Tyrphostins. 5. Potent Inhibitors of Platelet-Derived Growth Factor Receptor Tyrosine Kinase: Structure−Activity Relationships in Quinoxalines, Quinolines, and Indole Tyrphostins

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A series of 3-indoleacrylonitrile tyrphostins, 2-chloro-3-phenylquinolines, and 3-arylquinoxalines were prepared and tested for inhibition of platelet-derived growth factor receptor tyrosine kinase (PDGF-RTK) activity. The potency of the inhibitors was found to be quinoxalines > quinolines > indoles. Lipophilic groups (methyl, methoxy) in the 6 and 7 positions and phenyl at the 3 position of quinoxalines and quinolines were essential for potency, in contrast to the hydrophilic catechol group in tyrphostins active against EGFR kinase inhibition at different sites. The inhibitors showed selectivity for PDGF and were not active against EGF receptor and HER-2/c-ErbB-2 receptor.

Introduction

Platelet-derived growth factor (PDGF) is a potent mitogen and mitogen for mesenchymal cells (for a recent review, see ref 1). The PDGF AA, AB, and BB isoforms interact differentially with PDGF α- and *β*-receptors. Both PDGF receptor types are closely related transmembrane tyrosine kinases whose activation by ligand binding is essential for cellular signaling. PDGF and its receptors have been shown to be involved in regulation of vital aspects of embryogenesis.[2,3] In the adult, most of the proposed functions of PDGF relate to different responses to injury as wound healing and inflammation.[4,5]

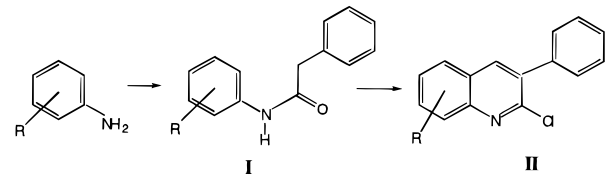
PDGF-induced cell proliferation is also likely to be involved in a number of pathophysiological conditions such as atherosclerosis, restenosis,[6] and fibrosis as well as in certain cancers such as gliomas. In atherosclerosis and restenosis, it is believed that PDGF, which is secreted by the injured wall of the blood vessel, induces the proliferation and migration of smooth muscle cells from the media to form the neointima.[6]

For various cancers it has been shown that the tumor cells express functional PDGF and PDGF receptors, suggesting that this autocrine loop may contribute to tumor growth.[7] Indeed, interference with PDGF signaling led, in various tumor cell lines expressing PDGF and PDGFR, to growth inhibition and reversion of the transformed phenotype.[8,9] Also, PDGF expression level has been found to correlate with malignancy in a number of human tumors.[10−15]

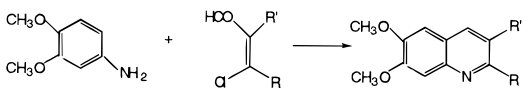
We and others have reported on the biological effects of PDGF tyrosine kinase blockers from the benzenemalononitrile family,[16] indole-containing blockers,[17] quinoline blockers,[18,19] quinoxaline blockers,[20,21] and aminopyrimidine blockers.[22] In this article we report on the properties of indole, quinoline, and a large number of quinoxaline compounds and on structure−activity relationships (SAR) which are important for inhibition of the PDGF receptor tyrosine kinase.

Chemistry

The tyrphostins of Table 1 were prepared by Knoevenagel condensation of the 3- or 5-formylindole with substituted acetonitriles. The quinolines **II** in Table 2 were prepared by Vilsmeyer reaction of amides **I**:[23]



I **II**



Quinolines **18** and **19** were obtained by condensation of 3,4-dimethoxyaniline with the substituted *β*-chloroacrolein (ref 24). Quinoxalines were synthesized mostly by condensation of the appropriate 1,2-diamino aromatics with the 1,2-keto aldehyde or diketo compounds. Several quinoxalines were prepared by exchange reaction of the bis-thiosemicarbazones[25] and condensation with 1,2-phenylenediamine.

Results and SAR

Our goal was to find inhibitors which would be both potent and selective as blockers of the PDGF receptor (PDGFR). Tyrphostins such as compound **1** and its analogs containing a catechol ring were moderately potent against PDGFR but not selective (Table 1).

Tyrphostins **2**−**4**, in which a 5-indole ring was introduced instead of a catechol ring, were either of low potency **(2),** not selective **(4)**, or even more potent

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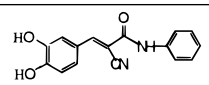
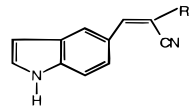
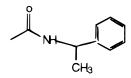
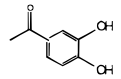
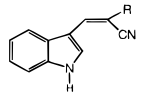
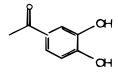
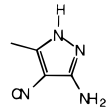
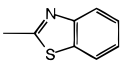
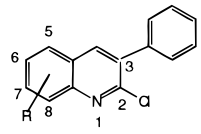
Table 1. Indole-Containing Tyrosine Kinase Inhibitors

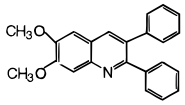
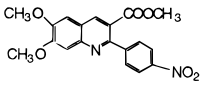
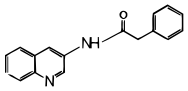
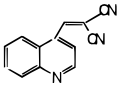
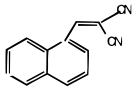
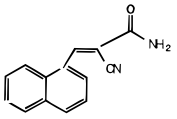
No		IC_{50}, μM PDGFR[a]	IC_{50}, μM EGFR[a]
1		10	10
			
	R		
2		100	100
3		>300	10
4		10	10
			
	R		
5		1	30
6		>100	>100
7		6.5	65
8		20	>300
9		20	300
10		6.5	65
11		100	100
12		100	100

[a] Inhibition of PDGFR and EGFR autophosphorylation in Swiss 3T3 membranes was conducted as described in ref 20. The IC_{50} values were determined in three independent experiments. IC_{50} values are reproducible within <7−8% between replicate experiments.

against EGFR **(3)** than toward the PDGFR. The 3-indole analog **(5)** exhibited both better selectivity and potency. We therefore prepared a series of 3-indole derivatives, with substituents that showed good efficacy against PDGFR. The best were compounds **7** and **8** (R = thioamide and amide, respectively), but both exhibited reduced efficacy and selectivity compared to tyrphostin **5**.

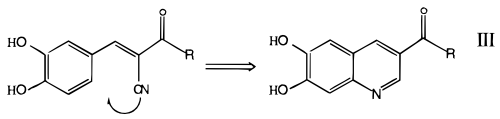
Table 2. Quinoline-Containing Tyrosine Kinase Inhibitors



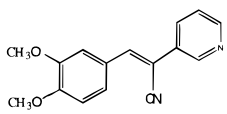
No	R	IC_{50}, μM PDGF[a]	IC_{50}, μM EGF[a]
13	6-OCH_3	>30	>30
14	6,7-methylene dioxy	>30	>30
15	5,6,7-$(OCH_3)_3$	>30	>30
16	5,8-$(OCH_3)_3$	>30	>30
17	6,7-$(OCH_3)_2$	1.5	>30
18		>30	>30
19		>30	>30
20		>30	>30
21		10	10
22		>100	>100
23		>100	>100

[a] Determined as in Table 1. IC_{50} values from a representative experiment. IC_{50} values are reproducible within <10% from experiment to experiment.

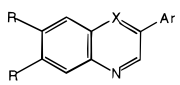
None of compounds **1**−**12** were active in intact Swiss 3T3 cells as blockers of PDGFR. We therefore tried to design more rigid tyrphostins. Recently two classes of rigidified tyrphostins were shown to exhibit reasonable inhibitory activity against $p56^{lck}$ **(III)**[26] and EGFR[16] in vitro against the isolated kinases. **III** and its analogs



III



IV



V

are obtained through incorporation of the cyanovinyl group of tyrphostins into a heteroaromatic ring. In **IV**[16] liphophilic dimethoxy (or dichloro) replaces dihydroxy groups, and the second aryl is connected directly and

not through the amide group to the cyanovinyl pharmacophore. The vinyl element also occurs in "stilbenic" tyrosine kinase inhibitors. We, therefore, decided to combine all these elements in our design to yield compounds of general structure **V**.

We first prepared a series of 2-chloro-3-phenylquinolines (**V**, X = CH), presented in Table 2. Only one compound in this series, **17**, with 6,7-dimethoxy substitution was both active and selective against PDGFR. Movement of the methoxy groups to different positions, exchanging chlorine in the 2 position for a bulky phenyl group (compound **18**), or exchanging the 3-phenyl for an ester or an amide group (compounds **19** and **20**) all resulted in loss of activity. The 4-quinolinylidenemalononitrile **21** inhibits both PDGFR and EGFR with identical potency (IC_{50} = 10 μM). The removal of the nitrogen ring to yield naphthalene analogs **22** and **23** abolishes activity.

The next logical step was to try the related quinoxaline system (**V**, X = N), with the additional nitrogen added at the 4 position. A large series of quinoxalines was synthesized and is described in Table 3.

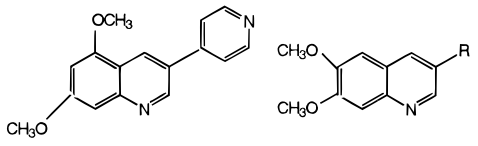
Inspection of the quinoxalines showed that the most potent inhibitors of PDGFR are **25, 26**, **54**, and **66** with IC_{50} = 0.4, 0.6, 0.8, and 0.9 μM, respectively. These compounds possess lipophilic dimethoxy, dimethyl, or benzo groups at the 6 and 7 positions and phenyl at the 3 position. Interestingly, the 6,7-dichloro analog **52** was inactive. Substitution of the 6 and 7 positions with hydrophilic dihydroxy or hydroxymethoxy groups (**43** and **39**, with IC_{50} = 100 and 100 μM) abolished activity. Attaching dihydroxy groups on the other side of the molecule, on the 3-phenyl, did not reduce potency (compound **54** with IC_{50} = 0.6 μM compared to compound **25** with IC_{50} = 0.4 μM). Polar groups at the 6 position lowered the IC_{50} (**27**, 6-COOH, has IC_{50} > 50 μM; **44**, 6-NO_2, IC_{50} = 25 μM compared to **24**, IC_{50} = 10 μM), while the large 6-benzoyl derivative yields somewhat better inhibition (**45**, IC_{50} = 6 μM).

Phenyl at the 3 position (compound **24**) is much better than 2-furan (**32**), 3-indole (**33**), or 2′-thiophene (**31**) and its derivatives, while the 3-CH_3 analog is inactive.

Adding to 3-phenylquinoxaline almost any substituent at the 2 position (methyl, Ph, furan, pyridyl, CHO, ethyl ester, dimethylamino, or phosphate) reduced or abolished activity; however, the 2-carboxy analog **56** retained potency (IC_{50} = 1.0 μM).

The quinoxalines were found to be inactive against EGFR and Flk-1/KDR when tested in cell free system or intact Swiss 3T3 cells (data not shown, but see ref 20). Several compounds (**59**−**69**) related to quinoxalines were also prepared, but none had improved IC_{50} values compared to its appropriate quinoxaline analog. Also three dimeric compounds (**67**−**69**), designed in the hope they could interact with both sites on two PDGF receptors, were not active. Compound **66** which is a close analog of **25** and **26** was found to be active (IC_{50} = 0.9 M).

Recently, quinoline inhibitors were described by other groups[18,19] One group (ref 19) tested a series of 3-(4-pyridinyl)quinolines **VI** and found that the 5,7-dimethoxy derivative is the most potent (IC_{50} = 0.08 μM) while 6,7-dihydroxy yields IC_{50} = 36 μM. These findings contrast with our results where the most potent inhibitor in the quinoline series (**17**, IC_{50} = 1.5 μM) possesses 6,7-dimethoxy groups, while substitution at the 5 or 8 position in compounds **15** and **16** reduced potency.



VI VII

R = 3-Py, 3-Thiophene

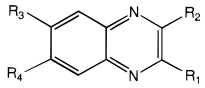
Another group[18] described quinolines **VII** in which the most potent inhibitors (IC_{50} values in the low nanomolar range) had either 3-pyridyl, 3-thienyl, styryl, or 4-substituted phenyl at the 3-quinoline position and 6,7-dimethoxy groups. Here, too, 5,7-dimethyl derivatives were potent, while the 6,7-dihydroxy group abolished activity.

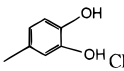
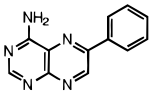
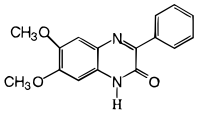
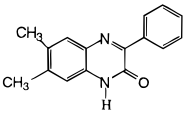
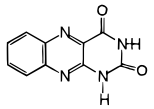
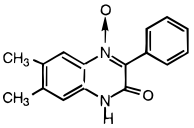
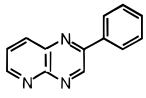
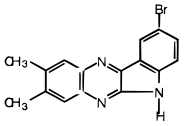
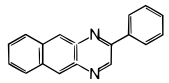
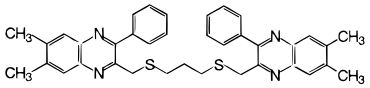
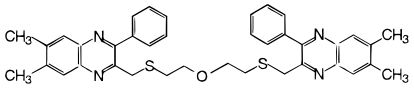
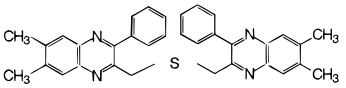
Discussion

In a previous study[20] we have demonstrated that two quinoxalines, compounds **25** (AG 1295) and **26** (AG 1296), were very potent and selective inhibitors of PDGFR but inactive against EGF receptor kinase. The selective potencies of AG 1295 and AG1296 were demonstrated both in a cell free system and in intact Swiss 3T3 cells. In the present study we report in more detail on the potency of 46 quinoxalines as inhibitors of the PDGFR kinase in intact cells. These compounds are inactive against EGFR in biochemical assays and cellular assays (ref 20 and data not shown). We show the evolution of the rationale moving from first-generation *cis*-arylidenemalononitrile (BMN) tyrphostins to closed structures, the quinolines, moving on to quinoxalines. Indeed, BMN kinase inhibitors exemplified by compound **1**[27] show no selectivity and block PDGFR and EGFR equipotently in a biochemical assay, using the Swiss 3T3 membrane preparation.[20] Indole-containing tyrphostins show some improvement in some cases (compounds **5**, **7**, and **8**, Table 1), but most compounds are either inactive or possess low potency (Table 1). Closing the pharmacophore of the tyrphostin into a second-ring heterocycle, thus eliminating the *cis*-cyano group in the arylidenemalononitrile, generates quinolines which yielded a lead structure (compound **17**, Table 2) with good efficacy against the PDGFR and promising selectivity vis-a-vis the EGFR. The insertion of another nitrogen into the quinoline ring yielding the quinoxaline moiety generated several compounds (**25**, **26**, **54**, **56**, and **66**) with excellent efficacy ($IC_{50} \leq 1.0$ μM) toward PDGFR in intact cells. These compounds were all ineffective against the EGFR at 50−100 μM (not shown). Elimination of the nitrogen atom from the quinoline (compounds **22** and **23**, Table 2) yielded naphthalene compounds with no kinase inhibitory activity.

Only a portion of the other compounds were tested on Swiss 3T3 membranes, whereas all the quinoxalines (Table 3) were tested for PDGFR inhibition in intact cells. Thus one cannot exclude the possibility that some of the compounds may exert their inhibitory effects via indirect mechanisms. An interesting point is that the most potent quinoxaline PDGFR inhibitors, **25**, **26**, and **66**, are lipophilic compounds, in contrast to the hydrophilic BMN tyrphostins, which contain the catecholic

Table 3. Inhibition of PDGFR Kinase in Intact Cells by Quinoxalines



No	R1	R2	R3	R4	IC_{50}, μM [a]
24	H	Ph	H	H	10.0
25	H	Ph	CH_3	CH_3	0.4
26	H	Ph	OCH_3	OCH_3	0.8
27	H	Ph	COOH	H	>50
28	CH_3	CH_3	H	H	>100
29	Ph	Ph	H	H	>50
30			H	H	>100
31	H		H	H	100
32	H		H	H	>100
33	H		H	H	>100
34	H		H	H	>100
35	H		H	H	>100
36	H		H	H	>100
37	H		H	H	>100
38	CH_3	Ph	H	H	>100
39	H	Ph	OCH_3	OH	100
40	H	Ph	CH_3	H	90
41			H	H	100
42			CH_3	CH_3	>100
43	H	Ph	OH	OH	>100
44	H	Ph	NO_2	H	25
45	H	Ph		H	6
46	CH_2Br	Ph	CH_3	CH_3	>50
47	CH_3	Ph	CH_3	CH_3	>50
48	Cl	Ph	CH_3	CH_3	>50
49	$CH_2PO(OEt)_2$	Ph	CH_3	CH_3	>50
50	$CH_2PO(OH)_2$	Ph	CH_3	CH_3	>50
51	CH_2OEt	Ph	CH_3	CH_3	>50
52	H	Ph	Cl	Cl	>50
53	CH_3	CH_3	CH_3	CH_3	>50
54	H		CH_3	CH_3	0.6
55	COOEt	Ph	CH_3	CH_3	>50
56	COOH	Ph	CH_3	CH_3	1.0
57	CHO	Ph	CH_3	CH_3	>50
58	CH_2-N$(CH_3)_2$	Ph	CH_3	CH_3	>50
59					>50
60					>50
61					10
62					>50
63					>50
64					35
65					>50
66					0.9
67					>50
68					>50
69					>50

[a] IC_{50} values in repeated experiments vary <10% from experiment to experiment. The most potent compounds, **25** and **26**, were run in parallel to each set of compounds. The IC_{50} values for compounds **25** and **26** in repeated experiments vary <6−8% of the value quoted in the table.

ring. Exchanging the dimethoxy groups at 6 and 7 positions of **26** to dihydroxy groups yields compound **43** with greatly reduced potency. Quinoxalines **54** and **56** possess the 5,6-dimethyl lipophilic region, which seems to be the critical pharmacophore for potency and selectivity of the PDGFR. These inhibitors possess also additional polar groups on the other side of the molecule.

The quinoxaline pharmacophore seems, so far, to give the most potent and most selective PDGFR inhibitors. In this regard the quinoxaline compounds are superior to AG 17 (RG 50872) and AG 370 (compound **2**) we reported previously. AG 17 is highly potent in its antimitogenic activity against PDGF-stimulated growth of rabbit vascular smooth muscle cells as well as a PDGFR kinase blockers in intact cells. Interestingly, the most potent and selective inhibitors of the EGFR kinase are quinazoline derivatives, in which the 1,4-diaza structure of the quinaxoline is changed to 1,3-diazanaphthalene. These derivatives block the EGFR kinase in intact cells with $IC_{50} \sim 1-400$ nM and are devoid of activity against the PDGFR kinase (Gazit et al., unpublished; Ben Bassat et al., unpublished). This difference in selectivity between quinoxalines and quinazolines probably reflects the structural difference between the kinase domains of PDGFR and EGFR.

It remains to be seen whether relatively nonselective PTK inhibitors such as AG 17 (RG 50872) or selective PDGFR blockers will be more effective as agents to combat various disorders in which PDGF is involved.

Experimental Section

Methods and Materials. General directions have appeared previously.[27] Microanalyses were performed by The Hebrew University Analytical Services and are given in the Supporting Information.

Synthetic Methods. For each class of compounds, a general procedure is given followed by yield and melting points (mp) for the compounds in that class. NMR and MS data for these compounds is given in the Supporting Information. Starting materials for which no procedure or ref is given were obtained from Aldrich.

Compounds **1** and **2** were described by us previously (ref 27). Compound **10** was prepared according to literature (ref 28).

Compound 3. 5-Formylindole (0.3 g, 2 mM) and 0.4 g, 2 mM, of α-methylbenzyl cyanoacetamide (compound **43** in ref 27) in 15 mL of ethanol and 2 drops of piperidine were refluxed for 3 h. Water and HCl were added, and the reaction mixture was extracted with EtAc to yield a viscous oil. Chromatography on silica gel gave 0.42 g, 66% yield, of a pale-yellow solid: mp 76 °C; NMR ($CDCl_3$) δ 8.44 (1H, s), 8.25 (1H, s, vinyl), 7.88 (1H, dd, $J = 8.6$, 2.0 Hz), 7.68 (1H, d, $J = 2.0$ Hz), 7.4−7.2 (5H, m), 6.66−6.50 (2H, m), 5.26 (1H, quin, $J = 7.1$ Hz, CH), 1.60 (3H, d, $J = 7.1$ Hz, CH_3); MS *m/e* 315 (M^+, 24), 196 ($M - NCH(CH_3)C_6H_5$, 22), 195 (25), 188 (21), 173 (24), 168 (13), 149 (57), 145 (100), 134 (92), 116 (53).

Compound 4. 5-Formylindole (130 mg, 1 mM), 180 mg, 1 mM, of 2-cyano-3′,4′-dihydroxyacetophenone (ref 27), and 30 mg of β-alanine were refluxed for 3 h; water was added and the reaction mixture extracted with EtAc to give an oily solid. Chromatography gave an orange solid: 86 mg, 28% yield; mp 185 °C; NMR (acetone-d_6) δ 8.40 (1H, d, $J = 1.6$ Hz, H_4), 8.18 (1H, s, vinyl), 8.03 (1H, dd, $J = 8.6$, 1.6 Hz, H_6), 7.63 (1H, d, $J = 8.6$ Hz, H_7), 7.54−7.40 (3H, m, H_3, $H_{2'5'}$), 7.0 (1H, d, $J = 8.5$ Hz, H_6), 6.69 (1H, d, $J = 3.2$ Hz, H_2); MS *m/e* 304 (M^+, 8), 177 (29), 137($C_6H_3(OH)_2CO^+$, 100), 117 (12), 116 (indole$^+$, 15), 109 (93).

Compound 5. 3-Formylindole (105 mg, 0.7 mM), 130 mg, 0.7 mM, of 2-cyano-3′,4′-dihydroxyacetophenone, and 20 mg of β-alanine in 30 mL of ethanol were refluxed for 5 h. Water was added and the reaction mixture extracted with ethyl acetate. Evaporation yielded an oily solid which was triturated with CH_2Cl_2 and filtered to give 160 mg, 72% yield, of an orange solid: mp 228 °C; NMR (acetone-d_6) δ 8.77 (1H, s), 8.54 (1H, s, vinyl), 7.90−7.0 (7H, m); MS *m/e* 304 (M^+, 29), 137 (69), 130 (10), 117 (100), 109 (16).

Compound 7. 3-Formylindole (435 mg, 3 mM), 350 mg, 3.5 mM, of cyanothioacetamide, and 20 mg of β-alanine in 20 mL of ethanol were refluxed for 6 h. Cooling and filtering gave 0.415 g, 61% yield, of a yellow-orange solid: mp 238 °C; NMR (acetone-d_6) δ 8.74 (1H, s), 8.69 (1H, s), 7.92 (1H, m), 7.60 (1H, m), 7.30 (2H, m); MS *m/e* 227(M^+, 80), 226 (100), 194 (30), 193 (25), 140 ($M - CSNH_2 - HCN$, 25).

The yields and melting points for the analogous compounds were **6**, 81%, 228 °C; **8**, 77%, 293 °C; **9**, 87%, 194 °C; **10**, 47%, 242 °C;[28] **12**, 80%, 302 °C.

Compound 11. 3-Formylindole (0.29 g, 2 mM), 0.29 g, 2 mM, of 3-amino-4-cyano-5-(cyanomethyl)-1,2-pyrazole (ref 29), and 20 mg of β-alanine in 30 mL of ethanol were refluxed 4 h. Cooling and filtering gave 0.34 g, 62% yield, of a yellow solid: mp 281 °C; NMR (acetone-d_6) δ 8.52 (1H, s, vinyl), 8.42 (1H, s, H_2), 7.79 (1H, m), 7.57 (1H, m), 7.27 (2H, m), 6.17 (1H, br, s, NH); MS *m/e* 274 (M^+, 100), 219 (14), 91 (35).

Compound 17 (2-Chloro-3-phenyl-6,7-dimethoxyquinoline). a. To 3 g, 19.6 mM, of 3,4-dimethoxyaniline and 3.5 g, 22.6 mM, of phenylacetyl chloride in 60 mL of CH_2Cl_2 was added 5 mL of pyridine. After 2 h stirring at room temperature, water was added and the organic phase was evaporated to give a violet solid. Trituration with ethanol gave 3.0 g, 57% yield, of a white solid: mp 147 °C; NMR ($CDCl_3$) δ 7.40 (5H, m), 7.0 (1H, m), 6.75 (2H, m), 3.86, 3.84 (6H, 2 s, OCH_3), 3.73 (2H, s, CH_2Ph).

b. To 3 mL of DMF and 16 mL of $POCl_3$ was added 2.7 g, 10 mM, of amide from part a. The reaction was heated at 90 °C for 4 h, decanted on ice, filtered, and washed with water to give 2.9 g, 96% yield, of a white solid: mp 234 °C; NMR ($CDCl_3$) δ 8.26 (1H, s, H_4), 8.0 (1H, s, H_8), 7.51 (5H, s, Ph), 7.15 (1H, s, H_5), 4.13, 4.05 (6H, 2s, OCH_3); MS *m/e* 301, 299 (M^+, 33, 100), 286, 284 ($M - CH_3$, 2, 6), 258, 256 (6, 18), 220 (9), 215, 213 (4, 13).

The yields and melting points for the analogous quinolines were **13**, 20%, 120 °C; **14**, 84%, 167 °C; **15**, 85%, 103 °C; **16**, 10%, 120 °C.

Compound 18 (2,3-Diphenyl-6,7-dimethoxyquinoline). 3,4-Dimethoxyaniline (0.76 g, 5 mM) and 1 g, 4 mM, of α-phenyl-β-chlorocinnamaldehyde (1:1 *Z*:*E* mixture) (ref 24) in 20 mL of ethanol were stirred overnight at room temperature. Filtering and washing with ethanol gave 6.8 g, 59% yield, of a bright yellow solid: mp 210 °C; NMR ($CDCl_3$) δ 8.58 (1H, br, s), 8.35 (1H, s), 7.50−7.19 (11H, m), 4.16, 4.08 (6H, 2s, OCH_3).

Compound 19 (2-(*p*-Nitrophenyl)-3-carbomethoxy-6,7-dimethoxyquinoline). 3,4-Dimethoxyaniline (0.35 g, 2.3 mM) and 0.54 g, 2 mM, of (*Z*)-*p*-nitro-1-carbomethoxy-2-chlorocinnamaldehyde (ref 24) in 30 mL of ethanol was stirred overnight at room temperature. Filtering gave 0.47 g, 66% yield, of a light-brown solid: mp 237 °C; NMR (acetone-d_6) δ 8.75 (1H, s, H_4), 8.36, 7.89 (4H, ABq, $J_{AB} = 8.8$ Hz), 7.55 (1H, s, H_5), 7.53 (1H, s, H_8), 4.07, 4.05 (6H, 2s, OCH_3), 3.97 (3H, s, $COOCH_3$).

Compound 20. To 0.86 g, 6 mM, of 3-aminoquinoline and 1.2 g, 7.7 mM, of phenylacetyl chloride in 40 mL of CH_2Cl_2 was added 1 mL of pyridine. After 2 h at room temperature, water was added and the reaction mixture extracted with CH_2Cl_2. Evaporation and trituration with ethanol gave 0.7 g, 50% yield, of a white solid: mp 147 °C; NMR ($CDCl_3$) δ 8.71 (1H, d, $J = 2.5$ Hz), 8.57 (1H, d, $J = 2.5$ Hz), 8.0 (1H, m), 7.80−7.40 (8H, m), 3.83 (2H, s, CH_2).

Compound 21 (4-Quinylidenemalononitrile). 4-Formylquinoline (0.54 g, 2.9 mM) and 0.3 g, 4.5 mM, of malononitrile in 20 mL of ethanol, under N_2, were refluxed for 3 h. Workup and extraction with CH_2Cl_2 gave 0.22 g, 37% yield, of a yellow solid: mp 136 °C; NMR ($CDCl_3$) δ 9.09 (1H, d, $J = 4.6$ Hz), 8.63 (1H, s,vinyl), 8.25 (1H, d, $J = 8.2$ Hz), 7.93−7.68 (4H, m); MS *m/e* 205 (M^+, 100), 204 (60), 179 ($M - CN$, 30), 178 ($M - CN$, 68), 152 ($M - CN - HCN$, 10), 151 (22).

The yields and melting points for compounds **22** and **23** were **22**, 94%, 175 °C; **23**, 83%, 194 °C.

Compound 25 (3-Phenyl-6,7-dimethylquinoxaline). Phenylglyoxal (2.4 g, 16 mM) and 2.2 g, 16 mM, of 4,5-dimethyl-1,2-phenylenediamine in 20 mL of ethanol were refluxed 1.5 h. Cooling and filtering gave 3.25 g, 88% yield, of a white solid: mp 124 °C; NMR ($CDCl_3$) δ 9.23 (1H, s, H_2), 8.19 (1H, d, $J = 1.7$ Hz), 8.15 (1H, d, $J = 1.7$ Hz), 7.90 (2H, d, $J = 9.0$ Hz), 7.57 (3H, m), 2.52 (6H, s, CH_3); MS *m/e* 234 (M^+, 100), 219 (M − CH_3, 11), 207 (M − HCN, 12), 165 (M − 2HCN − $CH_{3,2}$), 131 (M − Ph−CN, 3).

Compound 26 (3-Phenyl-6,7-dimethoxyquinoxaline). 4,5-Dimethoxy-1,2-dinitrobenzene (2.3 g, 10.1 mM) (ref 32) was hydrogenated over 0.2 g of Pd/C for 3 h, in 30 mL of acetic acid and 30 mL of ethanol. To the filtered light-red solution was added 1.6 g, 10.5 mM, of phenylglyoxal hydrate, and the reaction mixture was refluxed for 2 h. Ammonia was added to the cooled solution. Extraction with CH_2Cl_2 gave 2.1 g of a light-brown solid. Recrystallization of 0.4 g from ethanol gave 0.16 g of a white solid. Chromatography of 1.7 g of the brown solid gave 0.55 g of the white solid: yield 27%, mp 134 °C; NMR ($CDCl_3$) δ 9.13 (1H, s, H_2), 8.16 (1H, d, $J = 1.6$ Hz), 8.12 (1H, d, $J = 1.6$ Hz), 7.60−7.40 (5H, m), 4.09 (6H, s, OCH_3); MS *m/e* 266 (M^+, 100), 251 (M − CH_3, 12), 223 (M − CH_3 − CO, 13), 196 (M − CH_3 − CO − HCN, 5).

The yields and melting points for the quinoxalines were **24**, 46%, 65 °C; **27**, 47%, 278 °C; **28**, 63%, 102 °C; **29**, 62%, 121 °C (**29** is available form Aldrich); **30**, 42%, 128 °C; **38**, 51%, oil; **39**, 40%, oil; **40**, 31%, 114 °C; **41**, 91%, 179 °C; **42,** 78%, 192 °C; **44**, 90%, 203 °C; **45**, 69%, 133 °C; **46**, 51%, 144 °C; **47**, 62%, 98 °C; **52**, 86%, 155 °C; **53**, 70%, 192 °C; **59**, 49%, 282 °C; **62**, 88%, 278 °C, (**62** is available from Aldrich); **64**, 77%, 135 °C; **65**, 67%, >300 °C (from 5-bromoisatin).

Compound 34 (3-(3-Thienyl)quinoxaline). 3-Thiophene bis(thiosemicarbazone)[25] (1.7 g, 6 mM) and 0.8 g, 7.4 mM, of 1,2-phenylenediamine in 15 mL of acetic acid were refluxed for 6 h. Workup and chromatography gave 0.2 g of a light-yellow solid: mp 80 °C; 15% yield; NMR ($CDCl_3$) δ 9.25 (1H, s, H_2), 8.20−8.06 (3H, m), 7.92 (1H, dd, $J = 5.2$, 1.2 Hz), 7.75 (2H, m), 7.52 (1H, m); MS *m/e* 212 (M^+, 100), 185 (M − HCN, 71), 159 (9), 140 (19), 109 (38).

The yields and melting points for the quinoxalines obtained from the ketoaldehydes bis-thiosemicarbazones were **31**, 23%, 104 °C; **32**, 40%, 94 °C; **33**, 97%, 202 °C; **35**, 32%, 70 °C; **36**, 21%, 114 °C; **37**, 3%, 146 °C.

Compound 43 (3-Phenyl-6,7-dihydroxyquinoxaline). Compound **26** (0.15 g) in 5 mL of 48% HBr was refluxed for 23 h. Cooling and filtering gave a green-yellow solid, 95 mg, 53% yield, HBr salt by elemental analysis: mp 280 °C; NMR (DMSO-d_6) δ 9.25 (1H, s, H_2), 8.24 (1H, d, $J = 1.9$ Hz), 8.20 (1H, d, $J = 1.9$ Hz), 7.50 (3H, m), 7.35 (2H, m).

Mother liquid was neutralized with $NaHCO_3$. Extraction with EtAc gave 20 mg, 15% yield, of an orange solid: mp 305 °C; free base; NMR (acetone-d_6) δ 9.19 (1H, s, H_2), 8.29 (1H, d, $J = 1.5$ Hz), 8.25 (1H, d, $J = 1.5$ Hz), 7.6 (3H, m), 7.40 (2H, m).

Compound 48 (2-Chloro-3-phenyl-6,7-dimethylquinoxaline). Compound **61** (0.3 g) and 3 mL $POCl_3$ in 10 mL of trichlorethylene were refluxed for 3 h. Workup and chromatography gave 0.13 g, 40% yield, of a white solid: mp 128 °C; NMR ($CDCl_3$) δ 7.85 (1H, s), 7.79 (1H, s), 7.83 (2H, m), 7.50 (3H, m), 2.51 (3H, s), 2.49 (3H, s); MS *m/e* 268, 270 (M^+, 76, 25), 233 (M − Cl, 100), 183 (30), 77 (50).

Compound 49 (2-((Dimethoxyphosphinyl)methyl)-3-phenyl-6,7-dimethylquinoxaline). Compound **46** (0.7 g, 2.1 mM) and 0.5 g, 5 mM, of trimethyl phosphite in 15 mL of toluene were refluxed for 5 h. Evaporation and chromatography gave a viscous oil: 0.36 g, 45% yield; NMR ($CDCl_3$) δ 7.8−7.5 (7H, m), 3.72 (6H, d, $J = 11$ Hz), 3.70 (2H, d, $J = 22.0$ Hz), 2.51 (3H, s), 2.49 (3H, s).

Compound 50 (2-(Phosphonomethyl)-3-phenyl-6,7-dimethylquinoxaline). Compound **49** (0.4 g, 1.1 mM) and 0.5 g of trimethyl bromosilane in 30 mL of CH_2Cl_2 were refluxed for 4 h. Evaporation gave a viscous oil which solidified on standing. Trituration with CH_2Cl_2 gave 0.33 g, 90% yield, of a greenish-yellow solid: mp 168 °C; NMR (DMSO-d_6) δ 7.85 (1H, s), 7.80 (1H, m), 7.53 (5H, m), 3.46 (2H, d, $J = 22.0$ Hz), 2.48 (6H, s).

Compound 54 (3-(3′,4′-Dihydroxyphenyl)-6,7-dimethylquinoxaline). 4,5-Dimethyl-1,2-phenylenediamine (1.9 g, 14 mM) and 1.9 g, 10.2 mM, of α-chloro-3,4-dihydroxyacetophenone in 15 mL DMSO and 25 mL of ethanol were refluxed for 2 h. Cooling and filtering gave 0.76 g, 18% yield, of a deep-yellow solid: mp 278 °C; HCl salt; NMR (DMSO-d_6) δ 9.47 (1H, s, H_2), 9.31 (1H, s, OH), 9.29 (1H, s, OH), 7.79 (2H, s, $H_{5,8}$), 7.75 (1H, d, $J = 2.2$ Hz, $H_{2'}$), 7.62 (1H, dd, $J = 8.7$, 2.2 Hz, $H_{6'}$), 6.90 (1H, d, $J = 8.7$ Hz, $H_{5'}$), 2.44 (6H, s, CH_3).

Treatment of 0.5 g with Na_2CO_3 and extraction with ethyl acetate gave 0.4 g of a yellow-green solid: mp 276 °C; NMR (DMSO-d_6) δ 9.30 (1H, s, H_2), 7.81 (2H, s, $H_{5,8}$), 7.75 (1H, d, J = 2,2 Hz, $H_{2'}$), 7.62 (1H, dd, $J = 8.3$, 2.2 Hz, $H_{6'}$), 6.90 (1H, d, $J = 8.3$ Hz, $H_{5'}$), 2.44 (6H, s, CH_3).

Compound 55 (2-Carbethoxy-3-phenyl-6,7-dimethylquinoxaline). a. To 10 g of benzoylethyl acetate in 20 mL of acetic acid cooled with ice was added 3.7 g of $NaNO_2$. After 10 min 5 mL of water was added. After 3 h 100 mL of water was added and the solid filtered to give 7.7 g, 84% yield, of a white solid: mp 110 °C; NMR ($CDCl_3$) δ 7.90 (2H, m), 7.6−7.5 (3H, m), 4.30 (2H, q, $J = 7.4$ Hz), 1.24 (3H, t, $J = 7.4$ Hz).

b. Oxime (5 g, 22.6 mM) from part a and 3.1 g, 22.8 mM, of 4,5-dimethyl-1,2-phenylenediamine in 20 mL of ethanol and 5 mL of HCl were refluxed for 6 h. Workup (H_2O, $NaHCO_3$, CH_2Cl_2), chromatography, and trituration with hexane gave 2 g, 29% yield, of a white solid: mp 100 °C; NMR ($CDCl_3$) δ 7.96 (1H, s), 7.93 (1H, s), 7.7 (2H, m), 7.5 (3H, m), 4.30 (2H, q, $J = 7.0$ Hz), 2.53 (6H, s), 1.17 (3H, t, $J = 7.0$ Hz).

Compound 56 (2-Carboxy-3-phenyl-6,7-dimethylquinoxaline). Compound **55** (2.4 g) and 5 g of KOH in 20 mL of ethanol and 20 mL of H_2O were stirred for 20 h at room temperature. Acidification with HCl, filtering, and washing with water gave 2.1 g, 96% yield, of a light-yellow solid: mp 153 °C; NMR (acetone-d_6) δ 7.92 (1H, s), 7.90 (1H, s), 7.85 (2H, m), 7.50 (3H, m) 2.56 (6H, s).

Compound 57 (2-Formyl-3-phenyl-6,7-dimethylquinoxaline). Compound **46** (0.8 g) in 10 mL of DMSO was heated for 40 min at 90 °C. Workup and chromatography gave from first fractions 40 mg, 4% yield, of a white solid: mp 148 °C; 2-dibromomethyl derivative; NMR ($CDCl_3$) δ 8.03 (1H, s), 7.90 (1H, s), 7.60 (5H, m), 6.96 (1H, s, $CHBr_2$), 2.52 (6H, s).

Following fractions gave the 2-formylquinoxaline: white solid; mp 150 °C; 0.3 g, 47% yield; NMR ($CDCl_3$) δ 11.30 (1H, s, CHO), 8.05 (1H, s), 7.96 (1H, s), 7.67 (2H, m), 7.55 (3H, m), 2.55 (6H, s).

Compound 58 (2-((Dimethylamino)methyl)-3-phenyl-6,7-dimethylquinoxaline). Compound **46** (0.6 g, 1.8 mM) and 5 mL of 25% dimethylamine (in water) in 20 mL of acetonitrile were stirred for 14 h at room temperature. Workup (H_2O, CH_2Cl_2) and recrystallization with hexane gave 80 mg, 15% yield, of a white solid: mp 67 °C; NMR ($CDCl_3$) δ 7.86 (4H, m), 7.50 (3H, m), 3.70 (2H, s, CH_2N), 2.53 (6H, s, CH_3), 2.30 (6H, s, $N(CH_3)_2$).

Compound 60 (1,2-Dihydro-2-keto-3-phenyl-6,7-dimethoxyquinoxaline). Compound **26** (0.3 g, 1.13 mM) and 1.5 mL, 5 mM, of BBr_3 in 30 mL of CH_2Cl_2 under argon were stirred for 2 h at room temperature. Workup and chromatography gave 30 mg of a yellow solid, 11% yield: mp >300 °C; NMR ($CDCl_3$) δ 9.37 (1H, s), 8.51 (1H, s), 7.93 (2H, m), 7.54 (3H, m), 4.24 (3H, s, OCH_3), 4.12 (3H, s, OCH_3); MS *m/e* 282 (M^+, 11), 280 (36), 266 (M − O, 10), 167 (46), 149 (100), 133 (6), 125 (13), 119 (20).

Compound 61 (1,2-Dihydro-2-keto-3-phenyl-6,7-dimethylquinoxaline). 4,5-Dimethyl-1,2-diaminobenzene (0.56 g, 4 mM) and 0.6 g, 4 mM, of benzoylformic acid in 15 mL of ethanol were refluxed for 5 h. Cooling and filtering gave 0.8 g, 80% yield, of a yellow solid: mp 275 °C; NMR ($CDCl_3$) δ 8.38 (2H, m), 7.70 (1H, s), 7.51 (3H, m), 7.06 (1H, s), 2.40 (3H, s), 2.37 (3H, s); irradiation at 8.38 ppm gave a singlet at 7.51 ppm.

Compound 63 (1,2-Dihydro-2-keto-3-phenyl-6,7-dimethylquinoxaline 4-*N*-Oxide). a. To 3.6 g, 22 mM, of 2-nitro-4,5-dimethylaniline and 3.8 g, 25 mM, of phenylacetyl chloride in 50 mL of CH_2Cl_2 was added 5 mL of pyridine. After

2.5 h at room temperature, water was added. Evaporation of the organic phase, trituration with ethanol, and filtering gave 4.7 g, 76% yield, of a yellow solid: mp 166 °C; NMR ($CDCl_3$) δ 9.34 (1H, br s, NH), 8.57 (1H, s, H_3), 7.92 (1H, s, H_6), 7.40 (5H, m), 3.80 (2H, s), 2.32 (3H, s), 2.25 (3H, s).

b. Material (1 g) from part a and 0.5 g of KOH in 40 mL of ethanol were refluxed for 1 h. Workup (HCl, H_2O, CH_2Cl_2) gave an orange solid: 0.525 g, 56% yield; mp 103 °C; NMR ($CDCl_3$) δ 7.86 (1H, s, H_5), 7.30 (5H, br s, Ph), 6.58 (1H, s, H_8), 3.65 (1H, br s, NH), 2.22 (3H, s), 2.17 (3H, s).

Compound 66 (3-Phenyl-1,4-diazaanthracene). 2,3-Diaminonaphthalene (0.47 g, 3 mM) and 0.47 g of phenylgloxal hydrate in 20 mL of ethanol were refluxed for 1.5 h. Cooling and filtering gave 0.5 g, 65%, of a light-green solid: mp 163 °C; NMR ($CDCl_3$) δ 9.38 (1H, s, H_2), 8.71, 8.67 (2H, 2d, $H_{5,10}$), 8.25, 8.10 (4H, AA′ BB′ m, H_{6-9}), 7.58 (5H, m, Ph); MS *m/e* 256 (M^+, 100), 229 (M − CN, 12), 126 ($C_{10}H_6^+$, 71).

Compound 67. Compound **46** (0.33 g, 1 mM), 0.08 g, 0.74 mM, of 1,3-propanedithiol, and 0.1 g of KOH, in 25 mL of ethanol were stirred for 24 h at room temperature. Workup (H_2O, CH_2Cl_2) and trituration with hexane gave 0.18 g, 60% yield, of a white solid: mp 165 °C; NMR ($CDCl_3$) δ 7.85 (2H, s), 7.82 (2H, s), 7.70 (4H, m), 7.48 (6H, m), 3.96 (4H, s), 2.61 (4H, t, J = 7.6 Hz); MS *m/e* 328 (M − 272, 8), 248 (38), 247 (100), 232 (7), 189 (15).

Dimers **68** and **69** were prepared similarly: **68**, 41%, 216 °C; **69**, 55%, 153.

Biochemical Methods. Methods used are identical with those described in our previous publication.[20]

Membrane Autophosphorylation Assays. Membranes were prepared from confluent cultures of Swiss 3T3 cells as described.[30] For measuring receptor autophosphorylation, 10 μg of membrane protein/assay was incubated for 20 min on ice in the presence of 1.2 μg/mL EGF or 2 μg/mL PDGF, or both, 50 mM Hepes (pH 7.5), and 3 mM $MnCl_2$ (final concentrations) in a volume of 45 μL. As was previously demonstrated (ref 20 Figure 1), no PDGFR autophosphorylation occurs without PDGF. In order to test the effects of tyrphostins, these were added in a volume of 0.5 mL (in DMSO, final concentration 0.5%) 15 min before addition of the growth factors. Phosphorylation was initiated by addition of [γ-^{32}P]-ATP (5 mL, 3−5 μCi, final concentration 2 μM) and terminated after 2 min by addition of 10 mL of a solution containing 6% SDS, 30% β-mercaptoethanol, 40% glycerol, and 0.5 mg/mL bromophenol blue. The samples were heated for 5 min at 95 °C and subjected to SDS−PAGE according to the method of Laemmli[31] using 10% acrylamide gels. The gels were stained, dried, and subjected to autoradiographic analysis. Only a portion of the tyrphostins examined were tested on Swiss 3T3 membranes, and the results are published in ref 20.

For quantification of radioactivity in electrophoresis gels, a PhosphorImager (Molecular Dynamics, Fuji or BioRad) was used according to the instructions of the manufacturers. To obtain autoradiograms, objects were exposed to X-ray film (Fuji RX or Kodak X-OMAT) with intensifying screens at −70 °C.

Assay of Receptor Autophosphorylation in Intact Cells. Confluent Swiss 3T3 cells in 24-well plates (Nunc) were incubated for 20−24 h in serum free DMEM. Subsequently, tyrphostins were added at concentrations ranging from 0 to 100 μM (final DMSO concentration 0.5%), and the incubation was continued for 6−8 h. The cells were then stimulated with 100 ng/mL PDGF-BB for 5 min at room temperature. The growth factor treatment was terminated by washing twice with ice-cold PBS, and the cells were scraped off the wells in 60 mL of lysis buffer containing 20 mM Hepes, pH 7.4, 150 mM NaCl, 1% Triton X-100, 10 mM sodium pyrophosphate, 50 mM NaF, 2 mM sodium orthovanadate, 20 mM zinc acetate, 10 mM EDTA, 2 mM EGTA, 1 mM PMSF, and 5 μg/mL leupeptin. The cell lysates were clarified by centrifugation (cooled microfuge, 17 000 rpm, 15 min) and analyzed by SDS−PAGE (6.5% gels) and immunoblotting with anti-phosphotyrosine antibodies (either PY 20 or ICN and subsequently a peroxidase-coupled secondary antibody or RC20-peroxidase conjugate; Affiniti, Nottingham, U.K.). The blots were developed with a chemiluminescence detection system (Western light, Tropix or ECL, Amersham).

Supporting Information Available: NMR and MS data for tyrphostins (3 pages). Ordering information can be found on any current masthead page.

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