Tyrosine Kinase Inhibitors. 8. An Unusually Steep Structure−Activity Relationship for Analogues of 4-(3-Bromoanilino)-6,7-dimethoxyquinazoline (PD 153035), a Potent Inhibitor of the Epidermal Growth Factor Receptor

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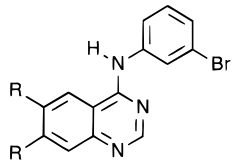
Received May 18, 1995[⊗]

4-(3-Bromoanilino)-6,7-dimethoxyquinazoline (**32**, PD 153035) is a very potent inhibitor (IC_{50} 0.025 nM) of the tyrosine kinase activity of the epidermal growth factor receptor (EGFR), binding competitively at the ATP site. Structure−activity relationships for close analogues of **32** are very steep. Some derivatives have IC_{50}s up to 80-fold better than predicted from simple additive binding energy arguments, yet analogues possessing combinations of similar phenyl and quinazoline substituents do not show this "supra-additive" effect. Because some substituents which are mildly deactivating by themselves can be strongly activating when used in the correct combinations, it is proposed that certain substituted analogues possess the ability to induce a change in the conformation of the receptor when they bind. There is some bulk tolerance for substitution in the 6- and 7-positions of the quinazoline, so that **32** is not the optimal inhibitor for the induced conformation. The diethoxy derivative **56** [4-(3-bromoanilino)-6,7-diethoxyquinazoline] shows an IC_{50} of 0.006 nM, making it the most potent inhibitor of the tyrosine kinase activity of the EGFR yet reported.

Introduction

Many of the tyrosine kinase enzymes which are early components of the growth signal transduction pathway in mammalian cells[1] are encoded by proto-oncogenes, and their transformation or overexpression has been shown to occur in a large percentage of clinical cancers.[2−5] These tyrosine kinase enzymes, particularly the receptors for growth factors such as EGF, bFGF, and PDGF, have thus become important targets for drug design.[1,6] Several classes of inhibitors have been described,[6−9] but most have proven to be of limited use in cellular assays and more advanced models. Following up on leads from our mass screening program, and concomitant patent publications from Zeneca,[10−12] we have recently reported[13,14] on the inhibitory properties of 4-anilinoquinazolines against the epidermal growth factor receptor (EGFR). Despite binding at the ATP site of EGFR (a site of close homology among different kinases),[12,15] members of this class are very potent and selective inhibitors of the EGFR. In particular, we have shown that 4-(3-bromoanilino)-6,7-dimethoxyquinazoline (**32**) has an IC_{50} of 25 pM (K_i 6 pM) for this enzyme.[13]

In a previous publication[14] we delineated the initial structure−activity relationship (SAR) for this series. The pyrimidine ring is mandatory, and a free NH linker is clearly optimal. Electron-withdrawing lipophilic substituents on the 3-position of the aniline are favorable, with chlorine and bromine optimal, as reported previously by others.[12] Electron-donating groups at the 6- and 7-positions (but not the 5- and 8-positions) of the quinazoline are preferred. However, the best of these only produce an increase in potency of about 10-fold over the unsubstituted 4-(3-bromoanilino)quinazoline parent compound **4**. This leaves the 6,7-dimethoxy derivative **32** another 2 orders of magnitude more potent than would be expected from completely additive effects of the two substituents. In the present paper we focus on some closely related analogues of **4**, delineate where in these SAR "supra-additive" substituent effects can be found, and speculate on their origin.



4 : R = H
32 : R = OMe (PD 153035)

Chemistry

The compounds were prepared by coupling the appropriate 4-chloroquinazolines with substituted anilines in refluxing 2-propanol as described previously.[14] The 6-nitro-7-(methylamino), -(dimethylamino), and -methoxy derivatives **49**, **50**, and **52** were prepared from the known[16] 7-chloro-6-nitroquinazolinone **70**. This was first converted to 4,7-dichloro-6-nitroquinazoline (**71**), which underwent selective displacement of the 4-chloro group with 3-bromoaniline to give **53**, followed by substitution of the 7-chloro group to give **49** and **50** (Scheme 1). Conversion of **70** to **72** with methoxide followed by chlorination to **73** and reaction of this with 3-bromoaniline gave **52**. The 6-NHMe derivative **35** was prepared by $LiAlH_4$ reduction of the methyl carbamate **37**, and the 7-NRR compounds **40**−**42** were prepared by fluoride displacement from the 7-fluoro analogue **75** (Scheme 2). The 7-substituted-6-NH_2 compounds **44**−**47** were prepared by reduction of the corresponding

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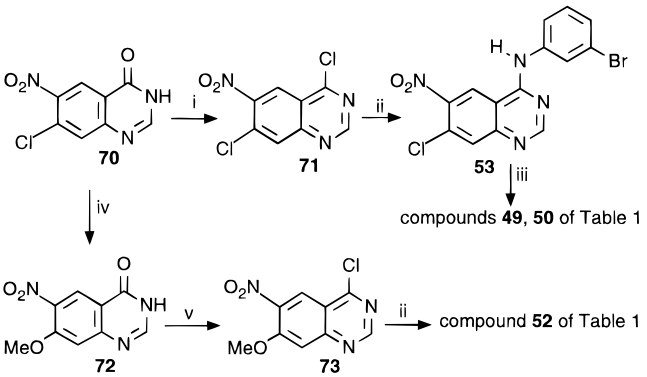
⊗ Abstract published in *Advance ACS Abstracts,* December 1, 1995.

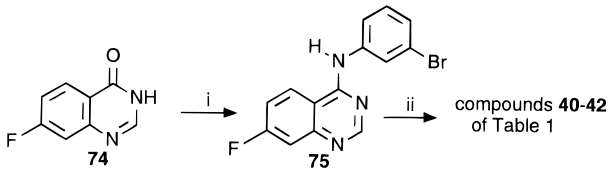
0022-2623/96/1839-0267$12.00/0

Scheme 1[a]



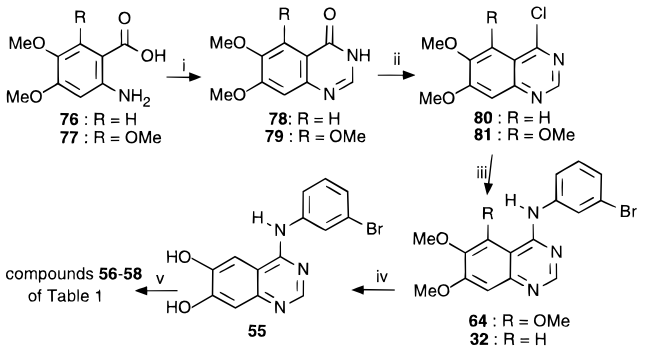
[a] (i) $SOCl_2$/DMF/reflux/1 h; (ii) 3-bromoaniline/HCl (trace)/iPrOH/reflux/30 min; (iii) R_2NH or RNH_2/EtOH/100 °C (pressure vessel)/1 h; (iv) MeOH/MeONa/100 °C (pressure vessel)/18 h; (v) $POCl_3$/reflux/30 min.

Scheme 2[a]



[a] (i) $POCl_3$/reflux/1 h and then iPrOH/3-bromoaniline/HCl (trace)/reflux/30 min; (ii) R_2NH or RNH_2/*N*-Me-pyrrolidone/130 °C/4 days.

Scheme 3[a]

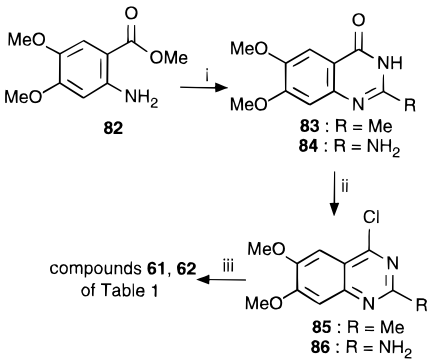


[a] (i) Formamidine hydrochloride/210 °C/15 min (R = H) or triazine/piperidine/MeOH/reflux (R = OMe); (ii) $(COCl)_2$/DMF (R = H) or $POCl_3$/reflux (R = OMe); (iii) 3-bromoaniline/iPrOH/25 °C/18 h or reflux/1 h; (iv) pyridine hydrochloride/205 °C; (v) RI/K_2CO_3 or RI/Cs_2CO_3/DMF/25 °C.

6-NO_2 compounds **49**, **50**, **52**, and **53** with Fe dust in 65% aqueous EtOH.

The normal cyclization of anthranilic acids with formamide at elevated temperatures (170−195 °C) was not efficient in forming the quinazolinone precursors for compounds **32** and **64**. In the former case, the quinazolinone **78** was formed by cyclizing the anthranilic acid **76** with an intimately ground mixture of formamidine hydrochloride at 210 °C for 15 min, and in the latter the trimethoxyquinazolinone **79** was formed from **77** by refluxing with triazine and a catalytic amount of piperidine in methanol (Scheme 3). The 6,7-dimethoxyquinazolinone **78** was converted in good yield to the 4-chloro compound **80** with oxalyl chloride/DMF (Vilsmeier procedure), while the 5,6,7-trimethoxy derivative **79** was converted to **81** using $POCl_3$ in 1,2-dichloroethane buffered with Hunig's base. Demethylation of **32** to **55** was achieved by fusion with pyridinium hydrochloride at 200 °C. Extended reaction times (>2 h) were required for complete demethylation, but under these

Scheme 4[a]



[a] (i) RCN/HCl and then $NaHCO_3$; (ii) $POCl_3$/reflux and then 3-bromoaniline/2-methoxyethanol/HCl.

conditions competitive loss of the anilino side chain became appreciable. Conversion of **55** to the corresponding higher alkyl ethers **56**−**58** was accomplished by treatment with an excess of the corresponding alkyl iodide and K_2CO_3 or Cs_2CO_3 in DMF at 25 °C, followed by preparative TLC (Scheme 3).

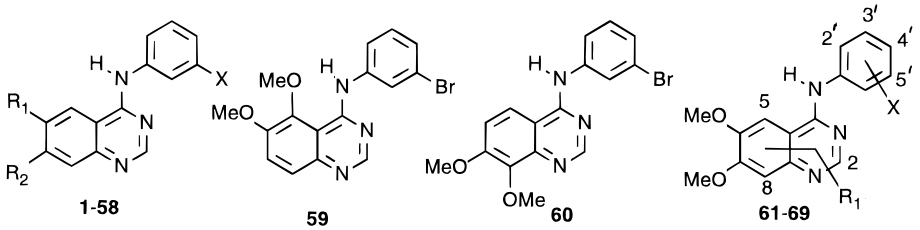
The 2-substituted analogues **61** and **62** were prepared as shown in Scheme 4. Reaction of methyl 4,5-dimethoxyanthranilate (**82**) with either acetonitrile or cyanamide using HCl gas catalysis gave the quinazolinones **83** and **84**, respectively. In both cases the intermediate amidines cyclized spontaneously upon neutralization of the reaction mixture. The quinazolinones were converted to **61** and **62** by usual 4-chlorination ($POCl_3$) and displacement with 3-bromoaniline in 2-methoxyethanol at 90 °C.

Results and Discussion

The structures, physicochemical properties, and EGFR inhibitory potencies (IC_{50} in nanomolar) are given in Table 1. The IC_{50} values are the average of at least two independent determinations of full dose−response curves. The assay used initially was a purified full length EGFR, stimulated by EGF, phosphorylating a tyrosine-containing peptide based upon the major EGFR phosphorylation site on PLCγ.[13] Similar results were seen with representative compounds when the phosphopeptide substrate was substituted by a Glu-Tyr copolymer. The conditions under which this assay was used, described in detail elsewhere,[13] were critical. The use of less purified enzyme preparations gave unreliable results with these ATP competitive compounds, while performing reliably with literature standards which are probably not inhibitors of the same mechanistic class, such as several tyrphostins,[17] a sulfonylbenzoylnitrostyrene,[18] a thioindole,[9] and erbstatin.[19]

Table 2 compares, for a limited set of quinazoline substituents (first column), the effect on activity of changes in the 3-halogen in the aniline side chain. For each quinazoline substituent pattern, the first row in Table 2 gives the compound number from Table 1 and the second row the actual IC_{50} value for the specified compound. The third row is the ratio of the IC_{50} value of the parent anilinoquinazoline (**1**, **7**, **9**, **12**, **15**, **17**, **23**, **29**) over the IC_{50} values for the corresponding 3-substituted side chain analogues [344/IC_{50}(3′-X)] and is therefore a direct measure of the effect of the 3′-H/3′-X substitution. The entry in the fourth row is the notional IC_{50} one would calculate for that compound assuming that the quinazoline and aniline substituents act com-

Table 1. Physicochemical and EGFR Tyrosine Kinase Inhibition Data for 4-Anilinoquinazolines



no.	R_1	R_2	X	mp (°C)	formula	anal.	IC_{50}[a] (nM)
1	H	H	H	ref 14			344
2[b]	H	H	F	257−260	$C_{14}H_{10}N_3F\cdot HCl$	C,H,N	56
3[b,c]	H	H	Cl	ref 14			23
4[b]	H	H	Br	ref 14			27
5[b]	H	H	I	ref 14			80
6[b]	H	H	CF_3	ref 14			577
7	OMe	H	H	260−261	$C_{15}H_{13}N_3O\cdot HCl$	C,H,N	55
8	OMe	H	Br	ref 14			30
9	NH_2	H	H	195−196	$C_{14}H_{12}N_4$	C,H,N	770
10[d]	NH_2	H	CF_3	180−181.5	$C_{15}H_{11}F_3N_4$	C,H,N	574
11	NH_2	H	Br	ref 14			0.78
12	NO_2	H	H	ref 14			5000
13	NO_2	H	Br	ref 14			900
14[d]	NO_2	H	CF_3	209−211	$C_{15}H_9F_3N_4O_2$	C,H,N	$>10^4$
15	H	MeO	H	265−267	$C_{15}H_{13}N_3O\cdot HCl$	C,H,N	120
16	H	MeO	Br	ref 14			10
17	H	NH_2	H	199−201	$C_{14}H_{12}N_4$	C,H,N	100
18	H	NH_2	F	271−274	$C_{14}H_{11}N_4F\cdot HCl\cdot 0.5C_3H_8O$	C,H,N	2.0
19	H	NH_2	Cl	311−313	$C_{14}H_{11}N_4Cl\cdot 2HCl$	C,H,N	0.25
20	H	NH_2	Br	ref 14			0.1
21	H	NH_2	I	259−261	$C_{14}H_{11}IN_4\cdot 0.4AcOH\cdot 0.5H_2O$	C,H,N	0.35
22	H	NH_2	CF_3	271−274	$C_{15}H_{11}N_4F_3\cdot HCl\cdot C_3H_8O$	C,H,N	3.3
23	H	NO_2	H	ref 14			1.2×10^4
24	H	NO_2	F	253−254	$C_{14}H_9N_4FO_2\cdot HCl$	C,H,N	6100
25	H	NO_2	Cl	311−313	$C_{14}H_9N_4ClO_2\cdot HCl$	C,H,N	810
26	H	NO_2	Br	ref 14			1000
27	H	NO_2	I	248−249 dec	$C_{14}H_9N_4IO_2\cdot HCl$	C,H,N	540
28	H	NO_2	CF_3	240−241 dec	$C_{15}H_9N_4F_3O_2\cdot HCl$	C,H,N	$>10^4$
29[d]	OMe	OMe	H	>250	$C_{16}H_{15}N_3O_2\cdot HCl$	C,H,N	29
30[d]	OMe	OMe	F	253−254	$C_{16}H_{14}N_3FO_2\cdot HCl\cdot H_2O$	C,H,N	3.8
31[d]	OMe	OMe	Cl	261−262	$C_{16}H_{14}N_3ClO_2\cdot HCl$	C,H,N	0.31
32	OMe	OMe	Br	ref 13			0.025
33	OMe	OMe	I	273	$C_{16}H_{14}N_3O_2I\cdot HCl$	C,H,N	0.89
34[d]	OMe	OMe	CF_3	274−276 dec	$C_{17}H_{14}N_3F_3O_2\cdot HCl$	C,H,N	0.24
35	NHMe	H	Br	141−144	$C_{15}H_{13}BrN_4$	C,H,N	4
36	NMe_2	H	Br	248−249	$C_{16}H_{16}BrN_4$	C,H,N	84
37	$NHCO_2Me$	H	Br	197−198.5	$C_{16}H_{13}BrN_4O_2$	C,H,N	12
38	H	OH	Br	290 dec	$C_{14}H_{10}BrN_3O$	C,H,N	4.7
39	H	NHAc	Br	351−353	$C_{16}H_{13}BrN_4O$	C,H,N	40
40	H	NHMe	Br	198−199	$C_{15}H_{13}BrN_4$	C,H,N	7
41	H	NHEt	Br	160−161.5	$C_{16}H_{15}BrN_4$	HRMS	12
42	H	NMe_2	Br	239−240	$C_{16}H_{15}BrN_4$	C,H,N	11
43	NH_2	NH_2	Br	ref 14			0.12
44	NH_2	NHMe	Br	238−240	$C_{15}H_{14}BrN_5$	C,H,N	0.69
45	NH_2	NMe_2	Br	174−175	$C_{16}H_{16}BrN_5$	C,H,N	159
46	NH_2	OMe	Br	210−211.5	$C_{15}H_{13}BrN_4O$	C,H,N	3.8
47	NH_2	Cl	Br	218.5−220.5	$C_{14}H_{10}BrClN_4$	C,H,N,Cl	6.5
48	NO_2	NH_2	Br	ref 14			53
49	NO_2	NHMe	Br	253−255	$C_{15}H_{12}BrN_5O_2$	C,H,N	68
50	NO_2	NMe_2	Br	240.5−243.5	$C_{16}H_{14}BrN_5O_2$	C,H,N	2000
51	NO_2	NHAc	Br	ref 14			28
52	NO_2	OMe	Br	206−207	$C_{15}H_{11}BrN_4O_3$	C,H,N	15
53	NO_2	Cl	Br	257−259	$C_{14}H_8BrClN_4O_2$	C,H,N	25
54	OCH_2O		Br	278.5−280	$C_{15}H_{10}BrN_3O_2$	C,H,N	15
55	OH	OH	Br	ref 14			0.17
56	OEt	OEt	Br	155−167	$C_{18}H_{18}N_3BrO_2\cdot H_2O$	C,H,N	0.006
57	OPr	OPr	Br	145−146	$C_{20}H_{22}BrN_3O_2\cdot 1.5H_2O$	C,H,N	0.17
58	OBu	OBu	Br	123−124	$C_{22}H_{26}BrN_3O_2$	C,H,N	105
59	5,6-diOMe			159−160	$C_{16}H_{14}BrN_3O_2$	C,H,N	1367
60	7,8-diOMe			250−252	$C_{16}H_{14}BrN_3O_2$	C,H,N	$>10^4$
61	2-Me		3′-Br	264−265	$C_{17}H_{16}BrN_3O_2\cdot HCl$	C,H,N	$>10^4$
62	2-NH_2		3′-Br	262−263	$C_{16}H_{15}BrN_4O_2\cdot 3HCl\cdot 0.25H_2O$	C,H,N	463
63	N^4-Me		3′-Br	218−219	$C_{17}H_{16}BrN_3O_2\cdot HCl\cdot 0.25C_3H_8O$	C,H,N	152
64	5-OMe		3′-Br	130−132	$C_{17}H_{16}BrN_3O_3$	C,H,N	0.67
65	8-OMe		3′-Br	212−213	$C_{17}H_{16}BrN_3O_3$	C,H,N	$>10^4$
66	H		2′-Br	230−231	$C_{16}H_{14}BrN_3O_2\cdot HCl$	C,H,N	128
67[b]	H		4′-Br	275	$C_{16}H_{14}N_3BrO_2\cdot HCl$	C,H,N	0.96
68	H		3′,4′-Br	254−264	$C_{16}H_{13}Br_2N_3O_2\cdot HCl$	C,H,N	0.072
69	H		3′,5′-diBr	>250	$C_{16}H_{13}Br_2N_3O_2\cdot 0.5H_2O$	C,H,N	113

[a] IC_{50} = concentration of drug (nM) to inhibit the phosphorylation of a 14-residue fragment of phospholipase Cγ1 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 10. [c] Reference 12. [d] Reference 11.

Table 2. Additivity of 3′-Halo and 6,7-Quinazoline Substituents in 4-Anilinoquinazolines

	substituent					
	3′-H	3′-F	3′-Cl	3′-Br	3′-I	3′-CF_3
compd no.	**1**	**2**	**3**	**4**	**5**	**6**
6,7-diH	344[a]	56	23	27	80	577
IC_{50} ratio[b]	1	6.1	15	12.7	4.3	0.60
$\Delta(\Sigma E)$ (kcal/mol)[c]	0	1.1	1.6	1.5	0.9	−0.30
compd no.	**17**	**18**	**19**	**20**	**21**	**22**
6-H, 7-NH_2	100	2.0	0.25	0.10	0.35	3.3
IC_{50} ratio	1 (3.44)	50	400	1000	286	30
calcd IC_{50}[d]	100	16	6.7	7.9	23	167
$\Delta(\Sigma E)$ (kcal/mol)	0 (0.74)	1.24	1.95	2.6	2.49	2.33
compd no.	**9**			**11**		**10**
6-NH_2, 7-H	770			0.78		574
IC_{50} ratio	1 (0.45)			990		1.35
calcd IC_{50}	770			61		1283
$\Delta(\Sigma E)$ (kcal/mol)	0 (−0.48)			2.6		0.48
compd no.	**29**	**30**	**31**	**32**	**33**	**34**
6,7-diMeO	29	3.8	0.31	0.025	0.89	0.24
IC_{50} ratio	1 (11.9)	7.6	94	1160	33	121
calcd IC_{50}	29	4.8	1.93	2.28	6.7	48.4
$\Delta(\Sigma E)$ (kcal/mol)	0 (1.47)	0.13	1.1	2.7	1.2	3.16
compd no.	**12**			**13**		**14**
6-NO_2, 7-H	5000			900		>10 000
IC_{50} ratio	1 (0.069)			5.6		<0.5
calcd IC_{50}	5000			390		
$\Delta(\Sigma E)$ (kcal/mol)	0 (−1.59)			−0.50		
compd no.	**23**	**24**	**25**	**26**	**27**	**28**
6-H, 7-NO_2	12 000	6100	810	1000	540	>10 000
IC_{50} ratio	1 (0.029)	1.97	14.8	12	22	≤1.2
calcd IC_{50}	12 000	1970	800	945	2790	1/1.67
$\Delta(\Sigma E)$ (kcal/mol)	0 (−2.1)	−0.65	0	0	0.98	∼0
compd no.	**7**			**8**		
6-MeO, 7-H	55			30		
IC_{50} ratio	1 (6.3)			1.8		
calcd IC_{50}	55			4.3		
$\Delta(\Sigma E)$ (kcal/mol)	0 (1.1)			−1.15		
compd no.	**15**			**16**		
6-H, 7-MeO	120			10		
IC_{50} ratio	1 (2.87)			12		
calcd IC_{50}	120			9.4		
$\Delta(\Sigma E)$ (kcal/mol)	0 (0.63)			∼0		

[a] Experimental IC_{50} value (nM) for the compound specified by the first row and column. [b] Ratio of IC_{50} values of the parent unsubstituted compound (**1**, 344 nM) over the IC_{50} values for the 3-substituted side chain analogues [=344/IC_{50}(aniline-3′-X)]. Figures in parentheses are ratios of the IC_{50} value of **1** over the IC_{50} values for the quinazoline-substituted compounds [=344/IC_{50}(quinazoline-R)]. [c] Difference in binding energy [$\Delta(\Sigma E)$ in kcal/mol] between the experimental and calculated IC_{50}s (see text). Compounds which bind more strongly than anticipated show a positive value, those that bind less well than expected a negative value. [d] Calculated IC_{50}, assuming additive effects of quinazoline and aniline substituents to binding energy (see text).

pletely independently of each other in their contributions to the binding constant (energetically additive). Finally, the fifth entry is the difference in binding energy [$\Delta(\Sigma E)$ in kcal/mol] between the experimental and calculated IC_{50}s, calculated from the Boltzmann equation ($\Delta G = -RT \ln K$) by making the reasonable assumption that enzyme inhibition is directly proportional to the binding energy of the inhibitor to the enzyme, so that IC_{50} differences are directly proportional to $\Delta(\Sigma E)$. Compounds which inhibit more strongly than anticipated show a positive "excess" binding energy; those which inhibit less well than expected show a negative value. For those where the quinazoline and anilino substituents act completely independently of each other, this value will be close to zero. The figures in parentheses in the first column are the ratios of the IC_{50} value of the parent unsubstituted compound (**1**) (344 nM) over the IC_{50} values for the compounds substituted only on the quinazoline ring [344/IC_{50}-(quinazoline-R)], and the corresponding $\Delta(\Sigma E)$ values, and are used to calculate "expected" binding energies.

From Table 1 it is apparent that the compounds, which vary little in structure, show an enormous range in potency (>10^6-fold, IC_{50}s from 12 000 to 0.006 nM). The large range in potencies among 4-anilinoquinazolines as EGFR inhibitors was also shown in the earlier SAR analysis,[14] but this was with a more structurally heterogeneous data set. The present investigation of the shape of the SAR around the anilinoquinazoline **4** fell into four distinct parts. The first part investigates the effects of individual substituents on either the quinazoline or anilino ring and establishes "base-line" values for them. The second part looks at the effects of combining certain quinazoline substituents with a series of halogens in the 3′-position. In the third part, the anilino substituent is held constant (as 3′-bromo) and the quinazoline substituents are altered. In the last part, compound **32** is used as a template and the effects of adding a substituent to the various available quinazoline positions are investigated.

General SAR for Quinazoline and Aniline Substituents. We initially investigated the effect of a series of substitutions either on the aniline ring or at the 6- and/or 7-position of the quinazoline rings (compounds **1**−**7**, **12**, **15**, **17**, **23**, and **29** of Table 1). These compounds, many of which have been reported previously

by ourselves and others,[10−14] provided base-line data for the effects of single substitutions on inhibitory potency. The data for these compounds in Tables 1 and 2 show that the effect of individual substitutions (top row and first column of Table 2) is generally not very great. All the 3′-halogens in the aniline ring are 4−15-fold activating, representing an increase of binding energy to the enzyme of 0.9−1.6 kcal. The similar CF_3 group appeared to be slightly detrimental, but the effect is small. For quinazoline substituents, nitro has a large deactivating effect (over a 2 kcal/mol loss of binding energy for 7-NO_2). However, as noted previously,[14] single electron-donating substituents at either the 6- or 7-position are usually moderately activating. The most potent compound in this group is the 6,7-diOMe (**29**), where the effects of the two methoxy substituents are almost additive, leading to a reasonable 12-fold increase in potency. Although these are significant substituent effects, they do not compare to what can happen when the two sets of substituents are combined.

Effects of Varying the 3′-Halogen on the Aniline Side Chain. The second part of the investigation examined the effects of varying the 3′-halogen on the aniline for three series of quinazoline-substituted analogues (7-NH_2, 7-NO_2, and 6,7-diOMe) (compounds **17**−**34**). The 7-NH_2 and 6,7-diOMe series were selected because these substituent patterns provided the most potent single- and double-substituted compounds found in the earlier study.[14] In a few other cases (6-NH_2, 6-NO_2, 6-OMe, and 7-OMe) just the 3′-Br and unsubstituted anilino compounds were compared. The binding data in rows 5−8 of Table 2 (for the 6- and 7-NO_2 and 6- and 7-OMe quinazoline substituents) suggest that the substituent groups are by and large independent of one another. Thus, four of these compounds (**17**, **25**, **26**, and **28**) show the IC_{50}s predicted by additivity arguments, three (**8**, **13**, and **24**) are 3−17 times less potent, and one (**27**) is 5-fold more potent than predicted. This is not an unusual amount of scatter, and as this data set includes the strongly deactivating nitro substituents, and the most strongly activating single substituent (6-OMe) when attached to **1**, the results might be expected to be broadly applicable.

However, the results for the 6-NH_2, 7-NH_2, and 6,7-diOMe substituents (rows 2−4 of Table 2) show a quite different pattern. An example of the large differences between closely related compounds is the 100-fold greater potency of **20** (3′-Br,7-NH_2) compared with **16** (3′-Br,7-OMe). Whereas the latter has exactly the IC_{50} predicted by simple additivity, the former is 80-fold more potent than expected (Table 2). Similar "supra-additive" effects are seen for the 3-bromoanilino derivatives bearing 6-NH_2 and 6,7-diOMe substituents (**11** and **32**). This effect is not restricted to bromine, being seen (where studied in the 7-NH_2 and 6,7-diOMe series) with the other three halogens. On average, the 3′-Br compounds show 2.6 kcal/mol of "excess" binding energy, the 3′-Cl and 3′-I compounds 1.6 kcal/mol, and the 3′-F compounds 0.7 kcal/mol. Most interesting is the 3′-CF_3 substituent, which is mildly deactivating in the parent compound **6**, is at least as activating as bromine in the 7-amino and 6,7-dimethoxy series, but only weakly activating in conjunction with the 6-NH_2 substituent.

SAR for Quinazoline Substituents with a 3′-Bromoaniline Side Chain. In the third part of the study, we kept the anilino side chain with the optimal 3′-Br substituent and varied the substituents on the quinazoline ring to determine the extent of the supra-additive effect (compounds **35**−**59**). Because the corresponding debromo derivatives were not made in this study, we cannot definitely say where the supra-additive effect was present, but we can make the implicit assumption that it is for the very potent compounds. None of the other single quinazoline substituents examined produced the potentiating effect of the 7-NH_2. Perhaps most surprising was the fact that both of the the NMe_2 derivatives **36** and **42** were around 100-fold less potent than the corresponding amino compounds **11** and **20**. The NHMe analogues **35** and **40** were of intermediate potency, making it hard to determine the status of these compounds. In line with the results noted above for nitro substituents, the less electron-donating NHAc and NHCOOMe substituents gave compounds (**37** and **39**) of modest activity.

In the 6,7-disubstituted compounds, the 6,7-diNH_2 derivative **43** had essentially the same potency as the 7-NH_2 derivative **20**, suggesting either that the electron donation of the 7-NH_2 is large enough to give the full effect available or that unfavorable steric or H-bonding effects occur between the *o*-amino groups (see below). The relatively low potency of the 6-amino-7-methoxy compound **44** makes it unlikely that a supra-additive effect is present in this case. Compounds **47** and **53** suggest that a 7-chloro substituent is moderately disadvantageous (8-fold) in conjunction with 6-NH_2 but highly beneficial (200-fold) in conjunction with 6-NO_2. In the other 6-NO_2-7-substituted compounds **48**−**53**, all of the electron-donating 7-groups (except the bulky dimethylamino) strongly mitigate (15−67-fold) the dystherapeutic effect of the 6-NO_2 substituent.

In the 6,7-diOR-substituted compounds **32** and **54**−**58**, combining the two OMe substituents of **32** into a methylenedioxy ring (**54**) resulted in enormous (600-fold) loss of potency. In compound **54**, the covalently bonded 5-membered ring does not place the oxygen lone pairs in an optimal orientation for overlap with the aromatic π system. Conversion of both methoxy substituents to hydroxyls was moderately deleterious, with diol **55** being 6-fold less potent than **32**. Conversely, making the alkyl side chains a little larger led to the the diethoxy analogue **56** which, with an IC_{50} of 0.006 nM, is the most potent quinazoline inhibitor of the EGFR tyrosine kinase activity we have found. The dipropoxy side chain (**57**, IC_{50} 0.17 nM) gave potency similar to diol **55**, and the dibutoxy compound **58** (IC_{50} 105 nM) appears too large to be tolerated well.

These results suggest that that there is significant bulk tolerance available in the region of the 6- and 7-positions of the quinazoline for further design. The limits of this area of bulk tolerance are demonstrated by compounds **59** and **60**, where moving the *gem*-diOMe substituents to either of the other allowed positions in the B-ring (5,6- or 7,8-positions) is clearly highly detrimental.

Additional Substituents on 4-(3-Bromoanilino)-6,7-dimethoxyquinazoline (32). In the fourth part of the SAR study, we looked briefly at a small series of compounds, based on **32** but either being positional isomers (**66** and **67**) or possessing additional substituents at various positions (**61**−**65**, **68**, and **69**). Placing an additional OMe group at the 5-position gave a subnanomolar inhibitor (**64**, IC_{50} 0.67 nM). By com-

parison with the 5,6-diOMe derivative **59**, adding a 7-OMe substituent led to a 2000-fold improvement in IC_{50}. This is very similar to the improvement seen on going from the 6-OMe to the 6,7-diOMe analogues (**7** and **32**), indicating that while a 5-OMe cannot act in concert with the 6-OMe to produce the supra-additive effect, it cannot prevent its occurrence (due to the 6,7-diOMe combination). However, in either case the 5-OMe substituent is about 25-fold detrimental. The 8-OMe substituent is intrinsically much more detrimental ($>10^5$-fold loss in affinity between **32** and **65**). Thus the 6,7,8-triOMe derivative **65** and the 7,8-diOMe analogue **60** both have very low potency (the assay was not carried to high enough inhibitor concentrations to establish the separation quantitatively).

Introduction of a methyl group to the 2-position (**61**) was enormously deactivating, with at least a 400 000-fold loss of potency. The corresponding amino compound **62** was still over 18 000-fold less active than **32**, so even small substituents at this position are highly disfavored in the quinazolines. This is consistent with our previous results with phthalazines and cinnolines, and especially with benzotriazines,[14] where the loss of potency upon replacing the 2-position ring methine with nitrogen was similarly enormous. Methylation of the N^4-linking nitrogen led to a 6000-fold drop in activity (**63**, IC_{50} 152 nM). In the parent anilinoquinazoline, this substitution was also highly disfavored, leading to a 300-fold loss of affinity.[14]

Finally, we looked briefly at the effects of Br substitution at different positions in the aniline ring. The 4′-Br substituent is reasonably activating on 6,7-dimethoxyquinazoline (30-fold increase in potency between **29** and **67**) and only moderately deactivating when combined with 3′-Br on the same nucleus (3-fold decrease in potency between **32** and **68**). In contrast both 2′-Br and 3′,5′-diBr substituents are about 5-fold deactivating on the same nucleus (compounds **66** and **69** compared with **29**), suggesting limits to the steric tolerance of the aniline-binding site.

Kinetic and Cellular Data. Figure 1 shows a double-reciprocal plot for inhibition of the EGFR by **4** with respect to ATP concentration. The converging line on the *y* axis, as well as best-fit analysis by nonlinear regression to Cleland's equations,[21] indicates that **4** behaves as a pure competitive inhibitor to ATP. Similar experiments with the peptide as the varying substrate (data not shown) indicated noncompetitive inhibition. Since tight-binding inhibitors do not conform to the equations derived for steady-state enzyme kinetics, a similar analysis could not be performed for **32**. A direct demonstration that this derivative is competitive with ATP was therefore not feasible, and other mechanisms cannot be ruled out, but it seems reasonable that the close analogues **4** and **32** have a similar mechanism.

Compounds **4** and **32** inhibited EGF-stimulated receptor autophosphorylation in A431 human epidermoid carcinoma cells, with IC_{50}s of 126 and 14 nM, respectively. Time course experiments indicated that inhibition of receptor autophosphorylation occurs immediately after the cells are exposed to either compound (data not shown), but there is a marked difference in the recovery of this property after the compound is washed out. Figure 2 shows an experiment in which cells were exposed to either **4** or **32** (1 μM) for 2 h, washed free of

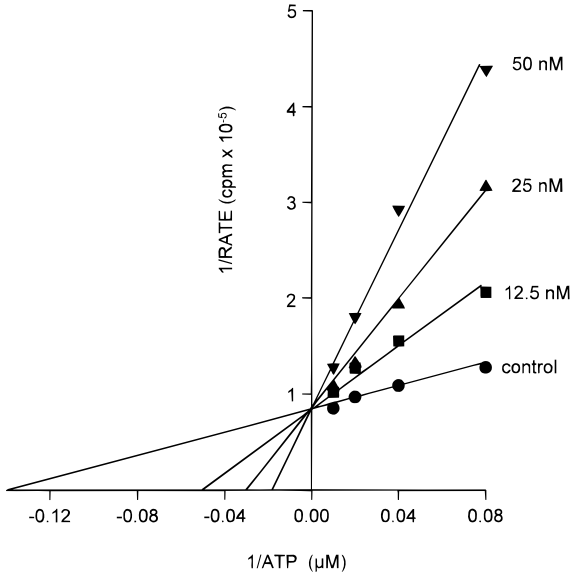


Figure 1. Double-reciprocal plot for inhibition of EGFR tyrosine kinase by compound **4**. Enzyme activity was determined as described in the Experimental Section but with the indicated concentrations of drug and ATP. Lines were determined by least-squares fitting.

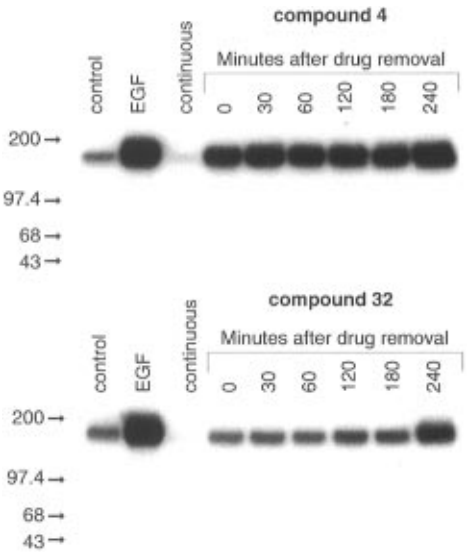


Figure 2. Time course for reversal of inhibition of EGFR autophosphorylation. Cells were treated with 1 μM **4** or **32** for 2 h and then washed free of drug. The cells were then exposed to EGF (100 ng/mL) for 5 min at varying times after the wash, and EGFR autophosphorylation was quantified by Western blotting as described in the Experimental Section.

compound, and then exposed to EGF for 5 min and the level of EGFR autophosphorylation quantitated at varying times. As would be expected for reversible inhibitors, there is a slow return of autophosphorylation activity, but this has a half-life of ca. 30 min following inhibition by **4** but becomes much longer (ca. 4 h) following inhibition by **32**. Data to be reported elsewhere (Mark Kunkel, personal communication) demonstrates that this reappearance of autophosphorylating activity is due to recovery of the original receptor population rather than synthesis of new protein. These data demonstrate that, while **32** is still a reversible inhibitor as would be expected from its structure, it has a very slow off-rate (consistent with very tight binding).

Origins of the Supra-additive Potentiation Seen with Some Substituent Patterns. Although deviations from strict additivity for substituent effects are

not uncommon (e.g., when substituents of two different parts of the molecule both bind in the same receptor pocket), the enormous potentiation shown by a subset of the compounds in this SAR is unusual. The most obvious explanation here is that particular combinations of substituents produce some dramatic steric or electronic change in the compounds in question. However, while there is probably good electronic communication between the quinazoline and aniline rings, as previously shown for 9-anilinoacridines,[22] a comparison of compounds which do and do not show the supra-additive effect makes any kind of unusual electronic "cross talk" between the two rings most unlikely. For example, electronic differences between the 6-OMe and 6-NH_2 3-bromoanilino compounds **8** and **11** are likely to be minimal. It is also difficult to see how steric interactions could play any part in this effect. Diarylamines such as these have only two rotatable bonds and will maintain a large dihedral angle between the rings. Additionally, the effect occurs with substituents which are well away from any possible interactions with the other ring, and, as discussed above, in the most likely case where a steric effect between the two rings could occur (compounds **59** and **64**), the supra-additive effect of the favorable groupings appears to still be present.

Another possible explanation is that the supra-additive combinations of substituents produce a change in binding mode of the inhibitor on the enzyme. Recent crystallographic data for the catalytic domain of the closely related insulin receptor show that the ATP-binding site is situated on the N-terminal lobe of the protein, where there is sufficient space to accommodate different inhibitor-binding modes.[23] However, while this hypothesis might explain why two activating substituents can combine together supra-additively on the enzyme, by binding to alternative acceptor sites, it is not adequate to account for the fact that two deactivating substituents can participate supra-additively with some of the already defined activating substituents (e.g., **11**, **22**, and **34**). If, for example, there are two different binding modes available to accommodate the 3′-CF_3 or 6-NH_2 group of the monosubstituted compounds **6** and **9**, the inhibitor should adopt the more favorable of these if it is capable of doing so. Normally this could only be prevented by an unfavorable steric interaction elsewhere in the molecule. However, here we see low activity (due to putative inhibition of occupancy) for the two monosubstituted compounds, before steric bulk (the other substituent in either case) is added. Therefore, we feel that this argument is also untenable and that one can only conclude that the reason that the parent 3′-CF_3 and 6-NH_2 compounds do not utilize a more favourable binding mode on the enzyme is that it is not there for those compounds.

In our view the most reasonable explanation is that the enzyme itself undergoes a conformational change when binding the inhibitors which contain supra-additive combinations of substituents. This concept of "induced fit" of ligands to protein substrates has been well-validated and is dramatically illustrated with the binding of the experimental antiviral drug disoxiril to the capsid proteins of picornavirus. Here, the X-ray crystallographic structure of the native virus shows no binding site for disoxiril, but the drug still binds, with the X-ray structure of the complexed virus showing how the protein alters conformation to accommodate the inhibitor.[24,25] In the present example, all of the data (see also below) can be explained by this concept, where the excess binding energy for the supra-additive compounds in effect comes from the protein, not the inhibitor. Under this hypothesis, the reason that the SARs are obviously discontinuous is that the inhibitors are binding to two different species and that intercomparisons are therefore inappropriate. Although no direct evidence is available as to the nature of the induced conformation changes with EGFR, the known X-ray structures of the closely homologous enzymes MAP kinase[26] and the insulin receptor kinase[23] suggest a possible scenario. In unactivated form, both of these show open conformations with the ATP-binding site completely exposed to solvent on one face of the N-terminal lobe, many angstroms from the catalytic machinery in the C-terminal lobe. In contrast, the X-ray structure of protein kinase A in active conformation, bound to both a substrate analogue (PK1) and ATP, shows that the lobes have fully closed down around the hinge and the domain surfaces have slid with respect to one another in the axis perpendicular to the major axis, trapping ATP completely inside the enzyme.[27] We suggest that the 4-anilinoquinazoline inhibitors discussed here differ in their ability to induce these kinds of motion in the EGFR protein. The very slow off-rates of the most potent inhibitors might then be a result of hydrophobic collapse of the enzyme in its most stable active conformation around an optimally shaped inhibitor, which now becomes a component of the hydrophobic core of the enzyme/inhibitor complex.

Conclusions

The above results for a large number of close analogues of the potent EGFR tyrosine kinase inhibitor **32** (PD 153035) show that the high potency of **32** is not unique, with derivatives possessing appropriate combinations of phenyl and quinazoline substitutions having similar IC_{50}s for inhibition of the isolated enzyme. Generally these highly potent analogues have IC_{50}s up to about 80-fold better than predicted from simple additivity arguments. However, other very similar compounds do not show this "supra-additive" effect when combined together on the anilinoquinazoline nucleus, and some substituents which are mildly deactivating by themselves can be strongly activating when used in the correct combinations. To explain these results, we propose that certain appropriately disubstituted analogues possess the ability to induce a change in the conformation of the tyrosine kinase domain when they bind. Using this hypothesis, some of the results can be interpreted to indicate differences between the "native" and "induced" binding sites. For example, there appears to be greater bulk tolerance at the quinazoline 6- and 7-positions at the induced binding site but less at the N^4 site. Because of the former property, **32** is not the optimal inhibitor in the induced conformation, with the diethoxy analogue **56** being superior. This compound, 4-(3-bromoanilino)-6,7-diethoxyquinazoline, has an IC_{50} of 0.006 nM, making it the most potent quinazoline inhibitor (and, to our knowledge, the most potent inhibitor of any type yet reported) of the tyrosine kinase activity of the EGFR.

Experimental Section

Analyses were performed by the Microchemical Laboratory, University of Otago, Dunedin, NZ, or Parke-Davis Pharmaceutical Research Analytical Department. Melting points were

determined using an Electrothermal Model 9200 or Gallenkamp digital melting point apparatus and are as read. NMR spectra were measured on Bruker AC-200 or AM-400 or Varian Unity 400 MHz spectrometers and referenced to Me_4Si. Mass spectra were recorded on a Varian VG 7070 spectrometer at nominal 5000 resolution or a Finnigan MAT 900Q spectrometer. Unless otherwise noted (below), the compounds of Table 1 were prepared from known[28] quinazolin-4(3*H*)-ones by conversion to the corresponding 4-chloroquinazolines with $POCl_3$, followed by reaction with substituted anilines in 2-propanol.[14]

4-[(3-Bromophenyl)amino]-7-chloro-6-nitroquinazoline (53): General Example of Coupling Procedure (Scheme 1). A solution of 7-chloro-6-nitro-4-quinazolin-4(3*H*)-one (**70**)[16] (7.6 g, 33.7 mmol) in $SOCl_2$ (100 mL) containing 2 drops of DMF was heated under reflux for 1 h. Excess $SOCl_2$ was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2 and washed with aqueous Na_2CO_3 solution. Removal of the solvent and recrystallization from light petroleum gave 4,7-dichloro-6-nitroquinazoline (**71**) (8.2 g, 99% yield): mp 197−210 °C (semimelt at 139−141.5 °C); ^{1}H NMR [$(CD_3)_2SO$] δ 8.60 (s, 1 H, ArH), 8.27 (s, 1 H, ArH), 7.85 (s, 1 H, ArH); ^{13}C NMR δ 159.0, 150.3, 144.8, 131.1, 121.4 (C), 149.2, 129.4, 124.2 (CH). Anal. ($C_8H_3Cl_2N_3O_3$) C, H, N, Cl.

A mixture of **71** (7.9 g, 32 mmol) and 3-bromoaniline (7.9 mL, 70 mmol) in iPrOH (250 mL) was heated to reflux and treated with 3 drops of concentrated HCl. Heating was continued for 30 min, sufficient Et_3N was added to basify the solution, and the solvent was then concentrated to give 4-[(3-bromophenyl)amino]-7-chloro-6-nitroquinazoline (**53**) (11.1 g, 91% yield): mp (EtOH) 257−259 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 10.36 (s, 1 H, NH), 9.43 (s, 1 H, H-5), 8.77 (s, 1 H, H-2), 8.16 (br s, 1 H, H-2′), 8.11 (s, 1 H, H-8), 7.86 (br d, J = 7.1 Hz, 1 H, H-6′), 7.42−7.36 (m, 2 H, H-4′,5′). Anal. ($C_{14}H_8BrClN_4O_2$) C, H, N.

4-[(3-Bromophenyl)amino]-7-(dimethylamino)-6-nitroquinazoline (50): General Example of Chloro Displacement. A solution of 4-[(3-bromophenyl)amino]-7-chloro-6-nitroquinazoline (**53**) (1.0 g, 26 mmol) in EtOH (100 mL) was heated at 100 °C with 40% aqueous dimethylamine (20 mL) in a closed pressure vessel for 1 h to give, on cooling, 4-[(3-bromophenyl)amino]-7-(dimethylamino)-6-nitroquinazoline (**50**) (0.75 g, 74% yield): mp (EtOH) 240.5−243.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.01 (s, 1 H, NH), 9.12 (s, 1 H, H-5), 8.58 (s, 1 H, H-2), 8.19 (s, 1 H, H-2′), 7.89 (d, J = 8.0 Hz, 1 H, H-6′), 7.36 (m, 2 H, H-4′,5′), 7.18 (s, 1 H, H-8), 2.93 (s, 6 H, NMe_2). Anal. ($C_{16}H_{14}BrN_5O_2$) C, H, N.

Similar reaction of **53** with methylamine gave compound **49**.

4-[(3-Bromophenyl)amino]-7-methoxy-6-nitroquinazoline (52). A solution of **70** (1.0 g, 4.4 mmol) in MeOH (150 mL) containing dissolved sodium metal (1.01 g, 44 mmol) was heated at 100 °C in a sealed pressure vessel for 18 h. The solution was neutralized with AcOH and diluted with water to give 7-methoxy-6-nitroquinazolin-4(3*H*)-one (**72**) (0.95 g, 97%): mp (EtOH) 287 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 12.51 (s, 1 H, NH), 8.53 (s, 1 H), 8.24 (s, 1 H), 7.43 (s, 1 H), 4.05 (s, 3 H, OCH_3). Anal. ($C_9H_7N_3O_4$) C, H, N.

A suspension of **72** (0.85 g, 3.8 mmol) in $POCl_3$ (50 mL) was heated under reflux for 30 min, when a clear solution was obtained. The $POCl_3$ was removed under reduced presssure, and the residue was dissolved in a mixture of CH_2Cl_2 and aqueous Na_2CO_3. The organic layer was dried and the solvent removed to give 4-chloro-7-methoxy-6-nitroquinazoline (**73**) (0.86 g, 93%): mp (hexane) 172−173 °C; ^{1}H NMR ($CDCl_3$) δ 9.06 (s, 1 H, H-5), 8.65 (s, 1 H, H-2), 7.56 (s, 1 H, H-8), 4.13 (s, 3 H, OMe). Anal. ($C_9H_6ClN_3O_3$) C, H, N, Cl.

Reaction of **73** with with 3-bromoaniline in iPrOH as above gave 4-[(3-bromophenyl)amino]-7-methoxy-6-nitroquinazoline (**52**) (86% overall yield): mp (EtOAc/hexane) 206−207.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.13 (s, 1 H, NH), 9.25 (s, 1 H, H-5), 8.70 (s, 1 H, H-2), 8.18 (br s, 1 H, H-2′), 7.88 (d, J = 7.7 Hz, 1 H, H-6′), 7.49 (s, 1 H, H-8), 7.40−7.33 (m, 2 H, H-4′,5′), 4.07 (s, 3 H, OCH_3). Anal. ($C_{15}H_{11}BrN_4O_3$) C, H, N.

4-[(3-Bromophenyl)amino]-7-(methylamino)quinazoline (40): Example of Fluoro Displacement from Unactivated Quinazoline (Scheme 2). 7-Fluoroquinazolin-4(3*H*)-one[29] (**74**) was reacted sequentially with $POCl_3$ and 3-bromoaniline as above to give 4-[(3-bromophenyl)amino]-7-fluoroquinazoline (**75**): mp (MeOH) 201−203 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.96 (br s, 1 H, NH), 8.68−8.64 (m, 1 H, H-5), 8.66 (s, 1 H, H-2), 8.21 (br s, 1 H, H-2′), 7.89 (d, J = 7.9 Hz, H-6′), 7.63−7.55 (m, 2 H, H-6,8), 7.37 (t, J = 7.9 Hz, 1 H, H-5′), 7.32 (br d, J = 7.9 Hz, 1 H, H-4′). Anal. ($C_{14}H_9BrFN_3$) C, H, N.

A mixture of **75** (1.0 g, 3.1 mmol), methylamine hydrochloride (20 g, 0.3 mol), and Et_3N (30 mL, 0.3 mol) in *N*-methylpyrrolidone (50 mL) was heated at 130 °C in a sealed pressure vessel for 4 days, and the resulting mixture was partitioned between EtOAc and water. The organic layer was dried, the solvent was removed, and the residue was chromatographed on alumina gel, eluting with CH_2Cl_2, to give 4-[(3-bromophenyl)amino]-7-(methylamino)quinazoline (**40**) (0.54 g, 52%): mp (CH_2Cl_2) 198−199 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.45 (s, 1 H, NH), 8.43 (s, 1 H, H-2), 8.22 (br s, 1 H, H-2′), 8.19 (d, J = 9.1 Hz, 1 H, H-5), 7.86 (br d, J = 8.2 Hz, 1 H, H-6′), 7.31 (t, J = 8.1 Hz, 1 H, H-5′), 7.23 (br d, J = 7.9 Hz, 1 H, H-4′), 6.96 (dd, J = 9.1, 2.3 Hz, 1 H, H-6), 6.67 (t, J = 4.9 Hz, 1 H, NHMe), 6.57 (d, J = 2.3 Hz, 1 H, H-8), 2.81 (d, J = 4.9 Hz, 3 H, NCH_3). Anal. ($C_{15}H_{13}BrN_4$) C, H, N.

Similarly prepared were **41** and **42**.

7-Acetamido-4-[(3-bromophenyl)amino]quinazoline (39). Treatment of **30** with Ac_2O/pyridine gave 7-acetamido-4-[(3-bromophenyl)amino]quinazoline (**39**): mp (EtOH) 351−353 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.42 (s, 1 H, NH), 9.76 (s, 1 H, NH), 8.59 (s, 1 H, H-2), 8.46 (d, J = 9.0 Hz, 1 H, H-5), 8.24 (s, 1 H, H-8), 8.15 (br s, 1 H, H-2′), 7.90 (d, J = 7.8 Hz, 1 H, H-6′), 7.74 (d, J = 8.5 Hz, 1 H, H-6), 7.35 (t, J = 8.0 Hz, 1 H, H-5′), 7.29 (d, J = 7.87 Hz, 1 H, H-4′), 2.14 (s, 3 H, CH_3). Anal. ($C_{16}H_{13}BrN_4O$) C, H, N.

4-[(3-Bromophenyl)amino]-6,7-dimethoxyquinazoline Hydrochloride (32) (Scheme 3). 2-Amino-4,5-dimethoxybenzoic acid (**76**) (29.58 g, 0.15 mol) and formamidine hydrochloride (20.20 g, 0.251 mol) were intimately ground together and then spread in an even layer around the bottom of a 1 L round bottom flask. This was blanketed with N_2 under an air condenser and rapidly heated to 210 °C on an oil bath. During heating a circular melting zone followed by resolidification passed from the outside to the center of the flask. After 15 min at 210 °C, the reaction mixture was allowed to cool to 80 °C and then sonicated with dilute NaOH solution (0.33 M, 150 mL). The resulting purple-gray solid was collected, rinsed with water (3 × 50 mL), and dried under vacuum at 70 °C to give 6,7-dimethoxyquinazolin-4(3*H*)-one (**78**) (24.50 g, 79%), mp 295−298 °C, which was used directly: ^{1}H NMR [$(CD_3)_2SO$] δ 12.05 (br s, 1 H, NH), 7.99 (s, 1 H, H-2), 7.44 (s, 1 H, H-5), 7.12 (s, 1 H, H8), 3.90, 3.87 (2s, 2 × 3 H, 2 × OCH_3).

DMF (13.16 g, 0.18 mol) was added dropwise over 20 min to a stirred solution of oxalyl chloride (22.85 g, 0.18 mol) in 1,2-dichloroethane (120 mL) under N_2 at 25 °C, resulting in an exotherm and gas evolution. When gas evolution ceased, **78** (24.76 g, 0.12 mol) was added with mechanical agitation, the mixture was heated to reflux for 4 h and cooled to 25 °C, then the reaction was quenched with dilute aqueous Na_2HPO_4 solution (0.5 M, 250 mL). The resulting mixture was stirred on an ice bath for 2 h, and the solid was collected, rinsed with water (2 × 50 mL), and dried at 50 °C under vacuum to give 4-chloro-6,7-dimethoxyquinazoline (**80**) (22.12 g, 82%) which was used directly: ^{1}H NMR ($CDCl_3$) δ 8.86 (s, 1 H, H-2), 7.38 (s, 1 H, H-5), 7.33 (s, 1 H, H-8), 4.07 (s, 6 H, OCH_3).

Reaction of **80** (22.12 g, 98 mmol) and 3-bromoaniline (16.34 g, 95 mmol) in stirred 2-propanol (1 L), under N_2 for 18 h gave 4-[(3-bromophenyl)amino]-6,7-dimethoxyquinazoline hydrochloride (**32**) (35.95 g, 92%): mp 264−266 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.61 (br s, 1 H, NH), 8.88 (s, 1 H, H-2), 8.43 (s, 1 H, H-5), 8.04 (s, 1 H, H-2′), 7.80 (br d, J = 7.4 Hz, 1 H, H-4′), 7.45 (br d, J = 8 Hz, 1 H, H-6′), 7.44 (br t, J = 7.5 Hz, 1 H, H-5′), 7.38 (s, 1 H, H-8), 4.03, 4.00 (2s, 2 × 3 H, 2 × OCH_3). Anal. ($C_{16}H_{14}BrN_3O_2$·HCl) C, H, N.

4-[(3-Bromophenyl)amino]-5,6,7-trimethoxyquinazoline (64) (Scheme 3). 2,3,4-Trimethoxy-6-nitrobenzoic acid[30] (7.72 g, 30 mmol) in MeOH (150 mL) was hydrogenated over Pd/C (600 mg). After 17 h, TLC showed 70% conversion, a further 200 mg of catalyst was added, and the reaction was

continued for a further 3.5 h. The suspension was filtered over Celite, and the filtrate was concentrated to near dryness. The resulting crude air-sensitive 6-amino-2,3,4-trimethoxybenzoic acid (**77**) was used immediately, by dissolving in MeOH (125 mL) and treating with *s*-triazine (2.505 g, 30 mmol) and piperidine (2.1 mL) according to the procedure of Kreutzberger and Uzbek.[31] The solution was heated under N_2 at 65 °C for 2.5 h and then concentrated to a solid residue that was purified by flash silica gel column chromatography. Elution with CH_2Cl_2 and then CH_2Cl_2/MeOH (9:1), followed by pooling and evaporation of appropriate fractions, resulted in a solid which was triturated in hot EtOAc to give 5,6,7-trimethoxyquinazolin-4(3*H*)-one (**79**) (5.00 g, 72%): mp 187−189° C; ^{1}H NMR ($CDCl_3$) δ 8.15 (br s, exchanges with D_2O, 1 H, NH), 7.27 (s, 1 H), 7.06 (s, 1 H), 4.02 (s, 3 H), 4.00 (s, 3 H), 3.95 (s, 3 H); CIMS (1% NH_3 in CH_4) *m*/*z* 221 (18), 237 (100, MH^+). Anal. ($C_{11}H_{12}N_2O_4$) C, H, N.

A suspension of **79** (2.36 g, 10 mmol) and $POCl_3$ (0.98 mL, 10.5 mmol) in 1,2-dichloroethane (20 mL) was treated dropwise with diisopropylethylamine (4.4 mL, 25 mmol). The resulting solution was heated at 80 °C under N_2 for 1.5 h and then cooled and concentrated to a solid that was diluted with CH_2Cl_2. The solution was washed with 5% aqueous $NaHCO_3$, dried ($MgSO_4$), and then filtered through a short column of silica gel, washing with CH_2Cl_2 and then CH_2Cl_2/EtOAc (3:1), to give 4-chloro-5,6,7-trimethoxyquinazoline (**81**) (1.29 g, 51%): mp (*tert*-butyl methyl ether) 115−116 °C; ^{1}H NMR ($CDCl_3$) δ 8.84 (s, 1 H), 7.23 (s, 1 H), 4.05 (s, 3 H), 4.03 (s, 3 H), 4.00 (s, 3 H); CIMS (1% NH_3 in CH_4) *m*/*z* 250 (31), 251 (100), 279 (12). Anal. ($C_{11}H_{11}ClN_2O_3$) C, H, Cl, N.

Reaction of **81** with 3-bromoaniline in 2-propanol under reflux for 1 h as above gave 4-[(3-bromophenyl)amino]-5,6,7-trimethoxyquinazoline (**64**) (82%): mp 130−132 °C; ^{1}H NMR ($CDCl_3$) δ 9.92 (br s, exchanges with D_2O, 1 H), 8.61 (s, 1 H), 8.11 (s, 1 H), 7.71−7.67 (m, 1 H, H-5′), 7.26−7.24 (m, 2 H, H-4′,6′), 7.08 (s, 1 H, H-8), 4.17 (s, 3 H), 4.01 (s, 3 H), 3.96 (s, 3 H); CIMS (1% NH_3 in CH_4) *m*/*z* 310 (65), 312 (21), 390 (100, MH^+), 392 (83), 418 (19), 420 (18). Anal. ($C_{17}H_{16}BrN_3O_3$) C, H, N.

4-[(3-Bromophenyl)amino]-6,7-dihydroxyquinazoline Hydrochloride (55) (Scheme 3). A mixture of **32** (198 mg, 0.5 mmol) and pyridinium hydrochloride (1.15 g, 10 mmol) was heated together under N_2 with stirring at 205 °C for 1 h. Upon cooling, the fused melt was sonicated with water (20 mL), and the residual solid was collected to give 4-[(3-bromophenyl)amino]-6,7-dihydroxyquinazoline hydrochloride monohydrate[14] (**55**) (25 mg, 13%): mp (EtOH at 0 °C) >320 °C.

4-[(3-Bromophenyl)amino]-6-(methylamino)quinazoline (35). A solution of 6-amino-4-[(3-bromophenyl)amino]-quinazoline[14] (**11**) (2.25 g, 7.14 mmol) in pyridine (20 mL) at 0 °C was treated dropwise with MeOCOCl (1.0 mL, 13 mmol), and the mixture was allowed to warm to room temperature over 2 h. Addition of water gave a precipitate of 4-[(3-bromophenyl)amino]-6-[(methoxycarbonyl)amino]quinazoline (**37**) (2.34 g, 98%): mp (EtOH) 197−198.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.00 (s, 1 H, NH), 9.88 (s, 1 H, NH), 8.56 (s, 1 H, H-2), 8.54 (br s, 1 H, H-5), 8.15 (br s, 1 H, H-2′), 7.85 (br d, J = 7.9 Hz, 1 H, H-6′), 7.77 (br s, 2 H, H-7,8), 7.34 (t, J = 8.0 Hz, 1 H, H-5,5′), 7.28 (br d, J = 8.2 Hz, 1 H, H-4′), 3.74 (s, 3 H, OCH_3). Anal. ($C_{16}H_{13}BrN_4O_2$) C, H, N.

A solution of **37** (1.0 g, 3 mmol) in dry THF (30 mL) was reacted with an excess of $LiAlH_4$ at reflux for 1 h. The mixture was neutralized with H_2O, the solvent was removed, and the residue was extracted into EtOAc. Chromatography on Al_2O_3, eluting with CH_2Cl_2, gave 4-[(3-bromophenyl)amino]-6-(methylamino)quinazoline (**35**) (0.24 g, 24%): mp (MeOH/H_2O) 141−144 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.41 (s, 1 H, NH), 8.39 (s, 1 H, H-2), 8.19 (br s, 1 H, H-2′), 7.93 (br d, J = 6.9 Hz, 1 H, H-6′), 7.55 (d, J = 9.0 Hz, 1 H, H-8), 7.35 (t, J = 8.0 Hz, 1 H, H-5′), 7.28−7.25 (m, 2 H, H-7,4′), 7.14 (d, J = 2.3 Hz, 1 H, H-5), 6.32 (q, J = 5.0 Hz, 1 H, NHMe), 2.86 (d, J = 5.0 Hz, 3 H, CH_3). Anal. ($C_{15}H_{13}BrN_4$) C, H, N.

4-[(3-Bromophenyl)amino]-6,7-diethoxyquinazoline (56). EtI (80 mL, 1 mmol) was added to a stirred suspension of crude **55** (81 mg, 0.25 mmol) and powdered K_2CO_3 (138 mg, 1.0 mmol) in DMSO (1 mL) under N_2 at 25 °C. After 4 h, the reaction mixture was put on a vacuum line overnight to remove all volatiles, and the residue was slurried in EtOH. Preparative TLC on silica gel, eluting twice with 4% $CHCl_3$/MeOH (19:1), gave a major band (R_f 0.53) that was extracted with $CHCl_3$/MeOH, and volatiles were removed under reduced pressure to give 4-[(3-bromophenyl)amino]-6,7-diethoxyquinazoline monohydrate (**56**) (43 mg, 42%) as a glass: mp 155−167 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.75 (br s, 1 H, NH), 8.56 (s, 1 H, H-2), 8.12 (m, 1 H, H-2′), 7.87 (s, 1 H, H-5), 7.86 (d, J = 8.0 Hz, H-6′), 7.37 (t, J = 8.0 Hz, H-5′), 7.31 (d, J = 8.4 Hz, 1 H, H-4′), 7.19 (s, 1 H, H-8), 4.24, 4.22 (2q, J = 6.7 Hz, 2 H, OCH_2), 1.45, 1.42 (2t, J = 6.7 Hz, 3 H, CH_3); CIMS *m*/*z* 388 (100, $^{79}BrMH^+$). Anal. ($C_{18}H_{18}N_3BrO_2 \cdot H_2O$) C, H, N.

4-[(3-Bromophenyl)amino]-6,7-dimethoxy-2-methylquinazoline Hydrochloride (61) (Scheme 4). HCl gas was bubbled through a solution of methyl 2-amino-4,5-dimethoxybenzoate (**82**) (0.210 g, 0.99 mmol) in CH_3CN (10 mL) for 1 h, and the resulting mixture was heated under reflux for 8 h and then cooled to room temperature. The resulting solid was collected and dissolved in water (10 mL), and the solution was neutralized with saturated aqueous $NaHCO_3$ solution to pH 7. Filtration and air-drying of the resulting solid gave 6,7-dimethoxy-2-methylquinazolin-4(3*H*)-one (**83**) (152 mg, 70%), which was used directly: ^{1}H NMR [$(CD_3)_2SO$] δ 7.39 (s, 1 H, H-5), 7.05 (s, 1 H, H-8), 3.87 (s, 3 H, OCH_3), 3.84 (s, 3 H, OCH_3), 2.30 (s, 3 H, CH_3).

Reaction of **83** (152 mg, 0.70 mmol) and $POCl_3$ (3 mL), under reflux for 8 h, followed by removal of the excess reagent $POCl_3$ under reduced pressure and partition of the residue between $CHCl_3$ and water gave 4-chloro-6,7-dimethoxy-2-methylquinazoline (**85**) (93 mg, 56%) as a white solid which was used directly: ^{1}H NMR [$(CD_3)_2SO$] δ 7.38 (s, 1 H, H-5), 7.36 (s, 1 H, H-8), 3.99 (s, 3 H, OCH_3), 3.97 (s, 3 H, OCH_3), 2.67 (s, 3 H, CH_3).

Reaction of this with 3-bromoaniline in 2-methoxyethanol at 90 °C for 30 min gave 4-[(3-bromophenyl)amino]-6,7-dimethoxy-2-methylquinazoline hydrochloride (**61**) (129 mg, 80%): mp 264−265 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.08 (br s, 1 H, H-5), 8.00 (br s, 1 H, H-8), 7.81 (d, J = 6.6 Hz, 1 H, H-6′), 7.48−7.44 (m, 2 H, H-4′,5′), 7.18 (s, 1 H, H-2′), 4.0 (s, 3 H, OCH_3), 4.0 (s, 3 H, OCH_3), 2.61 (s, 3 H, CH_3); CIMS *m*/*z* 373 (100, $^{79}BrMH^+$). Anal. ($C_{17}H_{16}BrN_3O_2 \cdot HCl$) C, H, N.

2-Amino-4-[(3-bromophenyl)amino]-6,7-dimethoxyquinazoline Trihydrochloride (62) (Scheme 4). Concentrated HCl (0.05 mL) was added dropwise to a solution of methyl 2-amino-4,5-dimethoxybenzoate (**82**) (0.217 g, 1.0 mmol) and cyanamide (67 mg, 1.6 mmol) in dioxane (10 mL) at 25 °C. The mixture was heated at 80 °C for 7.5 h and then cooled to 25 °C, and the resulting solid was collected and treated as above to give 2-amino-6,7-dimethoxyquinazolin-4(3*H*)-one (**84**) (174 mg, 79%), which was used directly: ^{1}H NMR [$(CD_3)_2SO$] δ 7.23 (s, 1 H, H-5), 6.67 (s, 1 H, H-8), 3.82 (s, 3 H, OCH_3), 3.77 (s, 3 H, OCH_3), 6.14 (br, 2 H, NH_2).

Reaction of **84** with $POCl_3$ as above gave 2-amino-4-chloro-6,7-dimethoxyquinazoline (**86**) (38%) as a yellow solid which was used directly: ^{1}H NMR [$(CD_3)_2SO$] δ 7.14 (s, 1 H, H-5), 6.89 (s, 1 H, H-8), 3.90 (s, 3 H, OCH_3), 3.86 (s, 3 H, OCH_3).

Reaction of this with 3-bromoaniline as above gave 2-amino-4-[(3-bromophenyl)amino]-6,7-dimethoxyquinazoline trihydrochloride (**62**) (7%): mp 262−263 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.44 (br s, 1 H, NH), 8.03 (d, J = 2.9 Hz, 2 H, H-2′,6′), 7.70 (s, 1 H, H-5), 7.35−7.27 (m, 2 H, H-4′,5′), 6.82 (s, 1 H, H-8), 6.56 (br s, 2 H, NH_2), 3.88 (s, 3 H, OCH_3), 3.86 (s, 3 H, OCH_3); CIMS *m*/*z* 374 (100, $^{79}BrMH^+$). Anal. ($C_{16}H_{15}BrN_4O_2 \cdot 3HCl \cdot 0.25H_2O$) C, H, N.

Enzyme Assay. EGFR 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.[13] The substrate used was based on a portion of phospholipase Cγ1, 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 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 incorporated label was assessed by scintillation counting in an aqueous fluor. Control activity (no drug) gave a count of ca. 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%.

EGFR Autophosphorylation in Human A431 Epidermoid Carcinoma Cells. Cells were grown to confluency in 6-well plates (35 mm diameter), exposed to serum-free medium for 18 h, and then treated with **4** or **32** for 2 h, followed by EGF (100 ng/mL) for 5 min. The monolayers were lysed in 0.2 mL of boiling Laemlli buffer (2% sodium dodecyl sulfate, 5% 2-mercaptoethanol, 10% glycerol, and 50 mM Tris, pH 6.8), and the lysates were heated to 100 °C for 5 min. Proteins in the lysate were separated by polyacrylamide gel electrophoresis and electrophoretically transferred to nitrocellulose. The membrane was washed once in 10 mM Tris, pH 7.2, 150 mM NaCl, and 0.01% azide (TNA) and blocked overnight in TNA containing 3% bovine serum albumin and 1% ovalbumin. The membrane was blotted for 2 h with antiphosphotyrosine antibody (UBI, 1 μg/mL in blocking buffer) and then washed twice in TNA, once in TNA containing 0.05% Tween-20 and 0.05% nonidet P-40, and twice in TNA. The membranes were then incubated for 2 h in blocking buffer containing 0.1 μCi/mmol [^{125}I]protein and then washed again as above. After the blots were dry they were loaded into a film cassette and exposed to X-AR X-ray film for 1−7 days. Band intensities were determined with a Molecular Graphics laser densitometer.

Acknowledgment. This work was supported in part by the Auckland Division of the Cancer Society of New Zealand.

Supporting Information Available: Melting points, crystallization solvents, and ^{1}H NMR data for the new compounds of Table 1 (8 pages). Ordering information is given on any current masthead page.

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JM9503613