Tyrosine Kinase Inhibitors. 5. Synthesis and Structure–Activity Relationships for 4-[(Phenylmethyl)amino]- and 4-(Phenylamino)quinazolines as Potent Adenosine 5′-Triphosphate Binding Site Inhibitors of the Tyrosine Kinase Domain of the Epidermal Growth Factor Receptor

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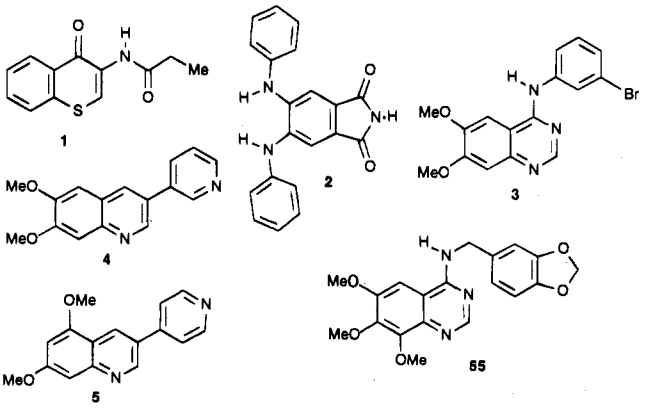
A series of 4-substituted quinazolines and related compounds have been prepared and evaluated for their ability to inhibit the tyrosine kinase activity of the epidermal growth factor receptor on a phospholipase C-γ1-derived substrate. The results show a narrow structure–activity relationship (SAR) for the basic ring system, with quinazoline being the preferred chromophore and benzylamino and anilino the preferred side chains. In the 4-anilino series, substitution on the 3-position of the phenyl ring with small lipophilic electron-withdrawing groups provided analogues with enhanced potency. Two series of compounds [4-(phenylmethyl)amino and 4-(3-bromophenyl)amino] were studied to determine SARs for quinazoline substituents. In the more active 4-(3-bromophenyl)amino series, electron-donating groups (NH_2, OMe) at the 6- or 7-position increased activity, in a pattern consistent with a requirement for high electron density in the vicinity of the 8-position of the quinazoline ring. The 6,7-dimethoxy derivatives were the most effective in both series, with the 4-(3-bromophenyl)amino derivative (**3**) having an IC_{50} of 0.029 nM, making it by far the most potent reported inhibitor of the tyrosine kinase activity of the epidermal growth factor receptor enzyme.

Introduction

In the past few years receptor tyrosine kinases have been intensively investigated because of their role in the transduction of proliferative signals in mammalian cells. Tyrosine phosphorylation appears to be the major pathway whereby mitogenic signals are transduced through the cell membrane to the nucleus.[1-3] Many of these enzymes are encoded by proto-oncogenes, and their mutational activation or overexpression is thought to be a major cause of malignant transformation.[4-6] In particular, human mammary, ovarian, and squamous cell head and neck carcinomas have been linked to overproduction of the epidermal growth factor receptor (EGFR)[7] or the closely homologous product of the *c-erbB2* oncogene.[4] As a result of this, there is great current interest in the development of compounds which can specifically inhibit the tyrosine kinase activity of these components of the signal transduction pathway in cancer cells.

The catalytic site in these enzymes contains neighboring domains for binding both the tyrosine-containing substrate and the adenosine 5′-triphosphate (ATP) cofactor, and many compounds are known which inhibit by competitive binding to the tyrosine site.[1-4,8-10] However, most of these inhibitors have low potency and moderate selectivity, with considerable ambiguity as to their mode of action. This, together with rather flat SARs, has made optimization of series difficult. In contrast, fewer studies have focused on ATP site inhibitors, out of concern that such compounds would not be selective,[3] given the high degree of homology for these sites among different kinases.[11] Because tyrosine phosphorylation is a primary mechanism of signal transduction in both normal and transformed cells,[12] compounds which can selectively inhibit specific enzymes are required. However, recent reports show that several classes of compounds which bind competitively with ATP, including benzothiopyranones (e.g., **1**)[13] and dianilinophthalimides (e.g., **2**)[14] do show good selectivity, not only between tyrosine and serine/threonine kinases, but also between different tyrosine kinases.



We have recently shown that a (phenylamino)-quinazoline (**3**), which was developed from leads identified through a mass screening program, is also a very highly selective inhibitor of the tyrosine kinase activity of EGFR and that analogues of **3** show competitive inhibition with respect to ATP.[15] This finding is of particular interest because of the very high potency of **3**, which has an IC_{50} of 0.029 nM against the isolated enzyme, and one of 15 nM for inhibition of EGF-stimulated tyrosine phosphorylation in NIH 3T3 cells.[15] Furthermore, **3** produces immediate inhibition in both

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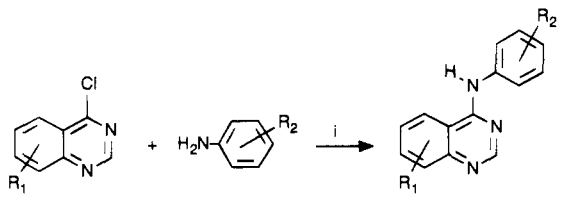
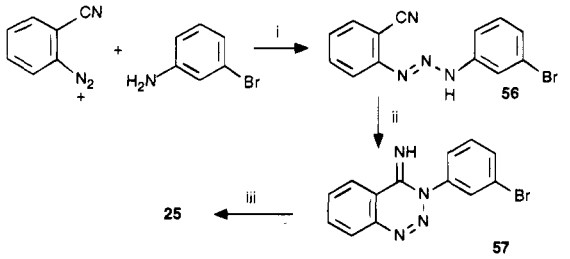
Scheme 1[a]



[a] (i) *i*-PrOH/heat.

Scheme 2[a]



[a] (i) 0 °C/2 h; (ii) EtOH/reflux/15 h; (iii) AcOH/reflux/1 h.

isolated enzyme and cellular-based assay systems and may represent a mechanistically novel class of inhibitors of EGFR.[15] Other recent reports have also been published on the activity of 4-(phenylamino)quinazolines[16,17] and dimethoxy-substituted pyridylquinolines (e.g, **4**, **5**)[18,19] as potent inhibitors of cellular tyrosine kinases. In this paper we report the synthesis of a series of substituted 4-(benzylamino)- and 4-(phenylamino)-quinazolines and discuss SARs for inhibition of EGFR tyrosine kinase activity by this new class of compounds.

Chemistry

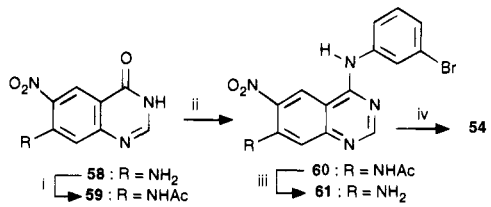
Compounds **6**–**24** were prepared by coupling the appropriate chloroheterocycle (4-chloroquinazoline,[20] 4-chloroquinoline,[21] 1-chloroisoquinoline,[22] 4-chlorocinnoline,[23] 4-chlorophthalazine,[24] or 2-chloroquinoxaline[25]) with the appropriate amines in refluxing 2-propanol (example given in Scheme 1). The benzotriazine derivative (**25**) was prepared using a reported procedure[26] (Scheme 2). Nitro- and methoxy-substituted quinazoline derivatives were prepared by coupling the appropriately substituted 4-chloroquinazoline with benzylamine or 3-bromoaniline. Hydroxy-substituted derivatives were prepared by demethylation of the analogous methoxy compounds either with sodium methanethiolate in DMA at 85 °C or with a pyridinium hydrochloride fusion at 200 °C. The 4-chloroquinazolines were prepared from the known quinazolin-4(3*H*)-ones [27–31] by treatment with $SOCl_2$ or $POCl_3$ and were often employed directly without isolation. Amine derivatives were prepared from the corresponding nitro compounds by reduction with Fe dust in aqueous ethanol or by hydrogenation over Pt/C.

The 6,7-diamino derivative (**54**) was prepared by activating 7-acetamido-6-nitroquinazolin-4(3*H*)-one (**59**) to the 4-chloro derivative and then coupling this with 3-bromoaniline to give **60**. Basic hydrolysis of the acetamido group to **61**, followed by reduction with Fe/aqueous EtOH/HCl, then gave **54** (Scheme 3).

Results and Discussion

The structures and physicochemical properties of the compounds prepared are given in Table 1. All the analogues were evaluated for their ability to inhibit

Scheme 3[a]



[a] (i) Ac_2O/AcOH/reflux/6 h; (ii) $POCl_3$/heat, then $ArNH_2$/*i*PrOH/heat; (iii) NaOH/MeOH; (iv) Fe/H^+/HCl/heat.

Scheme 3

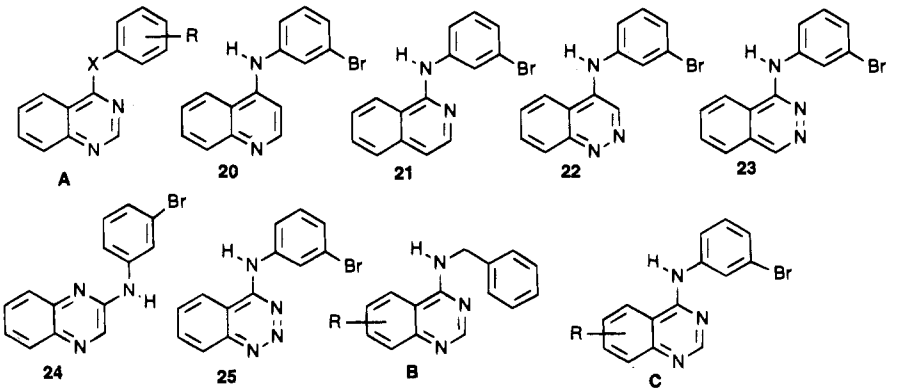
tyrosine phosphorylation of a polypeptide (a portion of phospholipase C-γ1) by full-length EGFR enzyme isolated from A431 cells.[15] Full dose–response curves were determined for each compound, and the resulting IC_{50}s listed in Table 1 are the average of at least two such determinations.

The results show that the nature of the linking group between the quinazoline chromophore and the phenyl 4-side chain has a substantial effect on inhibitory activity. The 2-phenylethyl derivative **6** was much less effective than either the benzylamino (**7**) or anilino (**10**) compounds (IC_{50} 4100 compared with 320 and 344 nM, respectively), and N-methylation of the amino group of **10** to give **11** completely abolished activity. Further elaboration of the benzyl side chain with either electron-withdrawing (4-Cl; **8**) or electron-donating (4-OMe; **9**) groups did not improve potency (IC_{50}s in the 7–10 μM range). In view of this, and of concurrent reports[16,17] of the utility of 3-substituted-anilino analogues as tyrosine kinase inhibitors, we focused on the latter series. The electron-withdrawing 3-OMe group (σ_m +0.12) did not prove favorable (**12**; IC_{50} 840 nM), but small, lipophilic, electron-withdrawing groups at the 3-position were beneficial, with the 3-Cl and 3-Br derivatives (**14**, **15**) being the most potent (IC_{50}s ca. 25 nM). An oxygen-linked 3-bromophenoxy analogue (**19**) was much less effective.

The requirement for the quinazoline structure was established by studying a small series of derivatives containing the 3-bromoanilino side chain. However, all alterations in the pattern of nitrogen substitution in the bicyclic ring system resulted in inactive compounds (quinoline **20**, isoquinoline **21**, cinnoline **22**, phthalazine **23**, quinoxaline **24**, and benzotriazine **25**; Table 1). Two series of analogues were prepared to study SARs for quinazoline ring substitution. The first series (compounds **26**–**37**) employed the unsubstituted benzylamino side chain. The four isomeric mononitro derivatives (**26**, **29**, **32**, and **35**) were all essentially inactive (IC_{50}s >6000 nM), as were the corresponding 5- and 8-monoamino compounds (**27** and **36**). The 6- and 7-amino analogues (**30** and **33**) were slightly more effective, but not as good as the parent. In contrast, while the 5- and 8-methoxy derivatives were also inactive, the 6- and especially the 7-analogue (**34**) (IC_{50} 58 nM) were more potent than **7**.

The high potencies shown by the methoxy derivatives prompted us to evaluate the 6,7-dioxygenated compounds (**38**–**40**). The methoxyhydroxy compounds (**38**, **39**) had IC_{50}s broadly similar to the corresponding monomethoxy derivatives, suggesting little effect of the hydroxy group. However, the 6,7-dimethoxy compound (**40**) proved the most potent of the benzylamino series,

Table 1. Physicochemical and Tyrosine Kinase Inhibitory Properties of 4-Anilino- and 4-(Benzylamino)quinazolines and Related Heterocycle



no.	type	R	X	mp (°C)	formula	analysis	IC_{50}[a](nM)
6	A	H	$NH(CH_2)_2$	169–170	$C_{16}H_{15}N_3 \cdot 0.5H_2O$	C,H,N	4100
7[b]	A	H	$NHCH_2$	169–170	$C_{16}H_{13}N_3$	C,H,N	320
8	A	4-Cl	$NHCH_2$	218–220	$C_{15}H_{12}ClN_3 \cdot 0.25H_2O$	C,H,N	7000
9	A	4-OMe	$NHCH_2$	155–156	$C_{16}H_{15}N_3O$	C,H,N	10^4
10[b]	A	H	NH	216–217	$C_{14}H_{11}N_3$	C,H,N	344
11[b]	A	H	NMe	245–247	$C_{15}H_{13}N_3 \cdot HCl$	C,H,N	10^5
12[c]	A	3-OMe	NH	216–218	$C_{15}H_{13}N_3O \cdot HCl \cdot 0.5EtOH$	C,H,N	842
13[b,c]	A	3-Me	NH	191–193	$C_{15}H_{13}N_3$	C,H,N	910
14[c]	A	3-Cl	NH	248–250	$C_{14}H_{10}ClN_3 \cdot HCl$	C,H,N	23
15[c]	A	3-Br	NH	216–218	$C_{14}H_{10}BrN_3$	H,N,Br; C[d]	27
16[c]	A	3-I	NH	228–229	$C_{14}H_{10}IN_3$	C,H,N	80
17[c]	A	3-CF_3	NH	208–209	$C_{15}H_{10}F_3N_3$	C,H,N	577
18[e]	A	3-aza	NH	180–182	$C_{13}H_{10}N_4$	C,H,N	>1000
19	A	3-Br	O	102	$C_{14}H_9BrN_2O$	C,H,N	756
20				196–198	$C_{15}H_{11}BrN_2$	C,H,N	5500
21				132–133	$C_{15}H_{11}BrN_2$	C,H,N	$>10^5$
22				276–278.5	$C_{14}H_{10}BrN_3 \cdot HCl$	C,H,N	$>10^4$
23				221–223	$C_{14}H_{10}BrN_3$	C,H,N	$>10^5$
24				145–146	$C_{14}H_{10}BrN_3$	C,H,N	$>10^5$
25				212–213.5	$C_{13}H_9BrN_4$	C,H,N	>1000
26	B	5-NO_2		85.5–86	$C_{15}H_{12}N_4O_2$	C,H,N	8000
27	B	5-NH_2		157–157.5	$C_{15}H_{14}N_4$	C,H,N	$>10^4$
28	B	5-OMe		92–93.5	$C_{16}H_{15}N_3O$	C,H,N	4900
29	B	6-NO_2		222–223	$C_{15}H_{12}N_4O_2$	C,H,N	$>10^4$
30	B	6-NH_2		191.5–195	$C_{15}H_{14}N_4 \cdot 2H_2O$	C,H,N	1400
31	B	6-OMe		150–151	$C_{16}H_{15}N_3O$	C,H,N	200
32	B	7-NO_2		201–202.5	$C_{15}H_{12}N_4O_2$	C,H,N	5900
33	B	7-NH_2		166–168	$C_{15}H_{14}N_4$	C,H,N	>1000
34	B	7-OMe		180	$C_{16}H_{15}N_3O \cdot 0.1HCl$	C,H,N	58
35	B	8-NO_2		144.5–145.5	$C_{15}H_{12}N_4O_2$	C,H,N	$>10^4$
36	B	8-NH_2		118–119	$C_{15}H_{11}N_4$	C,H,N	$>10^4$
37[f]	B	8-OMe		205–206	$C_{16}H_{15}N_3O$	C,H,N	$>10^4$
38	B	6-OMe,7-OH		245–246	$C_{16}H_{15}N_3O_2$	H,N;C[g]	588
39	B	6-OH,7-OMe		216–217	$C_{16}H_{15}N_3O_2$	C,H,N	56
40	B	6,7-$(OMe)_2$		226–229	$C_{17}H_{17}N_3O_2$	H,N;C[h]	10
41	C	5-NO_2		156–157.5	$C_{14}H_9BrN_4O_2$	C,H,N	355
42	C	5-NH_2		130.5–132	$C_{14}H_{11}BrN_4$	C,H,N	439
43	C	5-OMe		137–139	$C_{15}H_{12}BrN_3O$	C,H,N	139
44	C	6-NO_2		269.5–273	$C_{14}H_9BrN_4O_2$	C,H,N	897
45	C	6-NH_2		201.5–203.5	$C_{14}H_{11}BrN_4$	C,H,N	0.79
46	C	6-OMe		238–239	$C_{15}H_{12}BrN_3O \cdot 0.75HCl$	C,H,N	348
47	C	7-NO_2		228.5–230	$C_{14}H_9BrN_4O_2$	C,H,N	1000
48	C	7-NH_2		219.5–223	$C_{14}H_{11}BrN_4 \cdot 1.5H_2O$	C,H,N	0.1
49	C	7-OMe		178.5–179	$C_{15}H_{12}BrN_3O$	C,H,N	10
50	C	8-NO_2		226.5–228	$C_{14}H_9BrN_4O_2$	C,H,N	$>10^4$
51	C	8-NH_2		160.5–161.5	$C_{14}H_{11}BrN_4$	C,H,N	105
52	C	8-OMe		258–259	$C_{15}H_{12}BrN_3O$	C,H,N	974
53	C	6,7-$(OH)_2$		>320	$C_{14}H_8BrN_3O_2$	C,H,N	0.17
54	C	6,7-$(NH_2)_2$		137–141	$C_{14}H_{12}BrN_5$	C,H,N	0.12
3[i]	C	6,7-$(OMe)_2$		264–266	$C_{16}H_{14}BrN_3O_2 \cdot HCl$	C,H,N	0.029

[a] IC_{50}, concentration of drug (nM) to inhibit the phosphorylation of a 14-residue fragment of phospholipase C-γ1 by EGFR (prepared from human A431 carcinoma cell vesicles by immunoaffinity chromatography). See the Experimental Section for details. Values are the averages from at least two independent dose–response curves; variation was generally ±15%. [b] Reference 33. [c] Reference 16. [d] C out by 0.6%. [e] Reference 34. [f] Reference 35. [g] C out by 0.7%. [h] C out by 0.5%. [i] Reference 15.

with an IC_{50} of 10 nM. This limited study shows that quinazoline substitution with electron-donating groups can significantly improve potency in the benzylamino series (ca. 30-fold from **7** to **40**).

Essentially the same set of quinazoline substituents were then evaluated using a 4-(3-bromophenyl)amino side chain (compounds **15**, **41**–**52**). The mononitro derivatives (**41**, **44**, **47**, and **50**) were again relatively

nonpotent (although, with the exception of **50**, about 10-fold more effective than the corresponding benzylamino derivatives). However, the amino derivatives (**42**, **45**, **48**, and **51**) demonstrated much larger differences between the two series. In particular, the 6- and 7-amino compounds (**45**, **48**), which had IC_{50}s of 0.8 and 0.1 nM, respectively, were several thousand-fold more potent than their benzylamino counterparts. In contrast with the benzylamino series, the 6- and 7-methoxy derivatives (**46**, **49**) were less effective than the corresponding amino analogues. Three 6,7-disubstituted compounds (**53**, **54**, and **3**) were also evaluated in this series. The diamino derivative (**54**), while very potent (IC_{50} 0.12 nM), was no more active than the monosubstituted amino compounds (**45** and **48**). However, the 6,7-dimethoxy derivative **3** was much more effective than either of the monosubstituted methoxy compounds (and ca. 1000-fold more potent than the parent compound **7**), having the astounding IC_{50} of 0.029 nM as we have previously reported.[15]

These results demonstrate that 4-(phenylamino)-quinazoline is the primary pharmacophore for this class of EGFR inhibitors. Within this overall structure, there is a clear benefit from small lipophilic electron-withdrawing groups at the 3-position on the aniline, as previously noted by others.[16,17] In two series explored here, the benzylamino compounds were generally less effective than the corresponding (3-bromophenyl)amino derivatives. In the series with the latter side chain, the results of our limited exploration of monosubstitution on the quinazoline ring (compounds **41**–**52**) are consistent with a requirement for high electron density in the vicinity of the 8-position of the quinazoline ring. Thus substitution with the electron-withdrawing nitro group (NO_2 $\sigma_{o,p}$ +0.78, σ_m +0.71) at any ring position (but especially at C-8) decreases activity. Substitution with a 6-methoxy group (**46**) (OMe σ_m +0.12) also leads to a loss of activity, but a 7-methoxy group (**49**) (OMe $\sigma_{o,p}$ −0.27) enhances it, and substitution with amino groups at either position (NH_2 σ_m −0.16, $\sigma_{o,p}$ −0.66) gave large increases in potency.

However, this analysis is inadequate to explain the enormous increase in potency seen with the two 6,7-dioxygenated compounds **53** and **3** (60-fold and 350-fold respectively, compared with the 7-methoxy derivative **49**). As we have previously reported,[15] the 6,7-dimethoxy analogue **3**, with an IC_{50} of 0.029 nM, is by far the most potent inhibitor of EGFR tyrosine kinase activity yet known. The reason for the outstanding potency of **3** is not well understood and is under active investigation.

It is noted that several other studies have shown the efficacy of polymethoxy derivatives of similar compounds as enzyme inhibitors. For example, the 6,7-dimethoxyquinoline **4** is the most potent of a series of inhibitors of platelet-derived growth factor tyrosine kinase (but was not active against EGFR),[18] while the trimethoxyquinazoline **55** is a potent inhibitor of cyclic GMP phosphodiesterase.[31]

Conclusions

SAR in the 4-(phenylamino)quinazoline class of EGFR tyrosine kinase inhibitors indicate a requirement for small lipophilic electron-withdrawing groups at the 3-position on the aniline, and for electron-donating groups at the 6- and 7-positions of the quinazoline, with a possible more specific requirement for high electron density in the vicinity of the 8-position of the quinazoline ring. The clear SAR, together with the enormous potency and high selectivity seen for some analogues, notably the 6,7-dimethoxy derivative (**3**),[15] makes this an interesting class of compounds, and further structure–activity studies are in progress.

Experimental Section

Analyses were performed by the Microchemical Laboratory, University of Otago, Dunedin, NZ, or by Parke-Davis Pharmaceutical Research Analytical Department. Melting points were determined using an Electrothermal Model 9200 digital melting point apparatus and are as read. NMR spectra were measured on Bruker AC-200 or AM-400 spectrometers and referenced to Me_4Si. Mass spectra were recorded on a Varian VG 7070 spectrometer at nominal 5000 resolution.

4-[(3-Bromophenyl)amino]-1,2,3-benzotriazine (25). Preparation of this compound followed the general literature procedure.[26] A suspension of 2-aminobenzonitrile (5.9 g, 50 mmol) in 10 M HCl (25 mL) was cooled to 0 °C and treated with $NaNO_2$ (3.55 g, 51 mmol) in water (10 mL). The diazonium solution was then treated with an excess of NaOAc and stirred for 2 h at 0 °C with 3-bromoaniline (8.60 g, 50 mmol). The resulting red solid was kept at 4 °C overnight, collected, and recrystallized from benzene–petroleum ether to give 2-[3-(3-bromophenyl)triazenyl]benzonitrile (**56**) (5.75 g, 38%): mp 151–153 °C; ^1H NMR ($CDCl_3$) δ 8.30 (m, 1 H), 8.00 (d, J = 7.9 Hz, 1 H), 7.82 (td, J = 7.8, 1.3 Hz, 1 H), 7.74–7.70 (m, 2 H), 7.67 (d, J = 7.3 Hz, 1 H), 7.52–7.46 (m, 2 H), 6.66 (m, 1 H). Anal. ($C_{13}H_9BrN_4$) C, H, N.

A solution of **56** (3.50 g, 11.6 mmol) in 70% EtOH (200 mL) was heated under reflux for 15 h. Cooling and dilution with water gave 3,4-dihydro-4-imino-3-(3-bromophenyl)-1,2,3-benzotriazine (**57**) (2.8 g, 80%): mp (EtOH) 223 °C dec; ^1H NMR [$(CD_3)_2SO$] δ 10.02 (br s, 1 H, NH), 8.62 (d, J = 8.1 Hz, 1 H), 8.30 (br s, 1 H), 8.24 (dd, J = 7.7, 7.3 Hz, 1 H), 8.05 (dd, J = 7.7, 7.3 Hz, 1 H), 7.95 (d, J = 7.4 Hz, 1 H), 7.45–7.37 (m, 2 H). Anal. ($C_{13}H_9BrN_4$) C, H, N.

A suspension of **57** (1.5 g, 5 mmol) in AcOH (100 mL) was heated under reflux for 1 h to give 4-[(3-bromophenyl)amino]-1,2,3-benzotriazine (**25**) (1.4 g, 93%): mp (AcOH) 217.5–218 °C; ^1H NMR [$(CD_3)_2SO$] δ 10.00 (s, 1 H, NH), 8.62 (d, J = 8.1 Hz, 1 H, H-5), 8.30 (br s, 1 H, H-2′), 8.24 (d, J = 8.1 Hz, 1 H, H-8), 8.13 (t, J = 8.1 Hz, 1 H), 8.05 (t, J = 8.1 Hz, 1 H), 7.95 (d, J = 7.6 Hz, 1 H, H-6′), 7.44–7.37 (m, 2 H, H-4′,5′). Anal. ($C_{13}H_9BrN_4$) C, H, N.

4-[(3-Bromophenyl)amino]-7-nitroquinazoline (47): General Example of Coupling Procedure. A solution of 4-chloro-7-nitroquinazoline[28] (1.05 g, 5 mmol) in CH_2Cl_2 (20 mL) was combined with a solution of 3-bromoaniline (1.72 g, 10 mmol) in i-PrOH (50 mL), and the mixture was heated to boil off the CH_2Cl_2. 3-Bromoaniline hydrochloride (ca. 50 mg) was added to initiate the coupling reaction, and heating was continued for a further 15 min before the solution was concentrated to ca. 20 mL. A few drops of concentrated NH_3 were added to basify the solution, and further water was then added until the solution was just cloudy. After cooling, the solid was filtered off and dried to give 4-[(3-bromophenyl)-amino]-7-nitroquinazoline (**47**) (1.62 g, 94%): mp 228.5–230 °C; ^1H NMR [$(CD_3)_2SO$] δ 10.22 (s, 1 H, NH), 8.81 (d, J = 9.3 Hz, 1 H, H-5), 8.79 (s, 1 H, H-2), 8.52 (d, J = 2.3 Hz, 1 H, H-8), 8.40 (dd, J = 9.1, 2.4 Hz, 1 H, H-6), 8.22 (br s, 1 H, H-2′), 7.90 (d, J = 7.5 Hz, 1 H, H-6′), 7.42–7.35 (m, 2 H, H-4′,5′). Anal. ($C_{14}H_9BrN_4O_2$) C, H, N.

7-Amino-4-[(3-bromophenyl)amino]quinazoline (48): General Example of Nitro Reduction. A refluxing, stirred solution of 4-[(3-bromophenyl)amino]-7-nitroquinazoline (**47**) (1.10 g, 3.2 mmol) in aqueous EtOH (1:2, 250 mL) containing AcOH (2.5 mL) was treated with Fe powder (prewashed with dilute HCl and water) in portions. The mixture was heated under reflux for a further 1 h, and then concentrated ammonia solution (10 mL) was added to precipitate Fe salts. The resulting mixture was filtered through Celite, and the filtrate

was concentrated under reduced pressure to give 7-amino-4-[(3-bromophenyl)amino]quinazoline (**48**) (0.90 g, 90% yield): mp (H_2O) 219.5–223 °C; ^1H NMR [$(CD_3)_2SO$] δ 10.77 (s, 1 H, NH), 8.64 (s, 1 H, H-2), 8.50 (d, J = 9.2 Hz, 1 H, H-5), 8.06 (s, 1 H, H-2′), 7.77 (d, J = 7.0 Hz, 1 H, H-6′), 7.43–7.36 (m, 2 H, H-4′,5′), 7.04 (dd, J = 9.2, 2.2 Hz, 1 H, H-6), 6.93 (s, 2 H, NH_2), 6.80 (d, J = 2.2 Hz, 1 H, H-8). Anal. ($C_{14}H_{11}BrN_4 \cdot 1.5H_2O$) C, H, N.

Similar Fe dust reductions of the other nitro compounds gave the corresponding amino derivatives in good yields.

4-[(3-Bromophenyl)amino]-6,7-diaminoquinazoline (54). 7-Amino-6-nitroquinazolin-4(3*H*)-one (**58**) (5.90 g, 28.6 mmol) in a mixture of AcOH (300 mL)/Ac_2O (100 mL) was heated under reflux for 6 h. Water (100 mL) was added, and the solution was concentrated to a small volume to give 7-acetamido-6-nitroquinazolin-4(3*H*)-one (**59**) (5.37 g, 76%): mp (EtOH) 260.5–262.5 °C; ^1H NMR [$(CD_3)_2SO$] δ 10.51 (br s, 1 H, NH), 8.57 (s, 1 H, H-5), 8.24 (s, 1 H, H-2), 7.97 (s, 1 H, H-8), 2.15 (s, 3 H, CH_3). Anal. ($C_{10}H_8N_4O_4$) C, H, N.

Reaction of **59** (5.00 g, 20 mmol) in $POCl_3$ (250 mL) under reflux for 2 h, followed by removal of excess $POCl_3$ under reduced pressure and partitioning of the residue between CH_2Cl_2 and cold aqueous Na_2CO_3 solution, gave the crude 4-chloro derivative, which was coupled directly with 3-bromoaniline in 2-propanol as above to give 7-acetamido-4-[(3-bromophenyl)-amino]-6-nitroquinazoline (**60**) (3.60 g, 45%): mp (MeOH) 245.5–247.5 °C; ^1H NMR [$(CD_3)_2SO$] δ 10.56 (s, 1 H, NH), 10.29 (s, 1 H, NH), 9.34 (s, 1 H, H-5), 8.70 (s, 1 H, H-2), 8.19 (br s, 1 H, H-2′), 7.97 (s, 1 H, H-8), 7.88 (d, J = 6.0 Hz, 1 H, H-6′), 7.43–7.35 (m, 2 H, H-4′,5′), 2.13 (s, 3 H, CH_3). Anal. ($C_{15}H_{12}BrN_5O_2$) C, H, N.

Hydrolysis of **60** (1.50 g, 3.73 mmol) with KOH (2 g) in MeOH (190 mL) and H_2O (10 mL) under reflux for 30 min, followed by concentration, gave 7-amino-4-[(3-bromophenyl)-amino]-6-nitroquinazoline (**61**) (1.17 g, 87%): mp (MeOH) 325.5–328 °C; ^1H NMR [$(CD_3)_2SO$] δ 10.17 (s, 1 H, NH), 9.43 (s, 1 H, H-5), 8.43 (s, 1 H, H-2), 8.15 (br s, 1 H, H-2′), 7.86 (d, J = 7.1 Hz, 1 H, H-6′), 7.42 (s, 2 H, NH_2), 7.40–7.31 (m, 2 H, H-4′,5′), 7.12 (s, 1 H, H-8). Anal. ($C_{14}H_{10}BrN_5O_2$) C, H, N.

Reduction of **61** (0.5 g, 1.4 mmol) with Fe dust in 65% aqueous EtOH containing sufficient aqueous HCl to ensure solubility gave 4-[(3-bromophenyl)amino]-6,7-diaminoquinazoline (**54**) (0.30 g, 65%): mp (H_2O) 137–141 °C; ^1H NMR [$(CD_3)_2SO$] δ 9.14 (s, 1 H, NH), 8.27 (s, 1 H, H-2), 8.23 (br s, 1 H, H-2′), 7.85 (d, J = 8.0 Hz, 1 H, H-6′), 7.31–7.14 (m, 2 H, H-4′,5′), 7.29 (s, 1 H, H-5), 6.79 (s, 1 H, H-8), 5.73 (s, 2 H, NH_2), 5.13 (s, 2 H, NH_2). Anal. ($C_{14}H_{12}BrN_5$) C, H, N.

4-[(3-Bromophenyl)amino]-6,7-dihydroxyquinazoline (53). Fusion of **3** in pyridinium hydrochloride at 205 °C for 1 h followed by aqueous workup and recrystallization from EtOH gave 4-[(3-bromophenyl)amino]-6,7-dihydroxyquinazoline (**53**): mp >320 °C; ^1H NMR [$(CD_3)_2SO$] δ 12.6-11,8 (br s, 1 H, OH), 10.94 (slbr s, 1 H, NH), 10.40 (br s, 1 H, OH), 8.78 (s, 1 H, H-2), 8.02 (s, 1 H, H-5), 8.00 (t, J = 1.8 Hz, 1 H, H-2′), 7.72 (dt, J_d = 7.3 Hz, J_t = 1.8 Hz, 1 H, H-6′), 7.50-7.36 (m, 3 H, H-8, 4′, 5′). Anal. ($C_{14}H_8BrN_3O_2$) C, H, N.

7-Hydroxy-6-methoxy-4-[(phenylmethyl)amino]quinazoline (38) and 6-hydroxy-7-methoxy-4-[(phenylmethyl)amino]quinazoline (39). 4-[(Phenylmethyl)amino]-6,7-dimethoxyquinazoline (**40**) was heated with 140 mol % sodium methanethiolate in DMA at 85 °C for 4.5 h, followed by aqueous workup, to give an 8:1 mixture of monodemethylated compounds. Preparative TLC gave separately **38** (major isomer) and **39** (minor isomer). **38**: mp 245-246 °C; ^1H NMR [$(CD_3)_2SO$] δ 10.18 (s, 1 H), 8.40 (t, J = 5.6 Hz, 1 H), 8.25 (s, 1 H), 7.65 (s, 1 H), 7.40-7.18 (s, 5 H), 4.77 (d, J = 5.6 Hz, 2 H), 3.89 (s, 3 H). Anal. ($C_{16}H_{15}N_3O_2$) H, N; C: found, 67.6; calcd, 68.3. **39**: mp 216-217 °C; ^1H NMR [$(CD_3)_2SO$] δ 9.46 (s, 1 H), 8.31 (t, J = 6 Hz, 1 H), 8.26 (s, 1 H), 7.53 (s, 1 H), 7.35-7.2 (m, 4 H), 7.21 (t, J = 7 Hz, 1 H), 7.10 (s, 1 H), 4.73 (d, J = 6 Hz, 2 H), 3.93 (s, 3 H). Anal. ($C_{16}H_{15}N_3O_2$) C, H, N.

Enzyme Assay. Epidermal growth factor receptor was prepared from human A431 carcinoma cell shed membrane vesicles by immunoaffinity chromatography as previously described,[32] and the assays were carried out as reported previously.[15] Reactions were carried out in a total volume of 0.1 mL of 25 mM HEPES buffer (pH 7.4) containing 5 mM $MgCl_2$, 2 mM $MnCl_2$, 50 μM sodium vanadate, 0.5–1.0 ng of EGFR (which contained enough EGF to make a final concentration of 2 μg/mL), and 10 μM ATP containing 1 μCi of [^{32}P]-ATP, with varying concentrations of the drug under test and 200 μM of the substrate. The latter was based on a portion of phospholipase C-γ1 having the sequence Lys-His-Lys-Lys-Leu-Ala-Glu-Gly-Ser-Ala-Tyr^{472}-Glu-Glu-Val. The reaction was allowed to proceed for 10 min at room temperature and then was stopped by the addition of 2 mL of 75 mM phosphoric acid. The solution was then passed through a 2.5 cm phosphocellulose disk which bound the peptide. This filter was washed with 75 mM phosphoric acid (5×), and the incorporated label was assessed by scintillation counting in an aqueous fluor. Control activity (no drug) gave a count of approximately 100 000 cpm. At least two independent dose–response curves were done and the IC_{50} values computed. The reported values are averages; variation was generally ±15%.

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Supporting Information Available: ^1H NMR data for the compounds of Table 1 (6 pages). Ordering information is given on any current masthead page.

References

(1) Dobrusin, E. M.; Fry, D. W. Protein tyrosine kinases and cancer. *Annu. Rep. Med. Chem.* **1992**, *27*, 169–178.
(2) Workman, P.; Brunton, V. G.; Robins, D. J. Tyrosine kinase inhibitors. *Semin. Cancer Biol.* **1992**, *3*, 369–381.
(3) Burke, T. R. Protein-tyrosine kinase inhibitors. *Drugs Future* **1992**, *17*, 119–131.
(4) Levitzki, A. Tyrphostins: tyrosine kinase blockers as novel antiproliferative agents and dissectors of signal transduction. *FASEB J.* **1992**, *6*, 3275–3282.
(5) Chang, C.-J.; Geahlen, R. L. Protein-tyrosine kinase inhibition: mechanism-based discovery of antitumor agents. *J. Nat. Prod.* **1992**, *55*, 1529–1560.
(6) Halaban, R. Growth factors and tyrosine protein kinases in normal and malignant melanocytes. *Cancer Met. Rev.* **1991**, *10*, 129–140.
(7) El-Zayat, A. A. E.; Pingree, T. F.; Mock, P. M.; Clark, G. M.; Otto, R. A.; Von Hoff, D. D. Epidermal growth factor receptor amplification in head and neck cancer. *Cancer J.* **1991**, *4*, 375–380.
(8) Burke, T. R; Ford, H.; Osherov, N.; Levitzki, A.; Stefanova, I.; Horak, I. D.; Marquez, V. E. Aryl amides of hydroxylated isoquinolines as protein-tyrosine kinase inhibitors. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1771–1774.
(9) Burke, T. R; Lim, B.; Marquez, V. E.; Li, Z.-H.; Bolen, J. B.; Stefanova, I.; Horak, I. D. Bicyclic compounds as ring-constrained inhibitors of protein-tyrosine kinase p56^{lck}. *J. Med. Chem.* **1993**, *36*, 425–432.
(10) Rewcastle, G. W.; Palmer, B. D.; Dobrusin, E. M.; Fry, D. W.; Kraker, A. M.; Denny, W. A. Tyrosine kinase inhibitors. 3. Structure-activity relationships for inhibition of protein tyrosine kinases by nuclear-substituted derivatives of 2,2′-dithiobis(1-methyl-*N*-phenyl-1*H*-indole-3-carboxamide). *J. Med. Chem.* **1994**, *37*, 2033–2042.
(11) Hanks, S. K.; Quinn, A. M.; Hunter, T. The protein kinase family: conserved features and deduced phylogeny of of the catalytic domains. *Science* **1988**, *241*, 42–52.
(12) Fantl, W. J.; Johnson, D. E.; Williams, L. T. Signalling by receptor tyrosine kinases. *Annu. Rev. Biochem.* **1993**, *62*, 453–481.
(13) Geissler, J. F.; Roesel, J. L.; Meyer, T.; Trinks, U. P.; Traxler, P.; Lydon, N. B. Benzopyranones and benzothiopyranones: a class of tyrosine protein kinase inhibitors with selectivity for the v-*abl* kinase. *Cancer Res.* **1992**, *52*, 4492–4498.
(14) Trinks, U.; Buchdunger, E.; Furet, P.; Kump, W.; Mett, H.; Meyer, T.; Muller, M.; Regenass, U.; Rihs, G.; Lydon, N.; Traxler, P. Dianilinophthalimides: potent and selective, ATP-competitive inhibitors of the EGF-receptor protein tyrosine kinase. *J. Med. Chem.* **1994**, *37*, 1015–1027.
(15) Fry, D. W.; Kraker, A. J.; McMichael, A.; Ambroso, L. A.; Nelson, J. M.; Leopold, W. R.; Connors, R. W.; Bridges, A. J. A specific inhibitor of the epidermal growth factor receptor tyrosine kinase. *Science* **1994**, *265*, 1093–1095.

(16) Barker, A. J.; Davies, D. H. Therapeutic preparations containing quinazoline derivatives. European Patent Application Number 0 520 722 A1, December 30, 1992. Barker, A. J. Quinazoline derivatives. European Patent Application Number 0 566 226 A1, October 20, 1993.
(17) Ward, W. H. J.; Cook, P. N.; Slater, A. M.; Davies, D. H.; Holdgate, G. A.; Green, L. R. Epidermal growth factor receptor tyrosine kinase. Investigation of catalytic mechanism, structure-based searching and discovery of a potent inhibitor. *Biochem. Pharmacol.* **1994**, *48*, 659–666.
(18) Maguire, M. P.; Sheets, K. R.; McVety, K.; Spada, A. P.; Zilberstein, A. A new series of PDGF receptor tyrosine kinase inhibitors: 3-substituted quinoline derivatives. *J. Med. Chem.* **1994**, *37*, 2129–2137.
(19) Dolle, R. E.; Dunn, J. A.; Bobko, M.; Singh, B.; Kuster, J. E.; Baizman, E.; Harris, A. L.; Sawutz, D. G.; Miller, D.; Wang, S.; Faltynek, C. R.; Xie, W.; Sarup, J.; Bode, D. C.; Pagani, E. D.; Silver, P. J. 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. *J. Med. Chem.* **1994**, *37*, 2627–2629.
(20) Endicott, M. M.; Wick, E.; Mercury, M. L.; Sherrill, M. L. Quinazoline derivatives. I. The synthesis of 4-(4′-diethylamino-1′-methylbutylamino)quinazoline (SN 11,534) and the corresponding 2-phenylquinazoline (SN 11,535). *J. Am. Chem. Soc.* **1946**, *68*, 1299–1301.
(21) Hauser, C. R.; Reynolds, G. A. Relative ease of cyclization of 2-, 3- and 4-aminopyridine derivatives. Synthesis of naphthyridines. *J. Org. Chem.* **1950**, *15*, 1224–1232.
(22) Fisher, N. I.; Hamer, F. M. Cyanine dyes containing an isoquinoline nucleus. *J. Chem. Soc.* **1934**, 1905–1910.
(23) Leonard, N. J.; Boyd, S. N. Cinnolines. II. Synthesis of 4-hydroxycinnolines. *J. Org. Chem.* **1946**, *11*, 419–428.
(24) Atkinson, C. M.; Brown, C. W.; Simpson, J. C. E.; Some reactions in the phthalazine series. *J. Chem. Soc.* **1956**, 1081–1083.
(25) Gowenlock, A. H.; Newbold, G. T.; Spring, F. S. Synthesis of 2-monosubstituted and 2,3-disubstituted quinoxalines. *J. Chem. Soc.* **1945**, 622–625.
(26) Siddiqui, M. S. S.; Stevens, M. F. G. Triazines and related products. Part XI. Dimroth rearrangements of 3-substituted 3,4-dihydro-4-imino-1,2,3-benzotriazines in acetic acid. *J. Chem. Soc., Perkin Trans. I* **1974**, 609–610
(27) Elderfield, R. C.; Williamson, T. A.; Gensler, W. J., Kremer, C. B. Synthesis of *bz*-substituted quinazolines and antimalarials from them. A contribution to the chemistry of quinazoline. *J. Org. Chem.* **1947**, *12*, 405–421.
(28) Morley, J. S.; Simpson, J. C. E. The chemistry of simple heterocyclic systems. Part 1. Reactions of 6- and 7-nitro-4-hydroxyquinazoline and their derivatives. *J. Chem. Soc.* **1948**, 360–366.
(29) Baker, B. R.; Schaub, R. E.; Joseph, J. P.; McEvoy, F. J.; Williams, J. H. An antimalarial alkaloid from hydrangea. XIV. Synthesis of 5-, 6-, 7-, and 8-monosubstituted derivatives. *J. Org. Chem.* **1952**, *17*, 141–148.
(30) Osborn, A. R.; Schofield, K.; Short, L. N. Studies of the amino-isoquinolines, -cinnolines, and -quinazolines. (A) The basic strengths and ultraviolet absorption spectra. (B) The infrared spectra. *J. Chem. Soc.* **1956**, 4191–4206.
(31) Takase, Y.; Saeki, T.; Watanabe, N.; Adachi, H.; Souda, S.; Saito, I. Cyclic GMP phosphodiesterase inhibitors. 2. Requirement of 6-substitution of quinazoline derivatives for potent and selective inhibitory activity. *J. Med. Chem.* **1994**, *37*, 2106–2111.
(32) Gill, G. N.; Weber, W. Purification of functionally active epidermal growth factor receptor protein using a competitive antagonist monoclonal antibody and competitive elution with epidermal growth factor. *Methods Enzymol.* **1987**, *146*, 82–88.
(33) Girgis, N. S.; Moller, J.; Pedersen, E. B. Phosphorus pentoxide in organic synthesis. 25. New one step synthesis of 4-amino-quinazolines. Comparison between mass spectra of 4-amino-quinazolines and 6-aminopurines. *Chem. Scr.* **1986**, *26*, 617–621.
(34) Nagakami, K.; Yokoi, S.; Nishimura, K.; Nagai, S.; Honda, T.; Oda, K.; Fujii, K.; Kobayashi, R.; Kojima, M. Aminoquinazoline microbiocides. Japan patent 79 02,327; *Chem. Abstr.* **1979**, *90*, 198861f.
(35) Iyer, R. N.; Anand, N.; Dhar, N. I. Studies in potential amebicides. III. Synthesis of 4-substituted amino-8-hydroxy (and 8-methoxy) quinazolines and 3-substituted 8-hydroxy (and 8-methoxy)-4-quinazolines. *J. Sci. Ind. Res. (India)* **1956**, *15C*, 1–7.

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