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5,7-Dimethoxy-3-(4-pyridinyl)quinoline Is a Potent and Selective Inhibitor of Human Vascular β-Type Platelet-Derived Growth Factor Receptor Tyrosine Kinase

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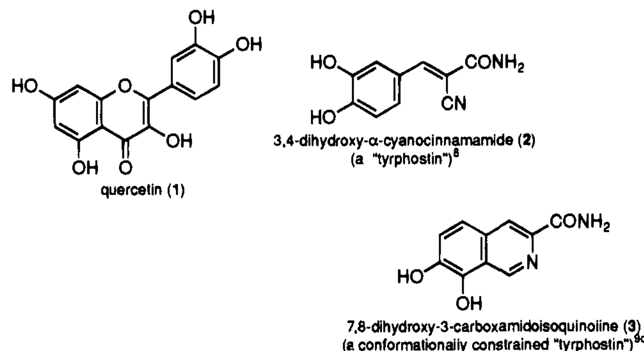
The major physiological role ascribed to the action of platelet-derived growth factor (PDGF) is in the regulation of normal cell proliferation.[1] Upon binding to its receptor, this cytokine induces receptor dimerization and autophosphorylation which serves to secure a bioactive conformation for PDGF receptor tyrosine kinase (PDGFrTK).[2] The activated receptor phosphorylates cytoplasmic substrates on the phenolic hydroxyl group of tyrosine residues facilitating second messenger function.[3] Small-molecule inhibitors of PDGFrTK may represent a novel class of therapeutic agents useful for the treatment of malignant and nonmalignant disease states involving excess cell proliferation.[1a] In addition to cancer,[4] these disease states include certain chronic inflammatory conditions (tissue fibrosis)[5] and atherosclerosis or restenosis.[6]

A survey of the literature for inhibitors of tyrosine kinases reveals three predominant generic structural families: the flavonoids,[7] exemplified by quercetin (**1**),



and the "acyclic" tyrphostins[8] and the "cyclic mimetic" tyrphostins,[9] represented by **2** and **3**. We conducted a substructure search of our proprietary compound file collection based on **1**–**3**. As a result of this search, a series of 3-(4-pyridinyl)quinolines[10] was discovered to exhibit selective inhibition of β-type PDGFrTK, with 5,7-dimethoxy-3-(4-pyridinyl)quinoline (**9**) emerging as one of the more potent inhibitors (IC_{50} = 80 nM). A subset of these inhibitors (**4**–**15**) and their activities are presented in Table 1.[11]

The parent compound, 3-(4-pyridinyl)quinoline (**4**) inhibits PDGF receptor tyrosine kinase activity with an IC_{50} = 1.5 μM. Mono- or dihydroxylation of the quinoline ring at positions 5, 6, or 7 [**5** (9.2 μM), **8** (1.3 μM), and **10** (36 μM)] typically leads to an attenuation of potency relative to **4**. In contrast, introduction of the more lipophilic methoxy or chlorine substituents in the quinoline nucleus results in enhanced inhibitory activity as exemplified by compounds **6**, **7**, **9**, **11**, and **12**. Each of these compounds has IC_{50} values <1.0 μM against PDGFrTK. The chlorine atom at ring position 7 (**13**: R_4 = Cl, 0.3 μM) is more effective than either the methoxy (**6**: R_4 = OMe, 0.6 μM) or the hydroxy (**5**: R_4 = OH, 9.2 μM) group at this position. Of most significance is the inhibitory potency of quinoline **9**, which possesses an IC_{50} = 80 nM against the enzyme.[12] It is evident that 5,7-disubstitution on the quinoline ring is a preferred substitution pattern, yielding the greatest inhibitory activity. Both the 6,7 and 5,6,7 substitution patterns afford compounds possessing less activity than **9**. Similar SAR's demonstrating a dependence on ring pattern substitution and tyrosine kinase activity and selectivity have been noted in the flavonoid and tyrphostin classes of inhibitors.[7c,8,9c] For example, Burke and co-workers have reported a 3800-fold difference in the potency of inhibition of $p56^{lck}$ by isoquinoline (**3**) versus its 6,7-dihydroxy analog.[9c]

3-(4-Pyridinyl)quinoline analogs bearing a methyl group at ring position 4 are devoid of PDGFrTK activity. This observation is supported by direct comparison of compound pairs **7** (R_1 = H; R_3 = OMe, 0.4 μM) and **14** (R_1 = Me; R_3 = OMe, >50 μM), and **6** (R_1 = H; R_4 = OMe, 0.6 μM) and **15** (R_1 = Me; R_4 = OMe, >50 μM). The poor tolerance of the C(4) methyl group may be due to an unfavorable nonbonded interaction between the inhibitor and the enzyme binding site or a more subtle, yet unfavorable, *ca.* 30° change in the dihedral angle between the quinoline–pyridine biaryl system.[13]

Inhibitors **9**–**11** and **13** were also examined for their selectivity against other tyrosine kinases including epidermal growth factor receptor tyrosine kinase (EGFrTK), erbB2, $p56^{lck}$, as well as the serine/threonine protein kinases A and C. With the exception of quinoline **10**, the compounds display greater selectivity (>100 times) for PDGFrTK and produced less than 50% inhibition at 10 μM against each of the other tyrosine kinases. In the case of inhibitor **10**, the dihydroxy congener of **11**, an IC_{50} of approximately 5 μM for inhibition of EGFrTK activity is observed.[14] Inhibitor **9** was further evaluated against several other receptors[15] and is free of ancillary receptor activity at concentrations ≤10 μM.

In further studies, several inhibitors from this series were also evaluated for their ability to inhibit PDGF-stimulated [^{3}H]thymidine incorporation into DNA of primary human vascular smooth muscle cells (Table 1). Compound **9** inhibits agonist-stimulated thymidine incorporation into DNA dose dependently with an IC_{50} = 0.9 μM.[16] In general, all other compounds are approximately 10 times less potent in this cell assay relative to their inhibition of purified PDGFrTK. However, there is an apparent correlation between potency

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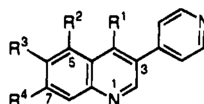
Table 1. Structure–Activity Relationship of the 3-(4-Pyridinyl)quinolines **4–15** against Human Vascular Smooth Muscle PDGFrTK



compd	R^1	R^2	R^3	R^4	IC$_{50}$, μM						
					PDGFrTK^{a,b}	EGFrTK^{c,d}	erbB2^d	p56$^{lck\,d}$	PK-A^d	PK-C^d	VSMC^{b,e}
4	H	H	H	H	1.5	*f*	*f*	*f*	*f*	*f*	*f*
5	H	H	H	OH	9.2	*f*	*f*	*f*	*f*	*f*	>10^h
6	H	H	H	OMe	0.6	*f*	*f*	*f*	*f*	*f*	6.5
7	H	H	OMe	H	0.4	*f*	*f*	*f*	*f*	*f*	*f*
8	H	OH	H	OH	1.3	*f*	*f*	*f*	*f*	*f*	5.5
9	H	OMe	H	OMe	0.08	>10	>10	>10	>10	>10	0.9^i
10	H	H	OH	OH	36	5^g	>10	>10	>10	>10	*f*
11	H	H	OMe	OMe	0.3	>10	>10	>10	>10	>10	1.8
12	H	OMe	OMe	OMe	0.4	*f*	*f*	*f*	*f*	*f*	3.5
13	H	H	H	Cl	0.3	>10	>10	>10	>10	>10	*f*
14	Me	H	OMe	H	>50	*f*	*f*	*f*	*f*	*f*	*f*
15	Me	H	H	OMe	>50	*f*	*f*	*f*	*f*	*f*	*f*

^{a} In vitro IC$_{50}$ determinations were performed in triplicate according to the assay protocol described in ref 18. ^{b} Standard deviations were <10%. ^{c} See ref 14. ^{d} Assays were performed according to ref 19. ^{e} Inhibition of [^{3}H]thymidine incorporation into intact vascular smooth muscle cells (VSMC). IC$_{50}$ determinations were performed in triplicate according to the assay protocol described in ref 16. ^{f} Data not determined. ^{g} Estimated from the percent inhibition at 10 μM, see ref 14. ^{h} 25% inhibition at 10 μM. ^{i} The IC$_{50}$ of **9** is 0.9 μM with a confidence interval of 0.76–1.02 (n = 9).

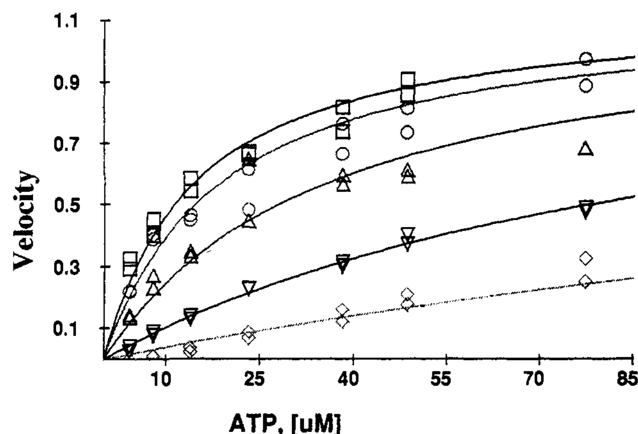


Figure 1. K_i determination of quinoline **9** in the PDGFrTK autophosphorylation assay. PDGF:PDGFr complexes were incubated with increasing concentrations of ATP in the absence (□) or presence of **9** (○, 0.5 nM; △, 25 nM; ▽, 100 nM; ◇, 316 nM). These data represent one of three identical experiments. The ATP substrate K_m = 11 ± 5 μM; the calculated K_i for quinoline **9** = 14 ± 2 nM.

of tyrosine kinase inhibition and the inhibition of this PDGF-mediated cellular event for this series of compounds.

It is established that autophosphorylation of the PDGF receptor is an obligatory first event for receptor activation.[2] In an enzyme kinetic study of PDGFrTK autophosphorylation using ATP as the substrate, **9** was found to effectively inhibit this reaction. The data presented in Figure 1 clearly show a dependence of IC$_{50}$ on ATP concentration with the kinetic data best fitting a competitive model of inhibition (K_i = 14 ± 2 nM). As a point of reference, ATP-γ-S, a nonhydrolyzable ATP analog, demonstrated a classical competitive inhibition profile against ATP in an identical PDGFrTK autophosphorylation kinetic study design.[17] The K_i for ATP-γ-S is 11.0 ± 0.5 μM, *ca.* 1000-fold less potent than **9**. Although the inhibition profile for **9** suggests that this compound interacts at the ATP binding site of PDGFrTK, **9** exhibits high enzyme affinity and on the basis of all other kinase data, exquisite selectivity for PDGFrTK.

In summary, 5,7-dimethoxy-3-(4-pyridinyl)quinoline (**9**) is an inhibitor of PDGFrTK and represents one of the most potent (IC$_{50}$ = 80 nM) and selective (>100-fold) inhibitors of this tyrosine kinase yet to be described. This discovery of **9** serves to support the original hypothesis of Gazit[8a] that it may be possible to identify potent, selective inhibitors of receptor tyrosine kinases. The results presented here and elsewhere,[9,10] suggest that the quinoline or isoquinoline bicyclic nucleus may be a common pharmacophore among the EGFr, PDGFr, and p56^{lck} tyrosine kinases.

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(11) Singh, B.; Lesher, G. Y. A Convenient Synthesis of 3-(4-Pyridinyl)-quinolines. *J. Heterocycl. Chem.* **1991**, *28*, 1453–1454. Analogs **5**, **8**, and **10** were derived from their respective methoxy congeners by treatment with 48% HBr under reflux conditions. All compounds exhibited acceptable C, H, N analysis.

(12) The IC_{50} values for **9** vary as a function of assay ATP concentration and the reported values are given where [ATP] > K_m. For example, the IC_{50} = 80 nM reported for **9** was the IC_{50} obtained at a high [ATP] of 20 × K_m. The K_i for **9** is calculated to be 14 ± 2 nM, assuming a competitive interaction at the ATP site (see Figure 1). The dependence of IC_{50} on ATP concentration is predicted to be observed for the other compounds in this series.

(13) The dihedral angle in an energy-minimized conformation of **6** (Macromodel) was determined to be 22°, while this angle is increased to 55° in **15**.

(14) Inhibitors **9**–**11** and **13** were assayed against human EGFrTK at a single concentration of 10 μM. The percent inhibition values found were as follows: **9**, 30% at 10 μM; 11, −8% at 10 μM; **13**, 1% at 10 μM and for **10**, 84% at 10 μM.

(15) Quinoline **9** produced <50% inhibition of binding of radioligands to the following receptors at 10 μM: general opioid, α_1, α_2, β_1, and β_2 adrenergic, central benzodiazepine, general serotonergic, dihydropyridine-sensitive Ca^{2+} channel, muscarinic cholinergic, histamine H_1, D_1 and D_2 dopamine, and A_1 and A_2 adenosine.

(16) Assay description: Inhibition of PDGF-stimulated [^{3}H]thymidine incorporation into DNA of primary human vascular smooth muscle cells (Clonetics) was quantitated in serum-starved (48 h) cells for multiple concentrations of various inhibitors. Cells were incubated with inhibitor for 2 h, followed by addition of PDGF-BB (10 ng/mL) for 24 h and of [^{3}H]thymidine (0.5 μCi/mL) for an additional 24 h. Following the 48-h incubation period, the medium was aspirated, and the cells were gently washed three times with 0.5 mL of ice-cold 5% trichloroacetic acid. The cells were extracted with 0.1 N aqueous NaOH (0.25 mL, 30 min, 25 °C), which was neutralized with 1.0 N aqueous HCl and counted. The percentage inhibition for each concentration of inhibitor was calculated by comparison to vehicle-treated (≤0.5% DMSO) cells. IC_{50} values were calculated and values represent mean ± SE of three or four experiments.

(17) Unpublished observation.

(18) PDGFrTK assay description: The PDGFrTK autophosphorylation assay employs an ELISA format measuring PDGK-stimulated kinase activity from wheat germ purified cell extracts. Essentially, PDGFrTK from vascular smooth muscle cells is incubated overnight at 4 °C in 96-well plates with PDGF-BB (150 ng/mL) in the presence of 4 mM $MnCl_2$, 50 mM HEPES, 10% glycerol, 0.2% Triton X-100, 0.1 mM sodium vanidate, and 0.5 mM dithiothreitol (total volume = 65 μL). All test compounds are dissolved in DMSO. Test compounds (10 μL aliquots) are incubated with the PDGF-PDGFr complex for 30 min at 4 °C prior to the addition of ATP. Autophosphorylation is initiated at 4 °C by the addition of 25 μL ATP (final [ATP] = 200 μM), and the assay is terminated after 10 min by the addition of 50 μL of EDTA (final [EDTA] = 20 mM); 100-μL aliquots of the reaction mixture are transferred to corresponding wells of a second plate previously coated with anti-PDGFr antibody. The phosphorylated tyrosine residues in each well are detected at 25 °C in a spectrophotometric plate reader (OD = 405 nm) using a biotinylated anti-phosphotyrosine/avidin-horse radish peroxidase/ABTS detection system.

(19) The EGFrTK, $p56^{lck}$, and erbB2 assays are carried out similarly to the PDGFrTK assay, except that no stimulating ligand is employed and the enzyme is captured onto antibody-coated plates before the reaction is initiated with ATP. The PKA and PKC assays are conducted using a modification of the literature procedures. Detailed experimental protocols for each of these assays will be reported separately.