction mixtures with cold, dilute aqueous HCl precipitated crude amide products, which were purified by hplc as described below. Compounds **2b** and **3b** were prepared by mixed anhydride coupling (ethyl chloroformate) of the free carboxylic acids with aniline. Acylated products were refluxed with aqueous methanolic HCl to provide the free dihydroxylated amides, which were purified by hplc using a Hamilton PRP - 1 reverse phase column (250 mm x 22 mm; 10 μ particle size) with an acetonitrile - H_2O gradient (20 mL / min). Overall yields of hplc purified products were in the range 10 - 15%. Except as noted products provided satisfactory C,H,N combustion analysis (**2b**: calcd; C, 54.37; found 53.87) and FABMS.
11. Burke, T. R., Jr.; Russ, P. L.; Marquez, V. E. *Heterocycles* **1992**, *34*, 757.