Discovery of a New Class of Anilinoquinazoline Inhibitors with High Affinity and Specificity for the Tyrosine Kinase Domain of c-Src

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Deregulated activity of the nonreceptor tyrosine kinase c-Src is believed to result in signal transduction, cytoskeletal and adhesion changes, ultimately promoting a tumor-invasive phenotype. We report here the discovery of a new class of anilinoquinazoline inhibitors with high affinity and specificity for the tyrosine kinase domain of the c-Src enzyme. Special attention was directed toward finding inhibitors selective against KDR tyrosine kinase in order to ensure that the in vivo profile of a specific Src inhibitor could be determined. The 4-aminobenzodioxole quinazoline series gave compounds with excellent potency and selectivity. The most interesting compounds were evaluated in vivo and displayed good pharmacokinetics following oral dosing. Compounds such as the aminobenzodioxoles were shown to be potent inhibitors of tumor growth in a c-Src-transformed 3T3 xenograft model in vivo, resulting in more than 90% growth inhibition at doses as low as 6 mg/kg po once daily. Src tyrosine kinase inhibitors such as these may provide a novel therapeutic modality for targeting cancer invasion and metastasis.

Introduction

The identification, more than 25 years ago, of v-Src tyrosine kinase as the transforming element of the oncogenic Rous Sarcoma retrovirus, and the subsequent findings demonstrating the oncogenic potential of the normal cellular homologue c-Src,[1] played a major role in the understanding of signal transduction pathways and oncogenic transformation. c-Src kinase, a nonreceptor tyrosine kinase, is the best understood member of a family of closely related kinases. Three members of this family, c-Src, c-Yes, and Fyn are ubiquitously expressed while other family members (six known) have a more restricted expression. c-Src is expressed at low levels in most cell types and, in the absence of appropriate extracellular stimuli, maintained in an inactive conformation through phosphorylation of a regulatory tyrosine domain at Tyr530. Activation of c-Src occurs through dephosphorylation of the Tyr530 site and phosphorylation of a second tyrosine, Tyr416, which is present within the kinase domain of the enzyme. Platelets, osteoclasts, and neural cells are the only normal cell types known to contain high levels of Src kinase. c-Src gene knock-out experiments in mice have shown that the only phenotypic consequence is osteopetrosis, a defect in osteoclast function.[2] In contrast to its highly regulated role in normal cells, there is significant evidence demonstrating deregulated, increased kinase activity of c-Src in several human tumor types, most notably colon and breast tumors.[3] The consequence of this deregulated activity in the genesis of cancer is still to be tested in clinical trials. Recent data suggest that deregulated c-Src tyrosine kinase activity is associated predominantly with adhesion and cytoskeletal changes in tumor cells, ultimately resulting in a change to a motile, invasive phenotype. c-Src tyrosine kinase activity has been shown to be an important component in the epithelial to mesenchymal transition that occurs in the early stages of invasion of carcinoma cells.[4] Increased c-Src tyrosine kinase activity results in breakdown of E-cadherin-mediated epithelial cell:cell adhesion,[5,6] which can be restored by Src inhibition.[6] c-Src activity is also known to be essential in the turnover of focal adhesions,[7] a critical cell motility component. In in vivo models of metastasis, c-Src inhibition markedly reduced the rate of lymph and liver metastasis.[6,8] Evidence from the clinic also supports a link between deregulated c-Src activity and increased invasive potential of tumor cells. In colon tumors, increased c-Src kinase activity has been shown to correlate with tumor progression, with the highest activity found in metastatic tissue.[3c] Increased Src activity in colon tumors has also been shown to be an indicator of poor prognosis.[9] In breast[3d,f] and ovarian[10] cancers, enhancement of Src kinase activity has been reported, and in transitional cell carcinoma of the bladder, c-Src activity peaked as superficial tumors became muscle invasive.[11]

Src kinase has been, and still is, one of the most studied cellular protein tyrosine kinases, yet no inhibitor has reached the market either for osteoporosis by inhibiting osteoclast function[12] or for cancer by targeting tumor growth,[13] cell adhesion, and mobility. In this paper, we describe the synthesis and structure−activity relationships of several new series of anilinoquinazolines as potent inhibitors of tyrosine phosphorylation by the c-Src kinase, with potential as therapeutic agents in invasive cancers.

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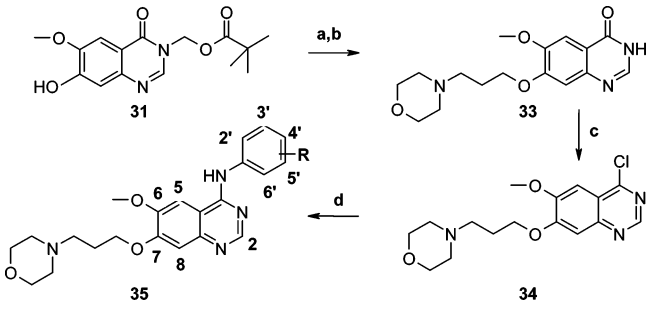
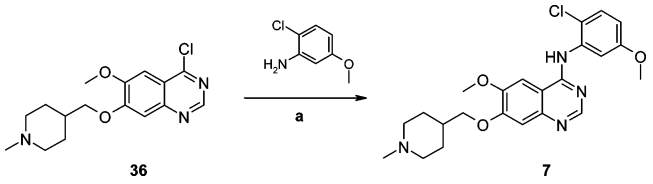
Scheme 1[a]



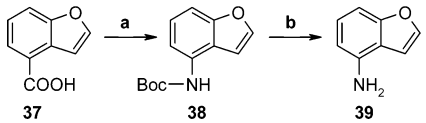
[a] Reagents: (a) PPh_3/(1-(3-hydroxypropyl)morpholine **30**/DEAD rt; (b) NH_3/methanol rt; (c) $SOCl_2$/DMF 80 °C; (d) Ar−NH_2/iPrOH/HCl gas 80 °C or Ar−NH_2/NaHMDS rt.

Scheme 2[a]



[a] Reagents: (a) 2-chloro-5-methoxyaniline/iPrOH/HCl gas 80 °C.

Scheme 3[a]



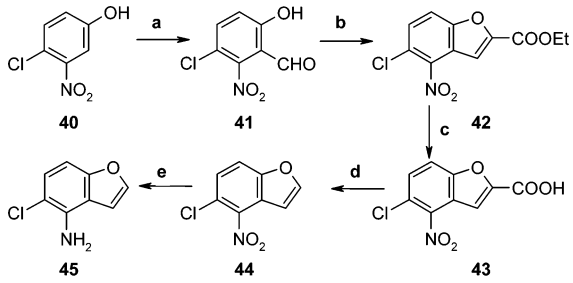
[a] Reagents: (a) DPPA/Et_3N/tBuOH reflux; (b) TFA 0 °C.

Chemistry

Most of the anilinoquinazolines (**1**−**6**, **8**−**24**) described here were prepared as shown in Scheme 1. The basic side chain in the C-7 position of the quinazoline was generally added on the quinazoline **31**[14] by Mitsunobu reaction followed by removal of the POM protecting group with ammonia. Chlorination of the quinazoline **33** gave the versatile 4-chloroquinazoline **34**, which was extensively used for optimization of the aniline. Introduction of the desired aniline was accomplished using a secondary alcohol as solvent and HCl gas as catalyst. The chloroquinazoline **36**,[15] having an *N*-methylpiperidine side chain, was reacted similarly with 2-chloro-5-methoxyaniline to give **7** (Scheme 2).

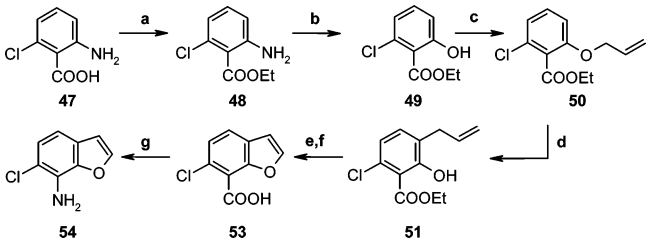
Benzofuran Syntheses. The 4-aminobenzofuran **39** was easily prepared from the known benzofuran-4-carboxylic acid **37**[16] by Curtius rearrangement followed by cleavage of the Boc group (Scheme 3). 4-Amino-5-chlorobenzofuran **45** was prepared by formylation of 4-chloro-3-nitrophenol **40** followed by cyclization with diethylbromomalonate, saponification of the remaining ester, decarboxylation, and reduction of the nitro group (Scheme 4). The 6-chloro-7-amino-benzofuran **54** was made by esterification of the 2-amino-6-chlorobenzoic acid **47** followed by diazotisation to give the phenol **49**, which was alkylated with allyl bromide before being submitted to a Claisen rearrangement to give **51**. Ozonolysis of the double bond gave the aldehyde, which was cyclized into the benzofuran **53**. Curtius rearrangement of **53** followed by deprotection of the Boc group gave the desired aniline **54** (Scheme 5). 3-Chloro-7-aminobenzofuran **58** was synthesized from the 7-nitrobenzofuran **55**[17] by addition of chlorine followed by elimination of HCl and reduction of the nitro group (Scheme 6).

Scheme 4[a]



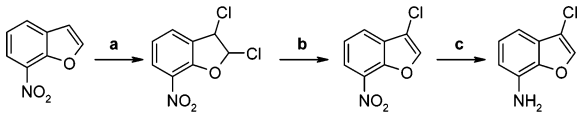
[a] Reagents: (a) hexamethylenetetramine/TFA/115 °C microwaves; (b) diethyl bromomalonate/K_2CO_3/Bu_4NBr reflux; (c) KOH/EtOH reflux; (d) CuO/quinoline 210 °C; (e) Raney Ni/hydrazine hydrate 55 °C.

Scheme 5[a]



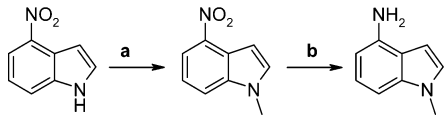
[a] Reagents: (a) NaH/EtI; (b) concd H_2SO_4/$NaNO_2$/water 0 °C; (c) allyl bromide/base rt; (d) 230 °C; (e) ozone/0 °C then H_3PO_4 100 °C; (f) KOH/water/reflux; (g) DPPA/Et_3N/tBuOH reflux then TFA 0 °C.

Scheme 6[a]



[a] Reagents: (a) AcOH/Cl_2 18 °C; (b) KOH/EtOH rt; (c) Raney Ni/hydrazine hydrate 60 °C.

Scheme 7[a]



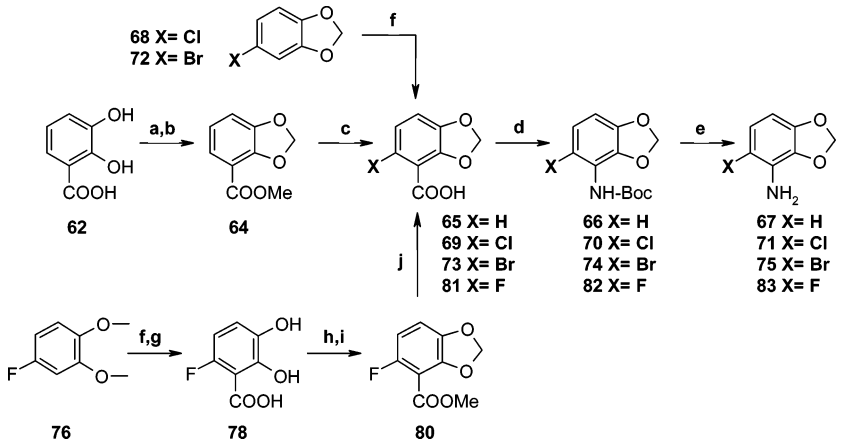
[a] Reagents: (a) NaH/MeI rt; (b) 10% Pd/C/H_2.

Indole Syntheses. Nitroindole **59** was simply *N*-methylated and hydrogenated to give 4-amino-1-methylindole **61** (Scheme 7). The other indoles used were commercially available or have been previously described in the literature (see Experimental Section).

Benzodioxole Syntheses. 2,3-Dihydroxybenzoic acid **62** was esterified with methanol and the catechol cyclized into a benzodioxole ring with dibromomethane. Saponification followed by Curtius rearrangement and deprotection of the Boc group gave the 2,3-methylenedioxyaniline **67** (Scheme 8). Similar routes were used for the 5-halogeno-3-benzodioxol-4-amines **71**, **75**, and **83**.

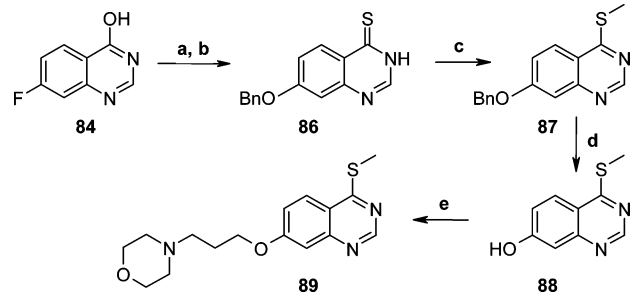
Quinazoline Syntheses. 7-Fluoro-3,4-dihydroquinazolin-4-one **84**[18] was reacted with sodium benzylate and then with phosphorus pentasulfide to give the thione **86**. Alkylation with methyl iodide followed by removal of the benzyl group and Mitsunobu coupling

Scheme 8[a]



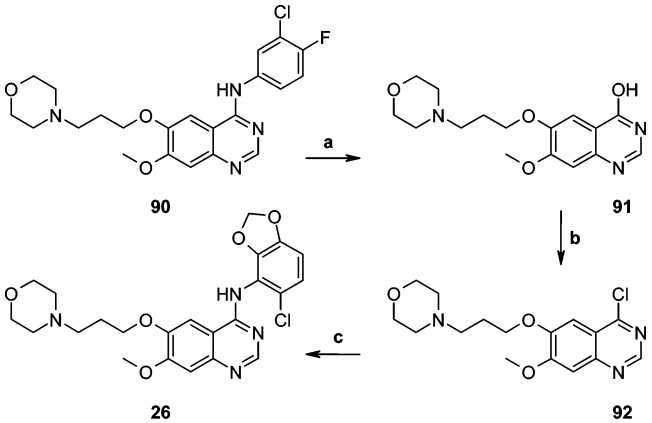
[a] Reagents: (a) H_2SO_4/MeOH 60 °C; (b) KF/CH_2Br_2; (c) NaOH rt; (d) DPPA/Et_3N/tBuOH reflux; (e) HCl or TFA 0 °C; (f) LDA/CO_2 −78 °C; (g) AcOH/HBr 130 °C; (h) $SOCl_2$/MeOH; (i) $CsCO_3$/CH_2BrCl 110 °C; (j) KOH aq rt.

Scheme 9[a]



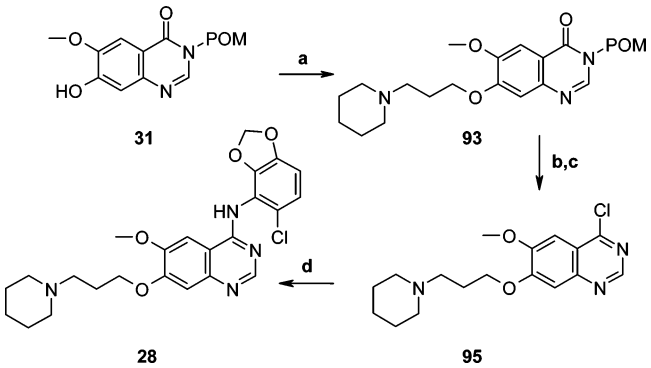
[a] Reagents: (a) Na/BnOH 80 °C; (b) P_2S_5/pyridine reflux; (c) NaOH/MeI rt; (d) TFA reflux; (e) DEAD/PPh_3/4-(3-hydroxypropyl)morpholine rt.

Scheme 10[a]



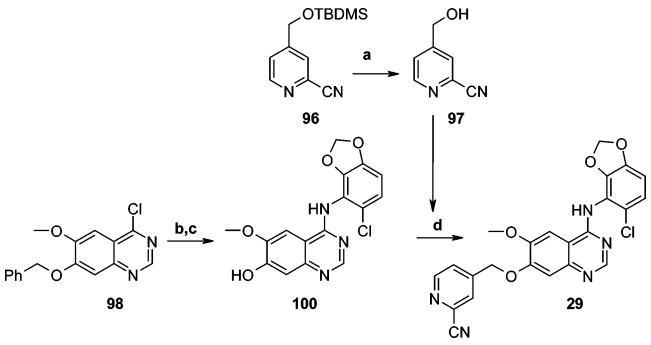
[a] Reagents: (a) HCl reflux; (b) $SOCl_2$ 80 °C; (c) aniline **71**/^{i}PrOH/HCl gas 100 °C.

Scheme 11[a]



[a] Reagents: (a) 1-(3-chloropropyl)piperidine/K_2CO_3 90 °C; (b) NH_3/MeOH rt; (c) $SOCl_2$ DMF reflux; (d) NaHMDS/aniline **71** rt.

Scheme 12[a]

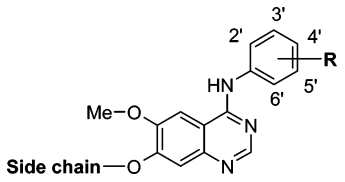


[a] Reagents: (a) Bu_4NF rt; (b) HCl/^{i}PrOH/aniline **71**; (c) TFA reflux; (d) DEAD/PPh_3/**96** rt.

with 4-(3-hydroxypropyl)morpholine[19] gave the desired quinazoline intermediate **89** ready for coupling with an aniline (Scheme 9). The 4-chloro-7-methoxy-6-(3-morpholinopropoxy)quinazoline **92** was prepared most conveniently by hydrolysis of compound **90**[20] to get the quinazolone **91** followed by chlorination with thionyl chloride (Scheme 10). Reaction of **92** with the 5-chloro-3-benzodioxol-4-amine **71** gave the desired anilinoquinazoline **26**. One of our lead compounds **28** (Scheme 11) was made by alkylation of the 7-hydroxyquinazolone **31**[14] with 1-(3-chloropropyl)piperidine[21] followed by deprotection of the POM group, treatment with thionyl chloride, and aniline coupling under basic conditions using NaHMDS as a base. Finally, coupling the aniline **71** onto the quinazoline **98**[14] followed by deprotection of the benzyl group gave **100** (Scheme 12). Deprotection of the TBDMS group on the cyanopyridine **96**[22] gave **97**, which was coupled with **100** via a Mitsunobu reaction to give **29**.

Biological Evaluation Overview. Compounds were tested sequentially in our in vitro cascade and had to meet the following criteria to progress to the next test: Src enzyme $<0.1\ \mu$M, KDR $\geq 1\ \mu$M (vide infra), and Src-3T3 and A549 cells $<1\ \mu$M. The Src-3T3 proliferation test uses a mouse fibroblast cell line that has been transfected with constitutively active human c-Src ki-

Table 1. Initial Lead Src Inhibitors



						IC_{50} (μM)[a]			
	R					enzyme inhibition		cell	
no.	2′	3′	4′	6′	side chains	Src	KDR	Src 3T3	A549
1		MeO			Me	0.253			
2		MeO			4-morpholinyl-$(CH_2)_3$	0.037	1.11	0.69	1.05
3	MeO				4-morpholinyl-$(CH_2)_3$	0.560			
4			MeO		4-morpholinyl-$(CH_2)_3$	0.477			
5		MeO		Cl	4-morpholinyl-$(CH_2)_3$	0.010	1.18	0.31	0.31
6		EtO		Cl	4-morpholinyl-$(CH_2)_3$	0.963			
7		MeO		Cl	4-*N*-methylpiperidinyl-CH_2	0.010	0.67	0.53	0.48

[a] Values are mean from at least three independent dose−response curves; variation was generally ±15% for Src and KDR enzymes and ±20% in 3T3 and A549 cells.

nase, allowing it to grow in a low serum concentration (0.5% fetal calf serum) incompatible with the growth of wild-type 3T3. In the second cell test, A549 (human non-small-cell lung cancer) cells were used in a migration assay. To prove that inhibition of cell migration was not due to a simple cytotoxic effect, cytotoxicity studies using MTT as a marker were routinely performed (see Experimental Section).

Results and Discussion

For clarity of discussion, data on only a limited, representative set of compounds are used to describe the structure−activity relationship. Where trends are exemplified by a single pair of compounds, it is to be understood that more examples[23] exist to support the structure−activity relationship described below. To make the relationship clearer we have deliberately chosen compounds with only one type of side chain (morpholinopropoxy) in the C-7 position of the quinazoline. Several of the compounds tested in our in vitro cascade met our criteria of Src enzyme <0.1 μM, KDR ≥1 μM (vide infra), and Src-3T3 and A549 cells <1 μM. When performing proliferation and migration cell assays, no toxicity was observed with any of our inhibitors at 10−20 μM.

Methoxy Anilines Series. High-throughput screening gave us our first lead (**1**, Table 1) belonging to the anilinoquinazoline family from which the EGFR-TKI (epidermal growth factor receptor-tyrosine kinase inhibitor) gefitinib (IRESSA, ZD1839)[24] and the angiogenesis inhibitor ZD6474[15] had previously been developed. Replacement of the methoxy group in the C-7 position of the quinazoline by polar side chains, such as a morpholinopropoxy (**2**), gave a significant increase in enzyme potency (**1**, 0.253 μM; **2**, 0.037 μM) and confirmed the *m*-methoxyanilino-quinazoline as being an interesting starting point. Directed robotic syntheses allowed us to explore the substitution pattern as well as the influence of other substituents on the aniline ring. The methoxy group in the meta position proved optimal (compare **2** with **3** and **4**), while the addition of a chlorine atom in the ortho position gave **5**, a very potent Src inhibitor selective versus KDR (0.010 μM, 100-fold selective versus KDR). A mixed Src-KDR inhibitor could be very advantageous, but only a selective agent could allow investigation of the in vivo effects of Src inhibition. Interestingly, the replacement of the methoxy group by an ethoxy group (**6**, 0.963 μM) substantially decreased the activity of the molecule. The enzymatic activity of **5** transposed well into our proliferation and migration cell assays (0.3 μM against both cell lines). C-7 side chain variations around **5** led to **7**, which gave high blood levels in mice up to 24 h (12 μM at 24 h from a 50 mg/kg oral dose). Compound **7** (also known as AZM475271) was used extensively to develop our in vivo cascade and to provide a benchmark profile for Src kinase inhibition.

Bicyclic Anilines. In an attempt to gain insight into the structural basis of the inhibitory activity of our methoxy aniline series, we performed molecular modeling and docking studies using the available 3D structure of activated Lck, a closely related Src family member.[25,26] On the basis of in-house studies and published experimental data, we found a binding mode consistent with the structure−activity relationship data (Figure 1).[14,27] In this model, the quinazoline ring occupies the adenine binding site. The highly conserved hydrogen bond is created by N1 of the quinazoline, which binds to the backbone amide of Met319 (Met341 in Src). The 3′-methoxy anilino group is buried in the hydrophobic pocket adjacent to the adenine site. It is clear from the model that the methoxy group adopts a particular conformation, coplanar with the aromatic ring and directed toward position 2′ rather than 4′ of the aniline. This latter orientation results in steric clashes with the proximal Ile314 and Lys273 (Ile336 and Lys295 in Src). From this observation, it was therefore tempting to make cyclic analogues such as benzofurans. The simple 4-anilinobenzofuran **8** (Table 2) proved to be more potent than the corresponding methoxy aniline **2**, but the introduction of a chlorine atom in position 5′ of this heterocycle (**9**) did not give the increase in activity observed between **2** and **5**. Interestingly, the 7-anilinobenzofuran **11** had the same level of activity as its regioisomer **9**, even though the oxygen atom was no longer in the same place as in the methoxyaniline **5**. This could reflect the favorable environment created by the proximal Thr316 (Thr338 in Src), which lies at the entrance to the selectivity pocket (Figure 1). Reduction of the chlorobenzofuran gave **12**, which showed reduced

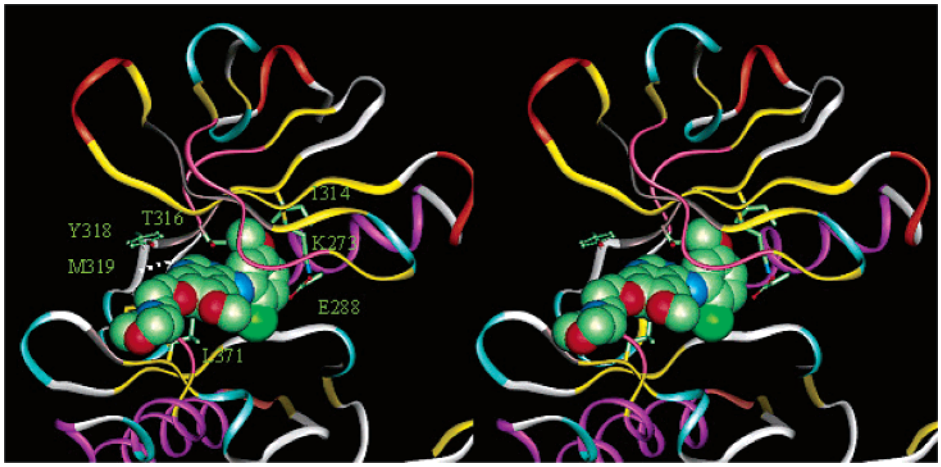
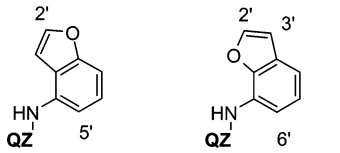


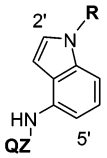
Figure 1. Stereoview of compound **5** docked into the ATP-binding site of Lck. The protein is represented as a ribbon diagram colored by secondary structure, with side chains of some selected residues shown in stick representation. The inhibitor is represented with van der Waals spheres colored by element. The quinazoline ring occupies the adenine binding site. The H-bond interaction between the quinazoline N1 and backbone NH of Met319 (Met341 in Src) is represented as a dotted line. The anilino moiety of **5** is buried into the hydrophobic pocket, characterized by a Thr residue at its entry (Thr316 in Lck, Thr338 in Src). It is clear from the model that the methyl of the 3′-OCH_3 points toward position 2′ rather than 4′ of the aniline, this latter orientation leading to steric clashes with the proximal Ile314 and Lys273 (Ile336 and Lys295 in Src).

Table 2. Benzofuran and Indole Inhibitors

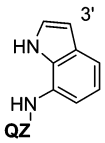


Benzofuran A

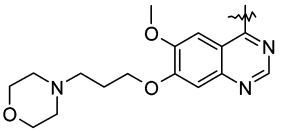
Benzofuran B



Indole A



Indole B



QZ

		IC_{50} (μM)[a]			
		enzyme inhibition		cell	
no.	H_2N-Ar	Src	KDR	Src 3T3	A549
8	benzofuran **A**, no substituent	0.016	0.25		
9	bnenzofuran **A**, 5-chloro	0.010	1.23	0.31	0.23
10	benzofuran **B**, no substituent	0.030	0.41		
11	benzofuran **B**, 6-chloro	0.010	1.38	0.34	0.26
12	benzofuran **B,** 2,3-dihydro	0.433			
13	benzofuran **B**, 3-chloro	<0.004	0.10		
14	indole **A**, R = H	0.177			
15	indole **A**, R = Me	0.040	0.42		
16	indole **B**, no substituent	0.260			
17	indole **B**, 3-chloro	0.020	2.32	0.38	0.98

[a] Values are mean from at least three independent dose–response curves; variation was generally ±15% for Src and KDR enzymes and ±20% in 3T3 and A549 cells.

activity compared with **10**, proving the need for a planar geometry in that region as suggested by the shape of the corresponding hydrophobic pocket. The study of this

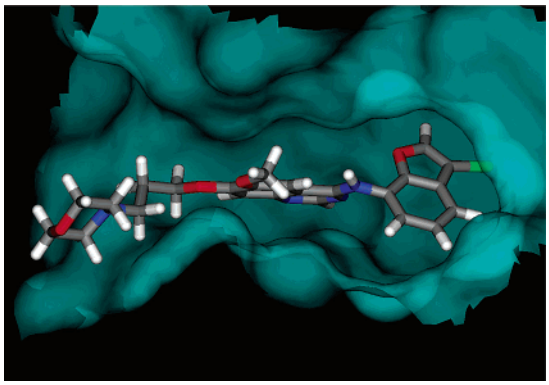


Figure 2. 3′-Chlorobenzofuran derivative **13**. The benzofuran and the benzodioxole series have been docked into the ATP binding site of Lck and fit well. The binding site is represented by its molecular surface,[28] and the inhibitors are illustrated in stick representation and colored by element.

pocket further suggested that small substituents at the 3′-position of the 7-anilinobenzofurans might be allowed (Figure 2), and indeed, introduction of the 3-chloro-7-aminobenzofuran on the quinazoline nucleus gave a remarkably potent molecule (**13**), still quite potent against KDR as well.

Benzofurans were then replaced by indoles (Table 2). The 4-aminoindole **14** gave a moderate level of Src inhibition, but *N*-methylation (**15**), to mimic the chlorine atom on the benzofuran **13**, significantly improved the potency. The 7-aminoindole regioisomer **16** behaved similarly to **14**, and the 3-chloro-7-aminoindole **17**, the most potent of the indole series, fell short of the activity found for the 3-chloro-7-aminobenzofuran **13**. Moreover, its IC_{50} against A549 cells was found to be higher than expected.

The work on the aminobenzofurans taught us two things: first, that the heterocyclic ring must be planar (cf. **12** vs **10**) and, second, that the oxygen atom was equally good in the 1′- or 3′-position (**8** vs **10** and **9** vs **11**). This encouraged the design of a benzodioxole heterocycle with two oxygen atoms and a pseudoplanar conformation. As expected, modeling of this compound

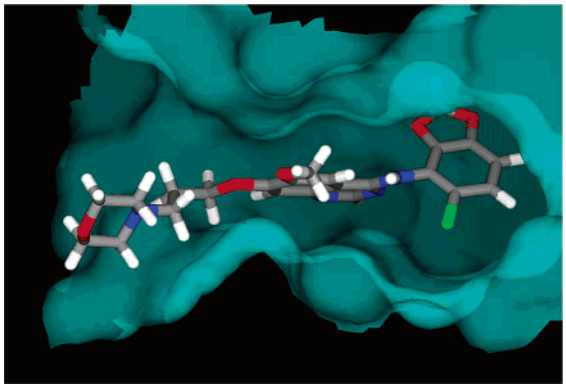
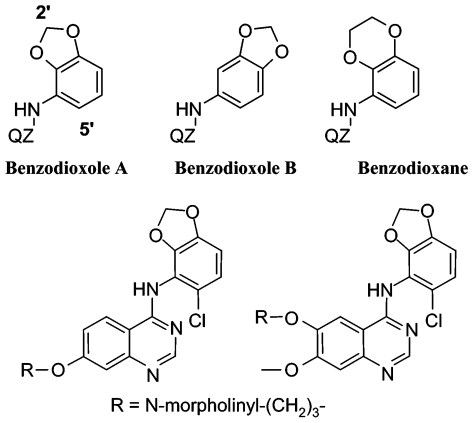


Figure 3. 5′-Chlorobenzodioxole derivative **19**. The binding site is represented by its molecular surface,[28] the inhibitors are illustrated in stick representation and colored by element.

Table 3. Benzodioxole Inhibitors



		IC_{50} (μM)[a]			
		enzyme inhibition		cell	
no.	H_2N-Ar	Src	KDR	Src 3T3	A549
18	benzodioxole **A**, no substituent	0.014	4.96	0.31	0.21
19	benzodioxole **A**, 5-chloro	<0.004	17.03	0.10	0.18
20	benzodioxole **A**, 5-bromo	<0.004	42.64	0.06	0.14
21	benzodioxole **A,** 5-fluoro	0.028	8.16	0.30	0.45
22	benzodioxole **A,** 2,2-difluoro	0.270			
23	benzodioxole **B**	0.760			
24	benzodioxane	0.370			
25		0.010	91.35	0.45	0.67
26		0.050	73.82	1.16	1.20

[a] Values are mean from at least three independent dose−response curves; variation was generally ±15% for Src and KDR enzymes and ±20% in 3T3 and A549 cells.

suggested it would fit particularly well into the active site (Figure 3). The first benzodioxole (**18**) made gave very encouraging results (Table 3). It was as potent as **5** and more selective for Src. The introduction of a chlorine atom in the 5′-position (**19**) increased the activity against Src still further and gave us even higher selectivity (KDR:Src ratio > 4000). Moreover, **19** was very potent in cells (3T3, 0.09 μM; A549, 0.18 μM). Superimposing our Lck model onto the crystal structure of KDR and comparing their amino acid sequences allowed us to rationalize the selectivity profile of this series.[29] In KDR the residue at the entrance to the selectivity pocket is a Val916, which gives less favorable contacts with the benzodioxole oxygens than the corresponding Thr316 in Lck or Thr338 in Src. Moreover, the chlorine in the 5′-position lies in a hydrophobic pocket lined by an alanine residue (Ala381 in Lck, Ala403 in Src), whereas in KDR this residue is a cysteine (Cys1045), which would be sterically and electronically less favorable.

Replacement of chlorine by bromine (**20**) again gave good results, but fluorine (**21**), being smaller, gave results similar to the parent benzodioxole (**18**). Replacement of the methylene bridge of the benzodioxole by a difluoromethylene (**22**) or changing the position of the amino group from the 4′- to the 5′-position (**23**) resulted in decreased activity. Enlargement of the dioxole into a dioxane ring (**24**) was also detrimental due to the loss of the planarity of the ring, similar to what we observed between the benzofuran **10** and the dihydrobenzofuran **12**. Removal of the C-6 methoxy group on the quinazoline (**25**) or switching around the C-7 side chain with the C-6 methoxy group on the quinazoline (**26**) gave compounds that were generally less potent against both Src enzyme and in cells.

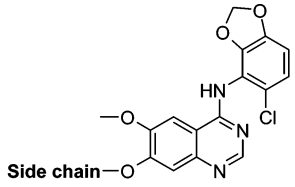
The C-7 quinazoline side chain was then varied in order to optimize the in vitro and in vivo potency and physical properties while the chlorobenzodioxole aniline was kept constant. Good in vitro potency, as well as selectivity, could be achieved with basic or nonbasic side chains and some representative examples are given in Table 4. As expected, basic side chains (**27**, **28**) gave compounds with a good solubility. The aminobenzodioxole series gave particularly high free drug levels in blood. For example, protein binding for the chloromethoxyaniline **5** results in 4.6% free drug in rat serum, whereas the chlorobenzodioxole **19** is 10.5% free. As previously observed in our research program on VEGF (vascular endothelial growth factor) tyrosine kinase inhibitors, the basic piperidines gave good and sustained blood levels in rats dosed orally at 20 mg/kg coupled with a good to excellent bioavailability (Table 5). Following iv administration, clearance of the compounds was moderate (equivalent to 50% and 68% hepatic blood flow), while the elimination half-life values (~5.8 h) were determined mainly by the high volume of distribution (15−20 L/kg).

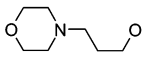
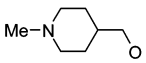
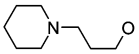
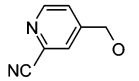
The best compounds were further investigated in a rat xenograft model based on the same 3T3 Src transfected cell line as in the in vitro assay. Results obtained with **28** provide a good example of the level of activity achieved with such compounds in rat xenografts. Inhibition of tumor growth was dose-dependent with almost complete inhibition achieved at doses as low as 6 mg/kg po once daily (Figure 4). Some of these compounds have also proven to be potent inhibitors of metastasis formation in several animal models and these results will be published elsewhere.

Conclusions

We have discovered several new anilinoquinazoline series that are potent Src inhibitors. Among these, the aminobenzodioxole quinazolines have been identified as providing the optimum profile of selectivity, enzyme inhibition, and cellular potency. These compounds gave high and prolonged blood levels when dosed orally and have been shown to markedly reduce the rate of Src-driven tumor cell growth in a rat xenograft model.

Table 4. Optimization of the C-7 Side Chain in the Chlorobenzodioxole Series



		enzyme inhibition IC_{50} (μM) [a]						cell IC_{50} (μM) [a]				
no.	side chains	Src	KDR	Flt	FTK	Tie-2	CSK	Src 3T3	A549	pKa	solubility (μM)	% free in rat serum
19		<0.004	17.0	>100	>100	17.1	7.3	0.09	0.18	7.2	71	10.5
27		0.005	15.6	>100	>100	11.3	2.0	0.12	0.26	9.4	>3300	9.8
28		<0.004	21.7	>100	>100	11.5	1.7	0.11	0.13	9.7	>2000	13.7
29		0.010	77.0	-	-	-	-	0.09	0.23	4.9	10	1.6

[a] Values are mean from at least three independent dose−response curves; variation was generally ±15% for Src and KDR enzymes and ±20% in 3T3 and A549 cells.

Table 5. Rat Pharmacokinetic Parameters in the Chlorobenzodioxole Series

	po (20 mg/kg)			iv (2 mg/kg)			
no.	C_{max} (μM)	$AUC_{0-\infty}$ (μg h/mL)	C_{24h} (μM)	term $t_{1/2}$ iv (h)	Cl (mL/min/kg)	V_{dss} (L/kg)	F (%)
27	0.91	2.5	0.06	5.7	49.0	17.0	37
28	2.96	7.9	0.08	5.8	36.0	14.4	86

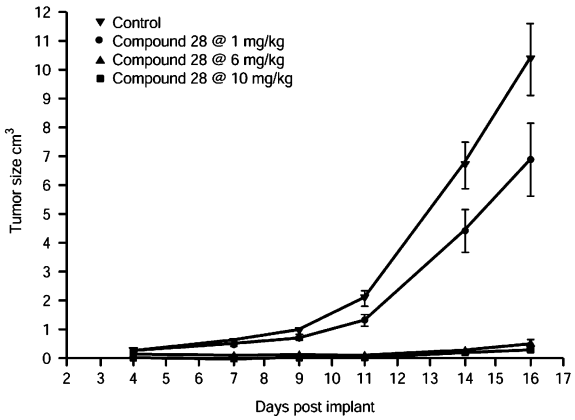


Figure 4. Compound **28** dosed orally once daily from day 0 to Src 3T3 tumor-bearing nude rats. Calliper measurements started on day 4.

Experimental Section

All experiments were carried out under an inert atmosphere and at room temperature unless otherwise stated. Flash chromatography was carried out on Merck Kieselgel 50 (Art. 9385). The purities of compounds for biological testing were assessed by analytical HPLC on a Hichrom S5ODS1 Spherisorb Column System set to turn isocratically with 60−70% MeOH + 0.2% CF_3COOH in water as eluent. TLCs were performed on precoated silica gel plates (Merck Art. 5715), and the resulting chromatograms were visualized under UV light at 254 nm. Purification by preparative HPLC/MS was done on a Waters LC/MS system using a Waters Symmetry column (C18, 5 μm, 19 mm diameter, 100 mm length) using a mixture of water with 1% acetic acid and acetonitrile (gradient from 5% to 100%) as solvent. The NMR spectra were obtained on a JEOL JNM EX 400 (400 MHz) spectrometer. Chemical shifts are expressed in unit of δ (ppm), and peak multiplicities are expressed as follows: s, singlet; d, doublet; dd, doublet of doublet; t, triplet; br s, broad singlet; m, multiplet. Mass spectrometry was done on an analytical Waters LC/MS system with positive and negative ion data collected automatically. NMR and mass spectra were run on isolated intermediates and final products and are consistent with the proposed structures. For the microanalysis, all the adducts mentioned were measured: water was measured by the Karl-Fisher method using a Mettler DL 18; HCl content was determined on a Metrohm 686 by titration using silver nitrate solution, and the organic adducts were measured by ^1H NMR. The following abbreviations have been used: ADDP, 1,1′-(azodicarbonyl)dipiperidine; Boc, *tert*-butoxycarbonyl; DEAD, diethyl-azodicarboxylate; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; DPPA, diphenylphosphoryl azide; Gold's reagent, [3-(dimethylamino)-2-azaprop-2-en-1-ylidene]dimethylammonium chloride; NaHMDS, sodium bis(trimethylsilyl)amide; POM, pivaloyloxymethyl; TFA, trifluoracetic acid.

6,7-Dimethoxy-*N*-(3-methoxyphenyl)quinazolin-4-amine (1). A mixture of 4-chloro-6,7-dimethoxyquinazoline[30] (0.177 g, 0.8 mmol), 3-methoxyaniline (0.106 mL, 0.95 mmol), 2-propanol (6 mL), and a saturated solution of HCl gas in 2-propanol (0.016 mL, 5 N solution) was stirred and heated at 90 °C for 2 h. Upon cooling to room temperature the formed precipitate was collected by filtration and washed with a mixture of ether:2-propanol (1:1) and then with ether. The solid was dissolved in dichloromethane and washed with a saturated aqueous solution of sodium bicarbonate. The organic phase was dried over magnesium sulfate and purified by flash chromatography using dichloromethane:methanol (98.5:1.5 then 98:2) as eluent. Evaporation of the solvent and trituration of the solid under ether gave 0.15 g of **1** (61%). ^1H NMR ($CDCl_3$): δ 3.85 (s, 3H), 4.05 (s, 6H), 6.70 (dd, 1H), 7.0 (s, 1H), 7.10 (s, 1H), 7.20 (d, 1H), 7.30 (t, 1H), 7.45 (s, 1H), 8.70 (s, 1H). MS-ESI *m*/*z* 312 $[MH]^+$. Anal. ($C_{17}H_{17}N_3O_3$) C, H, N.

***N*-(3-Hydroxypropyl)morpholine (30).** Morpholine (94 g, 1.08 mol) was added dropwise to a solution of 3-bromo-1-propanol (75 g, 0.54 mol) in toluene (750 mL) and the reaction mixture heated at 80 °C for 4 h. Upon cooling, a solid was removed by filtration, the volatiles were evaporated, and the

resulting yellow oil was purified by distillation at 0.4−0.7 mmHg to give **30** (40 g, 50%) as a colorless oil. bp 68−70 °C (∼0.5 mmHg). ^{1}H NMR (DMSO-d_6): δ 1.65−1.78 (m, 2H), 2.50 (t, 4H), 2.60 (t, 2H), 3.68 (t, 4H), 3.78 (t, 2H), 4.90 (br d, 1H).

[6-Methoxy-7-(3-morpholin-4-ylpropoxy)-4-oxoquinazolin-3(4*H*)-yl]methyl Pivalate (32). 7-Hydroxy-6-methoxy-3-((pivaloyloxy)methyl)-3,4-dihydroquinazolin-4-one **31**[14] (12 g, 39.2 mmol) was dissolved in dichloromethane (125 mL) under argon, and triphenylphosphine (13.3 g, 51 mmol) and 1-(3-hydroxypropyl)morpholine (6.25 g, 43 mmol) were added followed by dropwise addition of DEAD (8 mL, 51 mmol) in dichloromethane (20 mL). After being stirred for 3 h, the solvent was evaporated and the residue purified by flash chromatography using increasingly polar solvent mixtures starting with dichloromethane, then ethyl acetate:dichloromethane (95:5), then pure ethyl acetate, and ending with dichloromethane:ethyl acetate:methanol (8:1:1). Evaporation of the solvent gave 17 g (100%) of **32** as a white solid. ^{1}H NMR (DMSO-d_6): δ 1.15 (s, 9H), 1.95 (m, 2H), 2.40 (m, 4H), 2.50 (t, 2H), 3.60 (m, 4H), 3.90 (s, 3H), 4.20 (t, 2H), 5.90 (s, 2H), 7.15 (s, 1H), 7.50 (s, 1H), 8.35 (s, 1H).

6-Methoxy-7-(3-morpholin-4-ylpropoxy)quinazolin-4(3*H*)-one (33). A mixture of [6-methoxy-7-(3-morpholin-4-ylpropoxy)-4-oxoquinazolin-3(4*H*)-yl]methyl pivalate **32** (17 g, 39 mmol), methanol (65 mL) and a saturated solution of ammonia gas in methanol (350 mL) was stirred overnight. The white precipitate that formed was dissolved with dichloromethane, the solution was filtered to remove some insoluble material, the filtrate was concentrated, and the residue was triturated under ether:dichloromethane (95:5) and dried under vacuum to give 12.5 g of **33** (100%) as a white solid. ^{1}H NMR (DMSO-d_6): δ 1.95 (m, 2H), 2.4 (m, 4H), 2.5 (t, 2H), 3.6 (m, 4H), 3.90 (s, 3H), 4.2 (t, 2H), 7.15 (s, 1H), 7.45 (s, 1H), 8.0 (s, 1H).

4-Chloro-6-methoxy-7-(3-morpholin-4-ylpropoxy)-quinazoline (34). A mixture of 6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazolin-4(3*H*)-one **33** (12.5 g, 39 mmol), thionyl chloride (107 mL), and DMF (0.9 mL) was heated to 80 °C for 1.5 h. The volatiles were removed under vacuum and the remaining traces of thionyl chloride were eliminated by azeotropic distillation with toluene using a rotary evaporator. Dichloromethane was added and the solution was cooled to 0 °C prior to the addition of water and a saturated sodium bicarbonate solution until pH 8 was reached. The aqueous phase was extracted with dichloromethane, the organic phase was combined, washed in turn with water and brine, and dried over magnesium sulfate. The solvent was evaporated under vacuum and the residue was purified by column chromatography on silica using a dichloromethane:methanol (92:8) as eluent to give **34** as a white solid (6.9 g, 54%). ^{1}H NMR (DMSO-d_6): δ 1.95 (m, 2H), 2.40 (m, 4H), 2.50 (t, 2H), 3.60 (m, 4H), 4.05 (s, 3H), 4.30 (t, 2H), 7.40 (s, 1H), 7.45 (s, 1H), 8.9 (s, 1H).

6-Methoxy-*N*-(3-methoxyphenyl)-7-(3-morpholin-4-ylpropoxy)quinazolin-4-amine (2). Using a procedure similar to the one described for compound **1**, 4-chloro-6-methoxy-7-(3-morpholinopropoxy)quinazoline **34** (0.1 g, 0.3 mmol) and 3-methoxyaniline (0.04 mL, 0.35 mmol) were reacted to give after workup and purification 0.088 g of **2** (70%). ^{1}H NMR (DMSO-d_6): δ 1.95 (m, 2H), 2.35 (m, 4H), 2.45 (m, 2H), 3.6 (m, 4H), 3.80 (s, 3H), 3.95 (s, 3H), 4.20 (t, 2H), 6.70 (dd, 1H, J_1 = 1.8, J_2 = 7.8 Hz), 7.20 (s, 1H), 7.3 (t, 1H, $J_1 = J_2$ = 7.9 Hz), 7.40 (d, 1H, J_1 = 1.9, J_2 = 8.0 Hz), 7.50 (s, 1H, $J_1 = J_2$ = 2.2 Hz), 7.85 (s, 1H), 8.45 (s, 1H), 9.45 (s, 1H). Anal. ($C_{23}H_{28}N_4O_4\cdot 0.3H_2O$) C, H, N.

6-Methoxy-*N*-(2-methoxyphenyl)-7-(3-morpholin-4-ylpropoxy)quinazolin-4-amine (3) was prepared as described for the synthesis of **1**. 4-Chloro-6-methoxy-7-(3-morpholinopropoxy)quinazoline **34** (0.15 g, 0.44 mmol) was reacted with *o*-anisidine (0.06 mL, 0.53 mmol) in the presence of HCl in 2-propanol (0.1 mL, 0.49 mmol, 5 N solution) to give 0.125 g of **3** (66%). ^{1}H NMR (DMSO-d_6): δ 1.95 (m, 2H), 2.35 (m, 4H), 2.45 (m, 2H), 3.6 (m, 4H), 3.80 (s, 3H), 3.95 (s, 3H), 4.20 (t, 2H), 7.0 (t, 1H, $J_1 = J_2$ = 7.8 Hz), 7.15 (m, 2H), 7.25 (t, 1H, $J_1 = J_2$ = 7.8 Hz), 7.50 (d, 1H, J = 7.7 Hz), 7.80 (s, 1H), 8.30 (s, 1H), 9.10 (s, 1H). MS-ESI m/z 425 [MH]$^+$. Anal. ($C_{23}H_{28}N_4O_4\cdot 0.2H_2O$) C, H, N.

6-Methoxy-*N*-(4-methoxyphenyl)-7-(3-morpholin-4-ylpropoxy)quinazolin-4-amine (4) was prepared as described for the synthesis of **1**. 4-Chloro-6-methoxy-7-(3-morpholinopropoxy)quinazoline **34** (0.15 g, 0.44 mmol) was reacted with *p*-anisidine (0.06 mL, 0.53 mmol) in the presence of HCl in 2-propanol (0.1 mL, 0.49 mmol, 5 N solution) to give 0.135 g of **4** (72%). ^{1}H NMR (DMSO-d_6): δ 1.95 (m, 2H), 2.35 (m, 4H), 2.45 (m, 2H), 3.6 (m, 4H), 3.80 (s, 3H), 3.95 (s, 3H), 4.15 (t, 2H), 6.95 (d, 2H, J = 9.0 Hz), 7.15 (s, 1H), 7.60 (d, 2H, J = 9.0 Hz), 7.8 (s, 1H), 8.35 (s, 1H), 9.40 (s, 1H). MS-ESI m/z 425 [MH]$^+$. Anal. ($C_{23}H_{28}N_4O_4$) C, H, N.

***N*-(2-Chloro-5-methoxyphenyl)-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazolin-4-amine (5).** A mixture of 4-chloro-6-methoxy-7-(3-morpholinopropoxy)quinazoline **34** (6.9 g, 20 mmol), 2-chloro-5-methoxyaniline (3.9 g, 25 mmol), 2-propanol (100 mL), and a saturated solution of HCl gas in 2-propanol (4 mL) was stirred and heated at 80 °C for 2.5 h. Upon cooling to room temperature the formed precipitate was collected by filtration and washed with a mixture of ether: isopropyl alcohol (1:1) and then with ether. The solid was dried under vacuum overnight at 40 °C to give 10 g of **5** as a dihydrochloride (94%). ^{1}H NMR (DMSO-d_6 and CD_3CO_2D): δ 2.35 (m, 2H), 3.1 (m, 2H), 3.3 (m, 2H), 3.5 (m, 2H), 3.8 (s, 3H), 3.9 (m, 2H), 3.95 (m, 2H), 4.05 (s, 3H), 4.35 (m, 2H), 7.05 (dd, 1H, J_1 = 3.1, J_2 = 9.0 Hz), 7.15 (d, 1H, J = 3.0 Hz), 7.45 (s, 1H), 7.55 (d, 1H, J = 9.0 Hz), 8.3 (s, 1H), 8.8 (s, 1H). MS-ESI m/z 459 [MH]$^+$. Anal. [$C_{23}H_{27}ClN_4O_4\cdot 0.15H_2O\cdot 0.08$(2-propanol)$\cdot$ 2HCl] C, H, N.

***N*-(2-Chloro-5-ethoxyphenyl)-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazolin-4-amine (6)** was prepared as described for the synthesis of **1** and purified as described for **23**. 4-Chloro-6-methoxy-7-(3-morpholinopropoxy)quinazoline **34** (0.12 g, 0.35 mmol) was reacted with 2-chloro-5-ethoxyaniline (0.072 g, 0.42 mmol) in the presence of HCl in 2-propanol (0.08 mL, 0.40 mmol, 5 N solution) to give 0.105 g of **6** (63%). ^{1}H NMR ($CDCl_3$; CF_3CO_2D): δ 1.45 (t, 3H), 2.50 (m, 2H), 3.10 (m, 2H), 3.45 (m, 2H), 3.75 (m, 2H), 3.95−4.40 (m, 9H), 4.40 (m, 2H), 6.90 (dd, 1H, J_1 = 8.5, J_2 = 3.0 Hz), 7.35 (d, 1H, J = 3.0 Hz), 7.45 (d, 1H, J = 8.5 Hz), 7.55 (s, 1H), 7.70 (s, 1H), 8.70 (s, 1H). MS-ESI m/z 473 [MH]$^+$. Anal. ($C_{24}H_{29}ClN_4O_4$) C, H, N.

***N*-(2-Chloro-5-methoxyphenyl)-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinazolin-4-amine (7).** 4-Chloro-6-methoxy-7-(1-methylpiperidine-4-ylmethoxy)quinazoline **36**[15] (49 g, 152 mmol) and 2-chloro-5-methoxyaniline (35.5 g, 183 mmol) were reacted together using the same procedure as the one described for **2** to give 60 g of **7** (89%). ^{1}H NMR (DMSO-d_6): δ 1.3−1.4 (m, 2H), 1.78 (m, 3H), 1.9 (dd, 2H), 2.15 (s, 3H), 2.8 (d, 2H), 3.8 (s, 3H), 3.95 (s, 3H), 4.0 (d, 2H), 6.9 (d, 1H), 7.15 (m, 2H), 7.48 (d, 1H), 7.8 (s, 1H), 8.3 (s, 1H), 9.5 (bs, 1H). MS-ESI m/z 442 [MH]$^+$. Anal. ($C_{23}H_{27}ClN_4O_3$) C, H, N.

***tert*-Butyl 1-Benzofuran-4-ylcarbamate (38).** Benzofuran-4-carboxylic acid **37**[16] (0.5 g, 2.8 mmol), DPPA (1.2 mL, 5.6 mmol), triethylamine (0.79 mL, 5.6 mmol), and *tert*-butyl alcohol (1.5 mL) were heated to reflux for 18 h. Upon cooling to room temperature the mixture was poured into water and extracted with ethyl acetate. The organic phase was washed with water and brine, dried over magnesium sulfate, and filtered, and the solvent was evaporated. The resulting oil was purified by flash chromatography using dichloromethane as solvent. Evaporation of the solvent gave 0.8 g of **38** as an oil which still contained some DPPA. MS-ESI m/z 256 [MNa]$^+$.

4-Aminobenzofuran (39). *tert*-Butyl 1-benzofuran-4-ylcarbamate **38** (0.65 g, 2.7 mmol) was dissolved in dichloromethane (20 mL), TFA (2.5 mL) was added at 0 °C, and the mixture stirred for 1 h at the same temperature and then 1.5 h at room temperature. The volatiles were removed, and the residue was taken up in a saturated solution of sodium bicarbonate and extracted with ethyl acetate. The organic phase was dried over magnesium sulfate and filtered and the solvent evaporated. The resulting oil was purified by flash chromatography using dichloromethane:petroleum ether

(80:20) as solvent. Evaporation of the solvent gave 0.35 g of **39** (95% over the last two steps). ^{1}H NMR (DMSO-d_6): δ 5.50 (br s, 2H), 6.35 (d, 1H, J = 8.0 Hz), 6.70 (d, 1H, J = 8.0 Hz), 6.95 (dd, 1H, J_1 = 8.0, J_2 = 8.0 Hz), 7.0 (d, 1H, J = 2.4 Hz), 7.70 (d, 1H, J = 2.4 Hz). MS m/z 133 $[M]^+$.

***N*-(1-Benzofuran-4-yl)-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazolin-4-amine (8)** was prepared as described for the synthesis of **1** and purified as described for **23**. 4-Chloro-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazoline **34** (0.1 g, 0.3 mmol) was reacted with 4-aminobenzofuran **39** (0.059 g, 0.44 mmol) to give 0.99 g of **8** (76%). ^{1}H NMR (DMSO-d_6 and CF_3CO_2D): δ 2.35 (m, 2H), 3.15 (t, 2H), 3.35 (t, 2H), 3.50 (d, 2H), 3.80 (t, 2H), 4.0 (m, 2H), 4.05 (s, 3H), 4.35 (t, 2H), 6.90 (s, 1H), 7.40 (m, 3H), 7.65 (d, 1H, J = 8.2 Hz), 8.05 (d, 1H, J = 2.3 Hz), 8.35 (s, 1H), 8.80 (s, 1H). MS-ESI m/z 435 $[MH]^+$. Anal. ($C_{24}H_{26}N_4O_4$) C, H, N.

2-Nitro-3-chloro-6-hydroxybenzaldehyde (41). 4-Chloro-3-nitrophenol **40** (1 g, 5.8 mmol), TFA (9 mL) and hexamethylenetetramine (0.8 g, 5.8 mmol) were placed in a Teflon-lined reactor. Six identical reactors were placed in a microwave oven and heated at 115 °C for 1.5 h. Each reactor was then processed as follows: 4 N HCl (20 mL) was added and the mixture was extracted with dichloromethane. The organic phase was washed twice with brine and dried over magnesium sulfate and the solvent evaporated. Purification of the pooled samples by flash chromatography using dichloromethane gave 3 g of **41** (43%). ^{1}H NMR (DMSO-d_6): δ 7.25 (m, 1H), 7.85 (m, 1H), 10.25 (s, 1H).

2-(Ethoxycarbonyl)-5-chloro-4-nitrobenzofuran (42). A mixture of 2-nitro-3-chloro-6-hydroxybenzaldehyde **41** (3 g, 14.8 mmol), diethyl bromomalonate (2.78 mL, 16.3 mmol), potassium carbonate (3.1 g, 22.2 mmol), tetrabutylammonium bromide (0.477 g, 1.4 mmol), and toluene (50 mL) was heated to reflux in a Dean−Stark apparatus for 20 h. Upon cooling, the precipitate was removed by filtration and the solvent was evaporated. The residue was solubilized in ethyl acetate, washed with water and brine, dried over magnesium sulfate, and filtered, and the solvent was evaporated. The resulting oil was purified by flash chromatography using dichloromethane as solvent. Evaporation of the solvent gave 3 g of **42** (77%). ^{1}H NMR (DMSO-d_6): δ 3.35 (t, 3H), 4.45 (q, 2H), 7.85 (s, 1H), 7.90 (d, 1H, J = 8.1 Hz), 8.20 (d, 1H, J = 8.1 Hz). MS m/z 269 $[M]^+$.

2-Carboxy-5-chloro-4-nitrobenzofuran (43). 2-(Ethoxycarbonyl)-5-chloro-4-nitrobenzofuran **42** (3 g, 11.1 mmol) was suspended in ethanol (17 mL). KOH (2 N, 11.1 mL, 22.2 mmol) was added and the mixture refluxed for 1 h. The ethanol was evaporated and the residue redissolved in water. HCl (6 N) was added to adjust the pH to 2 and the resulting precipitate collected by filtration, washed with water, and dried under vacuum over phosphorus pentoxide to give 2.65 g of **43** (98%). ^{1}H NMR (DMSO-d_6): δ 7.25 (s, 1H), 7.70 (d, 1H, J = 8.8 Hz), 8.05 (d, 1H, J = 8.8 Hz).

5-Chloro-4-nitrobenzofuran (44). 2-Carboxy-5-chloro-4-nitrobenzofuran **43** (1.6 g, 6.6 mmol) and copper oxide (0.080 g, 1.0 mmol) were suspended in quinoline (14 mL) and heated at 210 °C for 30 min. Copper (0.020 g, 0.3 mmol) was then added and heating was increased to 230 °C for another 20 min. Upon cooling, the copper and unreacted starting material were removed by filtration, the filtrate was diluted with ether, washed with 2 N HCl (3×), and dried over magnesium sulfate, and the solvent was evaporated. The residue was purified by flash chromatography using ether:petroleum ether (15:85) as eluent. Evaporation of the solvent gave 0.36 g of **44** (28%). ^{1}H NMR (DMSO-d_6): δ 7.20 (d, 1H, J = 1.4 Hz), 7.70 (d, 1H, J = 8.8 Hz), 8.05 (d, 1H, J = 8.8 Hz), 8.35 (d, 1H, J = 1.4 Hz).

4-Amino-5-chlorobenzofuran (45). Methanol (7 mL), Raney nickel (0.020 g), and hydrazine hydrate (0.097 mL, 2 mmol) were warmed to 55−60 °C and 5-chloro-4-nitrobenzofuran **44** (0.1 g, 0.5 mmol) was added portionwise. The reaction mixture was then heated to reflux for 30 min. The catalyst was removed by filtration, the solvent evaporated, the residue dissolved in water and extracted with dichloromethane. The organic phase was dried over magnesium sulfate and the solvent evaporated. The crude product was purified using dichloromethane:petroleum ether (4:6) as eluent. Evaporation of the solvent gave 0.037 g of **45** (45%). ^{1}H NMR (DMSO-d_6): δ 5.80 (br s, 2H), 6.80 (d, 1H, J = 8.6 Hz), 7.10 (d, 1H, J = 8.7 Hz), 7.15 (d, 1H, J = 2.3 Hz), 7.80 (d, 1H, J = 2.0 Hz).

***N*-(5-Chloro-1-benzofuran-4-yl)-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazolin-4-amine Dihydrochloride (9).** 4-Chloro-6-methoxy-7-(3-morpholin-4-ylpropoxy)-quinazoline **34** (0.059 g, 0.17 mmol), 4-amino-5-chlorobenzofuran **45** (0.035 g, 0.2 mmol), 2-pentanol (4 mL), and HCl in 2-propanol (6 N, 0.038 mL, 0.19 mmol) were heated at 110 °C under anhydrous conditions for 5 h. Upon cooling, the precipitate was collected by filtration and washed with ether:2-propanol (1:1) and then with ether only. The solid was dissolved in methanol, MP carbonate resin (140 mg) was added, and the mixture stirred for 2 h. The resin was discarded and the methanol was evaporated. The crude product was purified by flash chromatography using first dichloromethane, then dichloromethane:methanol (95:5), and finally dichloromethane:methanol (92.5:7.5). The solvent was evaporated and dichloromethane was added followed by addition of a solution of HCl in ether. The precipitate was collected by filtration and dried under vacuum to give 0.15 g of **9** (24%). ^{1}H NMR (DMSO-d_6 and CF_3CO_2D): δ 2.35 (m, 2H), 3.15 (m, 2H), 3.35 (t, 2H), 3.55 (d, 2H), 3.70 (t, 2H), 4.05 (m, 5H), 4.35 (t, 2H), 6.95 (dd, 1H, J_1 = 2.4, J_2 = 0.9 Hz), 7.40 (s, 1H), 7.60 (d, 1H, J = 8.8 Hz), 7.80 (dd, 1H, J_1 = 8.8, J_2 = 0.9 Hz), 8.15 (d, 1H, J = 2.4 Hz), 8.25 (s, 1H), 8.80 (s, 1H). MS-ESI m/z 469 $[MH]^+$. Anal. ($C_{24}H_{25}ClN_4O_4 \cdot 0.4H_2O$) C, H, N.

7-Aminobenzofuran (46). 7-Nitrobenzofuran[17] (0.5 g, 3 mmol), methanol (9 mL), and Raney nickel (20 mg) were heated to 55 °C. Hydrazine hydrate (0.45 mL, 9 mmol) was added dropwise and the mixture heated to reflux for 30 min. The catalyst was removed by filtration, the solvent evaporated, water added, and the product extracted with dichloromethane. The organic phase was dried over magnesium sulfate and the solvent evaporated to give 0.4 g of **46** (100%) as an oil. ^{1}H NMR (DMSO-d_6): δ 5.25 (br s, 2H), 6.55 (d, 1H, J = 7.5 Hz), 6.80 (m, 2H), 6.9 (t, 1H, J = 7.5 Hz), 7.85 (d, 1H, J = 1.6 Hz).

***N*-(1-Benzofuran-7-yl)-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazolin-4-amine (10).** 7-Aminobenzofuran **46** (0.047 g, 0.35 mmol) was reacted with 4-chloro-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazoline **34** (0.1 g, 0.3 mmol) using a procedure similar to the one described for the synthesis of **5** and purified as described for **23** to give 0.11 g of **10** (84%). ^{1}H NMR (DMSO-d_6 and CF_3CO_2D): δ 2.35 (m, 2H), 3.15 (t, 2H), 3.35 (t, 2H), 3.55 (d, 2H), 3.75 (t, 2H), 4.05 (t, 3H), 4.10 (m, 2H), 4.35 (t, 2H), 7.10 (d, 1H, J = 1.9 Hz), 7.45 (m, 3H), 7.75 (dd, 1H, J_1 = 1.3, J_2 = 7.7 Hz), 8.05 (d, 1H, J = 1.9 Hz), 8.25 (s, 1H), 8.85 (s, 1H). MS-ESI m/z 435 $[MH]^+$. Anal. ($C_{24}H_{26}N_4O_4$) C, H, N.

Ethyl 2-Amino-6-chlorobenzoate (48). 2-Amino-6-chlorobenzoic acid **47** (18 g, 100 mmol) was dissolved in DMF, sodium hydride (4.6 g, 110 mmol, 60% in oil) was added, and the mixture was stirred for 30 min. Ethyl iodide (10 mL, 125 mmol) was added and the reaction mixture stirred for 2 days at room temperature. The solvent was evaporated, water was added, and the product was extracted with ethyl acetate. The organic phase was washed with water and brine, dried over magnesium sulfate, and filtered, and the solvent was evaporated. The crude product was purified by flash chromatography using ethyl acetate:petroleum ether (2:8) as solvent. Evaporation of the solvent gave 15.8 g of **48** (80%) as an oil. ^{1}H NMR (DMSO-d_6): δ 1.30 (t, 3H), 4.30 (q, 2H), 5.70 (br s, 2H), 6.60 (d, 1H), 6.70 (d, 1H), 7.10 (t, 1H).

Ethyl 2-Hydroxy-6-chlorobenzoate (49). Ethyl 2-amino-6-chlorobenzoate **48** (12.7 g, 63.6 mmol) was suspended in a mixture of water (38 mL), concentrated sulfuric acid (27.9 mL), and ice (76 g). Sodium nitrite (4.5 g in 100 mL water) was added dropwise over 5 min to this suspension. The reaction mixture was stirred at 0 °C for an additional 20 min, heated to 120 °C for 1 h, and poured into ice−water, and the product was extracted with ether. The organic phase was washed with water and brine, dried over magnesium sulfate, filtered, and

the solvent was evaporated. The resulting oil was purified by flash chromatography using dichloromethane:petroleum ether (2:8) as solvent. Evaporation of the solvent gave 9.8 g of **49** (77%). ^{1}H NMR (DMSO-d_6): δ 1.30 (t, 3H), 4.30 (q, 2H), 6.90 (d, 1H, J = 8.4 Hz), 6.95 (d, 1H, J = 8.4 Hz), 7.25 (t, 1H, J = 8.4 Hz), 10.45 (br s, 1H).

Ethyl 2-Allyloxy-6-chlorobenzoate (50). Ethyl 2-hydroxy-6-chlorobenzoate **49** (9.8 g, 48.8 mmol) was dissolved in acetonitrile (250 mL), and allyl bromide (5.5 mL, 63 mmol) was added followed by 1,5,7-triazabicyclo[4.4.0]dec-5-ene (10.4 g, 73 mmol). The reaction mixture was stirred for 20 h, the solvent was evaporated, and the crude product was purified by flash chromatography using petroleum ether:ether (85:15) as solvent. Evaporation of the solvent gave 10.3 g of **50** (88%). ^{1}H NMR (DMSO-d_6): δ 1.30 (t, 3H), 4.35 (q, 2H), 4.65 (d, 2H), 5.25 (d, 1H), 5.40 (d, 1H), 6.0 (m, 1H), 7.15 (m, 2H), 7.45 (t, 1H, J = 8.3 Hz).

2-Hydroxy-3-allyl-6-chlorobenzoate (51). Ethyl 2-allyloxy-6-chlorobenzoate **50** (10.3 g, 42.8 mmol) was heated at 230 °C for 1 h and then purified by flash chromatography using dichloromethane:petroleum ether (20:80) as solvent. Evaporation of the solvent gave 7.3 g of **51** (71%). ^{1}H NMR (DMSO-d_6): δ 1.30 (t, 3H), 3.30 (m, 2H), 4.35 (q, 2H), 5.05 (m, 2H), 5.95 (m, 1H), 6.95 (d, 1H, J = 8.2 Hz), 7.15 (d, 1H, J = 8.2 Hz), 9.70 (br s, 1H).

6-Chloro-7-(ethoxycarbonyl)benzofuran (52). Two different batches of ethyl 2-hydroxy-3-allyl-6-chlorobenzoate **51** were combined (8 g, 33 mmol), dissolved in methanol, and cooled at −78 °C. Ozone was bubbled through the solution for 30 min. Dimethyl sulfide was added (13 mL) and the solution was left to reach ambient temperature. The solvent was evaporated, and ether and water were added. The ether phase was washed with water and brine, dried over magnesium sulfate, and filtered and the solvent was evaporated. Evaporation of the solvent gave the desired aldehyde, which was immediately suspended in phosphoric acid (85%, 35 mL), and the mixture was heated to 100 °C for 20 min. Upon cooling to room temperature the acid solution was diluted with water and extracted with ether. The ether phase was washed with water and brine, dried over magnesium sulfate, and filtered, and the solvent was evaporated. The crude product was purified by flash chromatography using dichloromethane: petroleum ether (3:7) as solvent. Evaporation of the solvent gave 5.9 g of **52** (79%). ^{1}H NMR (DMSO-d_6): δ 1.35 (t, 3H), 4.45 (q, 2H), 7.10 (d, 1H, J = 2.3 Hz), 7.45 (d, 1H, J = 8.5 Hz), 7.85 (d, 1H, J = 8.7 Hz), 8.15 (d, 1H, J = 2.3 Hz).

6-Chloro-7-carboxybenzofuran (53). 6-Chloro-7-(ethoxycarbonyl)benzofuran **52** (5.9 g, 26.1 mmol) was dissolved in methanol, KOH (12.7 mL, 35% in water) was added, and the reaction mixture refluxed for 1 h. The methanol was evaporated, water was added, and the pH was adjusted to 2 with 6 N HCl. The precipitate was collected by filtration, washed with water, and dried under vacuum over phosphorus pentoxide to give 4.6 g of **53** (90%). ^{1}H NMR (DMSO-d_6): δ 7.05 (d, 1H, J = 2.2 Hz), 7.40 (d, 1H, J = 8.2 Hz), 7.75 (d, 1H, J = 8.5 Hz), 8.10 (d, 1H, J = 2.0 Hz). MS m/z 196 [M]$^+$.

6-Chloro-7-aminobenzofuran (54). 6-Chloro-7-carboxybenzofuran **53** (1 g, 5 mmol), DPPA (2.2 mL, 10 mmol), triethylamine (1.4 mL, 10 mmol), and *tert*-butyl alcohol (2.7 mL) were heated to reflux for 18 h. Upon cooling to room temperature the mixture was poured into water and extracted with ethyl acetate. The organic phase was washed with water and brine, dried over magnesium sulfate, and filtered, and the solvent was evaporated. The resulting oil was purified by flash chromatography using neutral alumina and increasingly polar solvent mixtures starting with dichloromethane: petroleum ether, then dichloromethane, then dichloromethane: ethyl acetate (9:1), and ending with dichloromethane:ethyl acetate (8:2). Evaporation of the solvent gave a mixture of 6-chloro-7-aminobenzofuran as well as the desired amide. This mixture was treated with TFA (1.2 mL) in dichloromethane (15 mL) at 0 °C for 1 h. The solvent was evaporated, and the residue was taken up in a saturated solution of $NaHCO_3$ and extracted with ethyl acetate. The organic phase was dried over magnesium sulfate and filtered, and the solvent was evaporated. The resulting oil was purified by flash chromatography using dichloromethane:petroleum ether (25:75) as solvent. Evaporation of the solvent gave 0.376 g of **54** (45%). ^{1}H NMR (DMSO-d_6): δ 5.50 (br s, 2H), 6.85 (m, 2H), 7.10 (d, 1H, J = 8.4 Hz), 7.95 (d, 1H, J = 2.2 Hz). MS m/z 167 [M]$^+$.

***N*-(6-Chloro-1-benzofuran-7-yl)-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazolin-4-amine (11).** NaHMDS (0.592 mL, 0.59 mmol, 1 M solution in THF, Aldrich) was added to 6-chloro-7-amino-benzofuran **54** (0.099 g, 0.59 mmol) in solution in DMF (5 mL) under argon. After 30 min at room temperature, 4-chloro-6-methoxy-7-(3-morpholin-4-ylpropoxy)-quinazoline **34** (0.1 g, 0.3 mmol) was added. The reaction mixture was stirred for 3 h and then water was added followed by ethyl acetate extraction. The organic phase was washed with water and brine, dried over magnesium sulfate, and filtered, and the solvent was evaporated. The crude product was purified as described for **23** to give 0.105 g of **11** (67%). ^{1}H NMR (DMSO-d_6 and CF_3CO_2D): δ 2.35 (m, 2H), 3.15 (m, 2H), 3.35 (m, 2H), 3.55 (d, 2H), 3.75 (t, 2H), 4.0 (m, 2H), 4.05 (s, 3H), 4.35 (t, 2H), 7.15 (d, 1H, J = 2.2 Hz), 7.45 (s, 1H), 7.55 (d, 1H, J = 8.5 Hz), 7.80 (d, 1H, J = 8.3 Hz), 8.10 (d, 1H, J = 2.2 Hz), 8.25 (s, 1H), 8.80 (s, 1H). MS-ESI m/z 469 [MH]$^+$. Anal. ($C_{24}H_{25}ClN_4O_4$) C, H, N.

***N*-(2,3-Dihydro-1-benzofuran-7-yl)-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazolin-4-amine (12).** 2,3-Dihydro-1-benzofuran-7-amine[31] (0.072 g, 0.53 mmol) was reacted with 4-chloro-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazoline **34** (0.15 g, 0.44 mmol) using a procedure similar to the one described for the synthesis of **5** and purified as described for **23** to give 0.155 g of **12** (81%). ^{1}H NMR (DMSO-d_6 and CF_3CO_2D): δ 2.35 (m, 2H), 3.15 (m, 2H), 3.30 (m, 4H), 3.55 (d, 2H), 3.80 (t, 2H), 4.0 (s, 3H), 4.05 (m, 2H), 4.35 (t, 2H), 4.6 (t, 2H), 6.95 (t, 1H, J = 7.8 Hz), 7.20 (d, 1H, J = 7.8 Hz), 7.30 (d, 1H, J = 7.8 Hz), 7.40 (s, 1H), 8.20 (s, 1H), 8.80 (s, 1H). MS-ESI m/z 437 [MH]$^+$. Anal. ($C_{24}H_{28}N_4O_4$) C, H, N.

***cis*- and *trans*-2,3-Dichloro-2,3-dihydro-7-nitrobenzofuran (56).** 7-Nitrobenzofuran **55**[17] (1.2 g, 7.3 mmol) was dissolved in AcOH (12 mL), and chlorine was bubbled through the solution for 30 min. The temperature was maintained at 18 °C during the reaction. The AcOH was evaporated and the residue was purified by flash chromatography using ethyl acetate:petroleum ether (1:1). Evaporation of the solvent gave 0.77 g of **56** (45%). MS m/z 233 [M]$^+$.

3-Chloro-7-nitrobenzofuran (57). Two different batches of *cis*- and *trans*-2,3-dichloro-2,3-dihydro-7-nitrobenzofuran **56** (0.85 g, 3.6 mmol) were combined and dissolved in ethanol (35 mL). A solution of KOH in ethanol (45.5 mL, 36 mmol, 0.8M solution) was added and the mixture stirred for 1.25 h. The mixture was concentrated to 1:10, water was added, the pH adjusted to 2 with 6 N HCl, and the product was extracted with ether. The organic phase was washed with water and brine, dried over magnesium sulfate, and filtered, and the solvent was evaporated to give 0.7 g of **57** (98%) as a white solid. ^{1}H NMR (DMSO-d_6): δ 7.65 (t, 1H, J = 8.1 Hz), 8.15 (d, 1H, J = 8.1 Hz), 8.30 (d, 1H, J = 8.4 Hz), 8.65 (s, 1H). MS m/z 197 [M]$^+$.

3-Chloro-7-aminobenzofuran (58). A mixture of hydrazine hydrate (0.81 mL, 16.7 mmol), Raney nickel (0.16 g), and methanol (30 mL) was heated to 60 °C. 3-Chloro-7-nitrobenzofuran **57** (0.66 g, 3.3 mmol) in solution in methanol (25 mL) was added dropwise over 5 min. The reaction mixture was heated to reflux for 5 min and cooled to room temperature, and the catalyst was removed by filtration. The solvent was evaporated, and the residue was diluted with water and extracted with dichloromethane. The organic phase was washed with water, dried over magnesium sulfate, and filtered, and the solvent was evaporated. The resulting oil was purified by flash chromatography using ethyl acetate:petroleum ether (1:1) as solvent. Evaporation of the solvent gave 0.41 g of **58** (75%). ^{1}H NMR (DMSO-d_6): δ 5.50 (br s, 2H), 6.65 (d, 1H, J = 7.7 Hz), 6.75 (d, 1H, J = 7.7 Hz), 7.05 (t, 1H, J = 7.7 Hz), 8.20 (s, 1H).

***N*-(3-Chloro-1-benzofuran-7-yl)-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazolin-4-amine (13).** 3-Chloro-7-aminobenzofuran **58** (0.062 g, 0.37 mmol) was reacted with 4-chloro-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazoline **34** (0.1 g, 0.3 mmol) using a procedure similar to the one described for the synthesis of **5** and purified as described for **23** to give 0.103 g of **13** (76%). ^{1}H NMR (DMSO-d_6 and CF_3CO_2D): δ 2.3 (m, 2H), 3.15 (t, 2H), 3.35 (t, 2H), 3.55 (d, 2H), 3.75 (t, 2H), 4.0 (s, 3H), 4.05 (m, 2H), 4.35 (t, 2H), 7.30 (s, 1H), 7.55 (t, 1H, J = 7.8 Hz), 7.60 (dd, 1H, J_1 = 7.8, J_2 = 1.2 Hz), 7.70 (dd, 1H, J_1 = 7.8, J_2 = 1.2 Hz), 8.20 (s, 1H), 8.40 (s, 1H), 8.85 (s, 1H). MS-ESI m/z 469 $[MH]^+$. Anal. ($C_{24}H_{25}ClN_4O_4$) C, H, N.

***N*-(1H-indol-4-yl)-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazolin-4-amine (14).** 4-Aminoindole (0.041 g, 31 mmol) was reacted with 4-chloro-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazoline **34** (0.08 g, 0.24 mmol) using a procedure similar to the one described for the synthesis of **5** and purified as described for **23** to give 0.075 g of **14** (72%). ^{1}H NMR (DMSO-d_6 and CF_3CO_2D): δ 2.35 (m, 2H), 3.15 (m, 2H), 3.35 (m, 2H), 3.55 (t, 2H), 3.80 (m, 2H), 4.0 (m, 2H), 4.05 (s, 3H), 4.35 (t, 2H), 6.35 (d, 1H), 7.15 (d, 1H, J = 7.8 Hz), 7.20 (t, 1H, J = 7.8 Hz), 7.40 (s, 1H), 7.42 (s, 1H), 7.5 (d, 1H, J = 7.8 Hz), 8.30 (s, 1H), 8.75 (s, 1H). MS-ESI m/z 434 $[MH]^+$. Anal. ($C_{24}H_{27}N_5O_3$) C, H, N.

1-Methyl-4-nitroindole (60). Sodium hydride (0.135 g) was added to a solution of 4-nitroindole **59** (0.5 g) in DMF (10 mL) cooled in an ice bath. Methyl iodide (0.19 mL) was added dropwise and the reaction stirred for 2 h. The solvent was removed under vacuum and the residue partitioned between ethyl acetate and water. The organic phase was dried over magnesium sulfate and the solvent evaporated to give 0.49 g of **60** (98%). ^{1}H NMR (DMSO-d_6): δ 3.80 (s, 3H), 7.00 (d, 1H), 7.34 (dd, 1H), 7.73 (d, 1H), 7.96 (d, 1H), 8.06 (d, 1H).

4-Amino-1-methylindole (61). 1-Methyl-4-nitroindole **60** (0.49 g) and 10% Pd/C (49 mg) in EtOH (20 mL) were stirred under an atmosphere of hydrogen overnight. The catalyst was filtered and concentrated under vacuum to give 0.43 g of **61** (99%) as an oil. ^{1}H NMR (DMSO-d_6): δ 3.63 (s, 3H), 5.10 (br s, 2H), 6.16 (dd, 1H), 6.23 (d, 1H), 6.55 (d, 1H), 6.80 (t, 1H), 7.00 (d, 1H).

6-Methoxy-*N*-(1-methyl-1H-indol-4-yl)-7-(3-morpholin-4-ylpropoxy)quinazolin-4-amine (15). 4-Chloro-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazoline **34** (0.08 g, 0.24 mmol) was reacted with 4-amino-1-methylindole **61** (0.045 g, 0.31 mmol) using a procedure similar to the one described for the synthesis of **5** and purified as described for **23** to give 0.057 g of **15** (53%). ^{1}H NMR (DMSO-d_6 and CF_3CO_2D): δ 2.35 (m, 2H), 3.15 (m, 2H), 3.35 (t, 2H), 3.55 (d, 1H), 3.8 (m, 2H), 3.85 (s, 3H), 4.0 (m, 2H), 4.05 (s, 3H), 4.35 (t, 2H), 7.20 (d, 1H, J = 7.8 Hz), 7.30 (t, 1H, J = 7.8 Hz), 7.38 (s, 1H), 7.41 (s, 1H), 7.45 (d, 1H, J = 7.8 Hz), 8.30 (s, 1H), 8.75 (s, 1H). MS-ESI m/z 448 $[MH]^+$. Anal. ($C_{25}H_{29}N_5O_3 \cdot 0.01CH_2Cl_2 \cdot 0.07EtOAc \cdot 0.2H_2O$) C, H, N.

***N*-(1H-indol-7-yl)-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazolin-4-amine (16).** 4-Chloro-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazoline **34** (0.15 g, 0.3 mmol) was reacted with 7-aminoindole (0.073 g, 0.56 mmol) using a procedure similar to the one described for the synthesis of **5** and purified as described for **23** to give 0.18 g (93%) of **16**. ^{1}H NMR (DMSO-d_6 and CF_3CO_2D): δ 2.35 (m, 2H), 3.15 (m, 2H), 3.35 (m, 2H), 3.55 (d, 2H), 3.8 (t, 2H), 4.0 (m, 2H), 4.05 (s, 3H), 4.35 (t, 2H), 6.55 (d, 1H, J = 3.2 Hz), 7.12 (t, 1H, J = 7.6 Hz), 7.18 (d, 1H, J = 7.6 Hz), 7.35 (d, 1H, J = 3.2 Hz), 7.4 (s, 1H), 7.6 (d, 1H, J = 7.6 Hz), 8.3 (s, 1H), 8.75 (s, 1H). MS-ESI m/z 434 $[MH]^+$.

***N*-(3-Chloro-1H-indol-7-yl)-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazolin-4-amine (17).** 4-Chloro-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazoline **34** (0.15 g, 0.44 mmol) was reacted with 7-amino-3-chloroindole[32] (0.110 g, 0.55 mmol) using a procedure similar to the one described for **5** and purified as described for **23** to give 0.166 g of **17** (81%). ^{1}H NMR (DMSO-d_6 and CF_3CO_2D): δ 2.35 (m, 2H), 3.15 (m, 2H), 3.35 (m, 2H), 3.55 (d, 2H), 3.8 (t, 2H), 4.0 (m, 2H), 4.05 (s, 3H), 4.35 (t, 2H), 7.25 (m, 2H), 7.4 (s, 1H), 7.55 (m, 2H), 8.25 (s, 1H), 8.75 (s, 1H). MS-ESI m/z 468 $[MH]^+$. Anal. ($C_{24}H_{26}ClN_5O_3 \cdot 1.37H_2O$) C, H, N.

Methyl 2,3-Dihydroxybenzoate (63). A mixture of 2,3-dihydroxybenzoic acid **62** (5 g, 32 mmol), methanol (50 mL), and concentrated sulfuric acid (10 drops) was stirred and heated at 60 °C for 24 h. The mixture was evaporated and the residue was taken up in ethyl acetate. The organic solution was washed with a saturated solution of sodium bicarbonate, dried over magnesium sulfate, and evaporated to give 2.19 g of **63** (40%). ^{1}H NMR ($CDCl_3$): δ 3.95 (s, 3H), 5.7 (s, 1H), 6.8 (t, 1H, J = 8.1 Hz), 7.15 (dd, 1H, J_1 = 8.1, J_2 = 1.5 Hz), 7.25 (s, 1H), 7.35 (dd, 1H, J_1 = 8.1, J_2 = 1.5 Hz).

Methyl 2,3-Methylenedioxybenzoate (64). After repetition of the previous reaction, a mixture of methyl 2,3-dihydroxybenzoate **63** (2.8 g, 16 mmol), potassium fluoride (4.8 g, 83 mmol), and DMF (45 mL) was stirred at ambient temperature for 30 min. Dibromomethane (1.28 mL, 18 mmol) was added and the mixture was heated to 120 °C for 3 h. The mixture was cooled to ambient temperature, poured into water, and extracted with diethyl ether. The organic phase was washed with water and then brine, dried over magnesium sulfate, and evaporated. The residue was purified by column chromatography using a 9:1 mixture of petroleum ether (bp 40−60 °C) and ethyl acetate as eluent. Evaporation of the solvents gave 2.3 g of **64** (80%). ^{1}H NMR ($CDCl_3$): δ 3.95 (s, 3H), 6.1 (s, 2H), 6.85 (t, 1H, J = 8.1 Hz), 7.0 (dd, 1H, J_1 = 8.1, J_2 = 1.5 Hz), 7.45 (dd, 1H, J_1 = 8.1, J_2 = 1.5 Hz).

2,3-Methylenedioxybenzoic Acid (65). Methyl 2,3-methylenedioxybenzoate **64** (2.8 g, 15 mmol), a 2 N aqueous potassium hydroxide solution (15.5 mL), and methanol (40 mL) were stirred at ambient temperature for 2 h. The solution was concentrated to about one-quarter of the original volume and cooled in an ice bath. The mixture was acidified to pH 3.5 by the addition of 2 N HCl. The resultant precipitate was collected by filtration, washed in turn with water and diethyl ether, and dried under vacuum to give 1.87 g of **65** (73%). ^{1}H NMR (DMSO-d_6): δ 6.1 (s, 2H), 6.9 (t, 1H, J = 7.9 Hz), 7.15 (dd, 1H, J_1 = 1.1, J_2 = 7.9 Hz), 7.3 (dd, 1H, J_1 = 1.1, J_2 = 7.9 Hz), 13.0 (br s, 1H).

***tert*-Butyl 2,3-Methylenedioxyphenylcarbamate (66).** 2,3-Methylenedioxybenzoic acid **65** (1.8 g, 10.8 mmol) was suspended in anhydrous dioxane (30 mL), and DPPA (2.45 mL, 11 mmol), triethylamine (1.6 mL, 11 mmol), and *tert*-butyl alcohol (9 mL) were added. The mixture was heated to reflux for 5 h. The mixture was cooled to ambient temperature, concentrated by evaporation, and diluted with ethyl acetate. The organic phase was washed in turn with a 5% aqueous citric acid solution, water, an aqueous sodium bicarbonate solution, and brine and dried over magnesium sulfate. The solvent was evaporated and the residue was purified by column chromatography on silica using a 19:1 mixture of petroleum ether (bp 40−60 °C) and ethyl acetate as eluent. Evaporation of the solvent gave 1.98 g of **66** (77%) as a solid. ^{1}H NMR ($CDCl_3$): δ 1.55 (s, 9H), 5.95 (s, 2H), 6.4 (br s, 1H), 6.55 (dd, 1H, J_1 = 8.1, J_2 = 1.1 Hz), 6.8 (t, 1H, J = 8.1 Hz), 7.45 (d, 1H, J = 8.1 Hz).

2,3-Methylenedioxyaniline (67). HCl (5 N, 30 mL) was added to a solution of *tert*-butyl 2,3-methylenedioxyphenylcarbamate **66** (1.9 g, 8 mmol) in ethanol (38 mL), and the reaction mixture was stirred at ambient temperature for 20 h. The ethanol was evaporated and the residual aqueous phase was washed with diethyl ether and neutralized to pH 7 by the addition of solid potassium hydroxide. The resultant mixture was filtered and the aqueous phase was extracted with diethyl ether. The organic phase was washed with brine and dried over magnesium sulfate, and the solvent was evaporated to give 1.0 g of **67** (91%). ^{1}H NMR ($CDCl_3$): δ 3.0 (br s, 2H), 5.9 (s, 2H), 6.3 (m, 2H), 7.25 (t, 1H, J = 8.1 Hz).

***N*-(1,3-Benzodioxol-4-yl)-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazolin-4-amine (18)** was prepared as described for the synthesis of **5**. 4-Chloro-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazoline **34** (0.1 g, 0.3 mmol) was reacted with 2,3-methylenedioxyaniline **67** (0.048 g, 0.36

mmol) to give 0.138 g of **18** (90%) as a dihydrochloride salt. ^{1}H NMR (DMSO-d_6 and CF_3CO_2D): δ 2.35 (m, 2H), 2.5 (m, 2H), 3.15 (m, 2H), 3.3 (m, 2H), 3.55 (d, 2H), 3.8 (t, 2H), 4.05 (s, 3H), 4.35 (t, 2H), 6.1 (s, 2H), 7.0 (m, 3H), 7.4 (s, 1H), 8.2 (s, 1H), 8.85 (s, 1H). MS-ESI *m*/*z* 439. Anal. ($C_{23}H_{26}N_4O_5$·2HCl·0.5H_2O) C, H, N.

5-Chloro-1,3-benzodioxole-4-carboxylic Acid (69). Diisopropylamine (4.92 mL, 35 mmol) in THF (100 mL) was cooled to −78 °C, *n*-butyl lithium (14 mL, 35 mmol, 2.5M) was added dropwise, and 15 min later 5-chloro-1,3-benzodioxole **68** (3.73 mL, 32 mmol) was added dropwise at the same temperature. Thirty minutes later, dry CO_2 was bubbled into the reaction mixture for another 30 min. The solution was left to reach ambient temperature, and 1 h later the reaction mixture was quenched with water. The solvent was evaporated off and the solution acidified to pH 2 with 2 N HCl. The solid was collected by filtration and washed with water and ether to give 5.4 g of **69** (84%). ^{1}H NMR (DMSO-d_6): δ 6.15 (s, 2H), 6.95 (d, 1H, J = 8.2 Hz), 7.0 (d, 1H, J = 8.5 Hz), 13.7 (br s, 1H). MS-ESI *m*/*z* 199 [M − H]$^-$.

***tert*-Butyl 5-Chloro-1,3-benzodioxol-4-ylcarbamate (70).** 5-Chloro-1,3-benzodioxole-4-carboxylic acid **69** (1 g, 5 mmol) was dissolved in 1,4-dioxane (15 mL). Anhydrous *tert*-butyl alcohol (4 mL), DPPA (1.12 mL, 5.2 mmol), and triethylamine (0.73 mL, 5.2 mmol) were added in turn under argon, and the mixture was heated to 100 °C for 4 h. The solvent was evaporated, and the residue was taken up in ethyl acetate and washed with 5% citric acid, water, saturated bicarbonate solution, and brine. The solid was removed by filtration and the solution dried over magnesium sulfate, filtered, and evaporated. The crude product was purified by flash chromatography using petroleum ether:ethyl acetate (9:1) as eluent and gave 1.1 g of **70** (81%). ^{1}H NMR (DMSO-d_6): δ 1.45 (s, 9H), 6.1 (s, 2H), 6.85 (d, 1H, J = 8.4 Hz), 6.95 (d, 1H, J = 8.4 Hz), 8.75 (s, 1H).

5-Chloro-1,3-benzodioxol-4-amine (71). *tert*-Butyl 5-chloro-1,3-benzodioxol-4-yl carbamate **70** (1.1 g, 4.0 mmol) was dissolved in dichloromethane (20 mL), TFA (6 mL) was added, and the solution was stirred for 3 h at room temperature. The solvent was evaporated off and the residue resolubilized in ethyl acetate. Water was added and the pH adjusted to 6.5 with a saturated solution of sodium bicarbonate. The organic phase was washed with brine, dried over magnesium sulfate, and filtered, and the solvent was evaporated to give 0.642 g of **70** (93%). ^{1}H NMR (DMSO-d_6): δ 5.15 (s, 2H), 6.0 (s, 2H), 6.25 (d, 1H, J = 8.4 Hz), 6.75 (d, 1H, J = 8.5 Hz).

***N*-(5-Chloro-1,3-benzodioxol-4-yl)-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazolin-4-amine (19)** was prepared as described for the synthesis of **1**. 4-Chloro-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazoline **34** (0.1 g, 0.3 mmol) was reacted with 5-chloro-1,3-benzodioxol-4-amine **71** (0.056 g, 0.32 mmol) to give 0.072 g of **19** (51%). ^{1}H NMR (DMSO-d_6 and CF_3CO_2D): δ 2.35 (m, 2H), 3.15 (m, 2H), 3.35 (m, 2H), 3.5 (m, 2H), 3.8 (m, 2H), 4.0 (m, 2H), 4.05 (s, 3H), 4.35 (m, 2H), 6.15 (s, 2H), 7.05 (d, 1H), 7.15 (d, 1H), 7.45 (s, 1H), 8.25 (s, 1H), 8.9 (s, 1H). MS-ESI *m*/*z* 471 and 473 [MH]$^+$. Anal. ($C_{23}H_{25}ClN_4O_5$·0.6H_2O) C, H, N.

5-Bromo-1,3-benzodioxole-4-carboxylic acid (73) was prepared as described for the synthesis of **69**. 5-Bromo-1,3-benzodioxole **72** (1 g, 5 mmol) gave 0.88 g of **73** (72%). ^{1}H NMR (DMSO-d_6 and CF_3CO_2D): δ 6.15 (s, 2H), 6.95 (d, 1H, J = 8.3 Hz), 7.1 (d, 1H, J = 8.3 Hz). MS-ESI *m*/*z* 243 [M − H]$^-$.

***tert*-Butyl 5-bromo-1,3-benzodioxol-4-ylcarbamate (74)** was prepared as described for the synthesis of **70**. 5-Bromo-1,3-benzodioxole-4-carboxylic acid **73** (0.88 g, 3.6 mmol) gave 1.1 g of **74** (96%). ^{1}H NMR (DMSO-d_6): δ 1.45 (s, 9H), 6.1 (s, 2H), 6.80 (d, 1H, J = 8.4 Hz), 7.1 (d, 1H, J = 8.4 Hz), 8.70 (s, 1H).

5-Bromo-1,3-benzodioxol-4-amine (75) was prepared as described for the synthesis of **71**. *tert*-butyl 5-bromo-1,3-benzodioxol-4-ylcarbamate **74** (1.1 g, 3.4 mmol) gave 0.6 g of **75** (81%). ^{1}H NMR (DMSO-d_6): δ 5.05 (s, 2H), 6.0 (s, 2H), 6.25 (d, 1H, J = 8.4 Hz), 6.9 (d, 1H, J = 8.4 Hz). MS-ESI *m*/*z* 216 and 218 [MH]$^+$.

***N*-(5-Bromo-1,3-benzodioxol-4-yl)-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazolin-4-amine (20)** was prepared as described for the synthesis of **1**. 4-Chloro-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazoline **34** (0.1 g, 0.3 mmol) was reacted with 5-bromo-1,3-benzodioxol-4-amine **75** (0.070 g, 0.32 mmol) to give 0.45 g of **20** (29%). ^{1}H NMR (DMSO-d_6 and CF_3CO_2D): δ 2.3 (m, 2H), 3.15 (m, 2H), 3.35 (t, 2H), 3.55 (d, 2H), 3.7 (t, 2H), 4.0 (s, 3H), 4.05 (m, 2H), 4.35 (t, 2H), 6.15 (s, 2H), 7.05 (d, 1H, J = 8.3 Hz), 7.3 (d, 1H, J = 8.3 Hz), 7.4 (s, 1H), 8.15 (s, 1H), 8.9 (s, 1H). MS-ESI *m*/*z* 517 [MH]$^+$. Anal. ($C_{23}H_{25}BrN_4O_5$·0.3H_2O) C, H, N.

6-Fluoro-2,3-dimethoxybenzoic Acid (77). In a flask containing diisopropylamine (49.6 g, 0.49 mol) in dry THF (1200 mL) cooled at −78 °C under nitrogen *n*-butyl lithium (196 mL, 2.5N solution in hexane, 0.49 mol) was added dropwise, keeping the internal temperature below −40 °C. The solution was stirred for an additional 20 min and then cooled to −60 °C. 1-Fluoro-3,4-dimethoxybenzene **76** (69.5 g, 0.445 mol) was added dropwise, the mixture was stirred at −60 °C for 1 h, and dry carbon dioxide was bubbled through the solution for 30 min. The temperature was left to rise to −5 °C and water was slowly added. The solvents were evaporated under vacuum, the residue was acidified to pH 1.5 with 2 N HCl and extracted twice with ethyl acetate. The organic layer was washed with brine and concentrated. The thick solid was triturated with pentane, filtered, washed with more pentane, and dried under vacuum to give 57.6 g of **77** (64%). ^{1}H NMR (DMSO-d_6): δ 3.46 (s, 3H), 3.50 (s, 3H), 6.53 (dd, 1H, $J_1 \sim J_2$ = 9 Hz), 6.54 (dd, 1H, J_1 = 9, J_2 = 5.3 Hz).

6-Fluoro-2,3-dihydroxybenzoic Acid (78). To a solution of acetic acid (750 mL) and aqueous hydrobromic acid (47%, 850 mL) heated at 130 °C (oil bath) was added 6-fluoro-2,3-dimethoxybenzoic acid **77** (57.6 g, 0.288 mol). The mixture was heated at 140 °C for 1 h. After cooling, the mixture was diluted with cold water (2.5 L) and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over magnesium sulfate, and evaporated. The residue was azeotroped with toluene to give 61 g of **78** (quant.). ^{1}H NMR (DMSO-d_6): δ 6.55 (dd, 1H, J_1 = 10, J_2 = 8.6 Hz), 6.9 (dd, 1H, J_1 = 8.6, J_2 = 5.6 Hz), 9.3 (br s, 2H).

Methyl 6-Fluoro-2,3-dihydroxybenzoate (79). Thionyl chloride (62.7 g, 0.527 mmol) was added dropwise to a solution of 6-fluoro-2,3-dihydroxybenzoic acid **78** (60.5 g, 0.351 mol) in methanol (520 mL) cooled at 0 °C. The temperature was allowed to increase to room temperature and the mixture was then heated at 70 °C for 18 h. The mixture was cooled and the solvents evaporated under vacuum. The residue was dissolved in ethyl acetate, washed with saturated aqueous sodium bicarbonate and brine, and dried over magnesium sulfate, and the solvent was evaporated. Purification over a short pad of silica using dichloromethane as eluent gave 39 g of **79** (60%). ^{1}H NMR ($CDCl_3$): δ 4.0 (s, 3H), 5.55 (s, 1H), 6.55 (dd, 1H, J_1 = 10.5, J_2 = 9 Hz), 7.0 (dd, 1H, J_1 = 9, J_2 = 5 Hz), 11.45 (s, 1H).

Methyl 5-Fluoro-1,3-benzodioxole-4-carboxylate (80). Bromochloromethane (40 g, 0.306 mol) and cesium carbonate (100 g, 0.306 mol) were added to a solution of methyl 6-fluoro-2,3-dihydroxybenzoate **79** (38 g, 0.204 mol) in dry DMF (450 mL) under nitrogen. The mixture was heated at 110 °C for 1.5 h. The mixture was cooled, the solids were filtered off, and the solvents were evaporated under vacuum. The residue was dissolved in ethyl acetate, washed with water, and dried over magnesium sulfate. After evaporation of the solvent, the residue was purified by flash chromatography using ethyl acetate:petroleum ether (2:8 up to 3:7) as eluent. Evaporation of the solvent gave 32.2 g of **80** (80%). ^{1}H NMR ($CDCl_3$): δ 3.92 (s, 3H), 6.09 (s, 2H), 6.57 (dd, 1H, J_1 = 11.4, J_2 = 8.8 Hz), 6.83 (dd, 1H, J_1 = 8.8, J_2 = 4 Hz).

5-Fluoro-1,3-benzodioxole-4-carboxylic Acid (81). An aqueous solution of potassium hydroxyde (2 N, 162 mL, 0.325 mmol) was added to a suspension of methyl 5-fluoro-1,3-benzodioxole-4-carboxylate **80** (32.2 g, 0.162 mol) in methanol (415 mL). The mixture was stirred for 3 h, and the solvents were evaporated under vacuum. Water was added and the pH

was adjusted to 2. The mixture was extracted with ethyl acetate, and the organic layer was washed with brine, dried over magnesium sulfate, and evaporated to give 29.1 g of **81** (97%). ^{1}H NMR ($CDCl_3$): δ 6.15 (s, 2H), 6.70 (dd, 1H, J_1 = 11.3, J_2 = 8.6 Hz), 7.05 (dd, 1H, J_1 = 8.6, J_2 = 4.2 Hz).

***tert*-Butyl 5-Fluoro-1,3-benzodioxol-4-ylcarbamate (82).** Dry *tert*-butyl alcohol (123 mL), triethylamine (16.7 g, 0.65 mol), and DPPA (45.5 g, 0.165 mol) were added to a solution of 5-fluoro-1,3-benzodioxole-4-carboxylic acid **81** (29 g, 0.157 mol) in dioxane (430 mL) under nitrogen. The mixture was heated at 100 °C for 4.5 h. Upon cooling, the cloudy mixture was filtered. The filtrate was evaporated under vacuum, diluted in ethyl acetate, washed with a 5% aqueous citric acid, a 5% aqueous sodium bicarbonate, water, and brine, dried over magnesium sulfate, and concentrated under vacuum to give 37.6 g of **82** (93%). ^{1}H NMR ($CDCl_3$): δ 1.5 (s, 9H), 5.95 (br s, 1H), 6.0 (s, 2H), 6.55 (m, 2H).

5-Fluoro-1,3-benzodioxol-4-amine (83). Trifluoroacetic acid (150 mL) was added to a solution of *tert*-butyl 5-fluoro-1,3-benzodioxol-4-ylcarbamate **82** (37.5 g, 0.147 mol) in dichloromethane (550 mL). The mixture was stirred for 3 h. After evaporation of the solvents, the residue was dissolved in ethyl acetate, washed with 5% aqueous sodium bicarbonate and brine, dried over magnesium sulfate, and concentrated. The residue was chromatographed on silica gel using dichloromethane as solvent to give 21.6 g of **83** (94%). ^{1}H NMR ($CDCl_3$): δ 3.60 (m, 2H), 5.95 (s, 2H), 6.20 (dd, 1H, J_1 = 8.3, J_2 = 3.5 Hz), 6.50 (dd, 1H, J_1 = 11.3, J_2 = 8.3 Hz).

***N*-(5-Fluoro-1,3-benzodioxol-4-yl)-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazolin-4-amine (21)** was prepared as described for the synthesis of **1**. 4-Chloro-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazoline **34** (0.2 g, 0.6 mmol) was reacted with 5-fluoro-1,3-benzodioxol-4-amine **83** (0.1 g, 0.65 mmol) to give 0.214 g of **21** (79%). ^{1}H NMR (DMSO-d_6 and CF_3CO_2D): δ 2.3 (m, 2H), 3.15 (m, 2H), 3.35 (m, 2H), 3.45 (m, 2H), 3.75 (t, 2H), 4.0 (s, 3H), 4.05 (m, 2H), 4.35 (t, 2H), 6.15 (s, 2H), 6.90 (dd, 1H, J_1 = 8.5, J_2 = 10.3 Hz), 7.0 (dd, 1H, J_1 = 8.5, J_2 = 4.4 Hz), 7.40 (s, 1H), 8.15 (s, 1H), 8.90 (s, 1H). MS-ESI *m*/*z* 455 $[M - H]^-$. Anal. ($C_{23}H_{25}FN_4O_5 \cdot 0.4H_2O$) C, H, N.

***N*-(2,2-Difluoro-1,3-benzodioxol-4-yl)-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazolin-4-amine (22)** was prepared as described for the synthesis of **1** and purified as described for **23**. 4-Chloro-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazoline **34** (0.15 g, 0.44 mmol) was reacted with 2,2-difluoro-1,3-benzodioxol-4-ylamine (0.092 g, 0.53 mmol) to give 0.10 g (48%) of **22**. ^{1}H NMR (DMSO-d_6 and CF_3CO_2D): δ 2.35 (m, 2H), 3.15 (m, 2H), 3.35 (m, 2H), 3.55 (d, 2H), 3.8 (t, 2H), 3.9 (m, 2H), 4.0 (s, 3H), 4.35 (t, 2H), 7.35 (m, 2H), 7.45 (m, 2H), 8.3 (s, 1H), 8.95 (s, 1H). MS-ESI *m*/*z* 475 $[MH]^+$. Anal. ($C_{23}H_{24}F_2N_4O_5$) C, H, N.

***N*-(1,3-Benzodioxol-5-yl)-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazolin-4-amine (23)** was prepared as described for the synthesis of **1**. 4-Chloro-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazoline **34** (0.074 g, 0.22 mmol) was reacted with 3,4-(methylenedioxy)aniline (0.032 g, 0.24 mmol) and purified by preparative HPLC/MS on a Waters/ZMD Micromass with a Beta Basic Hypercil 21 × 100 mm 5 μm column using an ammonium carbonate (2 g/L) aqueous solution:acetonitrile solvent gradient (8:2 up to 100% acetonitrile over a 7.5 min period, 25 mL/min). The solvents were evaporated under vacuum, the residue was dissolved into 1 mL of dichloromethane:methanol (8:2), and 10 mL of ether was added to precipitate the product, which was then collected by filtration and dried in a vacuum oven at 50 °C to give 0.082 g of **23** (76%). MS-ESI *m*/*z* 439 $[MH]^+$. Anal. ($C_{23}H_{25}N_4O_5 \cdot 0.75H_2O$) C, H, N.

***N*-(2,3-Dihydro-1,4-benzodioxin-5-yl)-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazolin-4-amine (24)** was prepared as described for the synthesis of **1**. 4-Chloro-6-methoxy-7-(3-morpholin-4-ylpropoxy)quinazoline **34** (0.1 g, 0.3 mmol) was reacted with 2,3-dihydro-1,4-benzodioxin-5-amine hydrochloride (0.068 g, 0.36 mmol) to give 0.075 g of **24** (55%). ^{1}H NMR (DMSO-d_6): δ 1.95 (m, 2H), 2.35 (m, 4H), 2.45 (t, 2H), 3.60 (m, 4H), 3.95 (s, 3H), 4.15 (t, 2H), 4.25 (m, 4H), 6.80 (dd, 1H, J_1 = 1.7, J_2 = 8.4 Hz), 6.85 (t, 1H, J = 8.0 Hz), 7.05 (dd, 1H, J_1 = 1.7, J_2 = 8.4 Hz), 7.15 (s, 1H), 7.80 (s, 1H), 8.20 (s, 1H), 9.15 (s, 1H). MS-ESI *m*/*z* 453 $[MH]^+$. Anal. ($C_{24}H_{28}N_4O_5 \cdot 0.5H_2O$) C, H, N.

7-Benzyloxy-3,4-dihydroquinazolin-4-one (85). Sodium metal (4.4 g, 191 mmol) was added to benzyl alcohol (100 mL) and the resultant mixture was stirred at ambient temperature for 30 min and then heated to 80 °C for 1 h. The mixture was cooled to 40 °C and 7-fluoro-3,4-dihydroquinazolin-4-one **84** (7.8 g, 47.6 mmol) was added. The reaction mixture was stirred at 130 °C for 4 h. The mixture was allowed to cool and was stirred for a further 18 h. The solution was quenched with water (800 mL) and acidified to pH 3 by the addition of concentrated HCl. The resultant precipitate was collected by filtration, washed with water and diethyl ether, and dried under vacuum for 4 h at 60 °C to give 7.02 g of **85** (59%).

7-Benzyloxy-3,4-dihydroquinazolin-4-thione (86). 7-Benzyloxy-3,4-dihydroquinazolin-4-one **85** (7.0 g, 28 mmol), phosphorus pentasulfide (12.5 g, 56 mmol), and pyridine (350 mL) were stirred under reflux for 8 h. After cooling, the mixture was poured into water (1 L), and the precipitate was collected by filtration and washed with water. The solid was dissolved in 6 N NaOH and the solution was filtered. The filtrate was acidified to pH 2 with 6 N HCl. The resultant precipitate was collected, washed with water, and dried under vacuum at 60 °C to give 7.42 g of **86** (99%). ^{1}H NMR (DMSO-d_6): 5.32 (s, 2H), 7.25 (d, 1H), 7.32 (m, 1H), 7.4 (m, 1H), 7.45 (t, 2H), 7.55 (d, 2H), 8.15 (s, 1H), 8.5 (d, 1H).

7-Benzyloxy-4-methylthioquinazoline (87). 7-Benzyloxy-3,4-dihydroquinazolin-4-thione **86** (3.45 g, 12.9 mmol) was dissolved in THF (13 mL) and 1 N NaOH (25.7 mL, 25.7 mmol) was added. Methyl iodide (0.97 mL, 15.6 mmol) was added dropwise and the mixture was stirred at ambient temperature for 30 min. The mixture was neutralized by the addition of 2 N HCl and the mixture was diluted by the addition of water. The resultant solid was collected, washed with water, and dried under vacuum to give 3.3 g of **87** (90%). ^{1}H NMR (DMSO-d_6): δ 2.67 (s, 3H), 5.32 (s, 2H), 7.3−7.45 (m, 5H), 7.5 (d, 2H), 8.05 (d, 1H), 8.9 (s, 1H).

7-Hydroxy-4-methylthioquinazoline (88). 7-Benzyloxy-4-methylthioquinazoline **87** (3 g, 10.6 mmol) and TFA (30 mL) were heated to reflux for 5 h. The acid was evaporated and the residue was suspended in water. Solid sodium bicarbonate was added until complete dissolution. The solution was extracted with diethyl ether and the aqueous layer was acidified to pH 2 by the addition of 2 N HCl. The obtained precipitate was collected by filtration, washed with water and diethyl ether, and dried under vacuum to give 2 g of **88** (97%). ^{1}H NMR (DMSO-d_6): δ 2.7 (s, 3H), 7.15 (d, 1H), 7.25 (m, 1H), 8.0 (d, 1H), 8.9 (s, 1H).

4-Methylthio-7-(3-morpholinopropoxy)quinazoline (89). DEAD (2.92 g, 17 mmol) was added dropwise to a stirred mixture of 7-hydroxy-4-methylthioquinazoline **88** (2.5 g, 12.9 mmol), 4-(3-hydroxypropyl)morpholine[19] (2.47 g, 17 mmol), triphenylphosphine (4.45 g, 17 mmol), and methylene chloride (65 mL). The reaction mixture was stirred at ambient temperature for 1 h. The mixture was evaporated and the residue was partitioned between a 1:1 mixture of ethyl acetate and diethyl ether and 1 N HCl. The aqueous layer was adjusted to pH 9 with solid sodium bicarbonate and extracted with methylene chloride. The organic layer was separated, washed with water and brine, dried over magnesium sulfate, and evaporated. The residue was purified by column chromatography on silica using increasingly polar mixtures of methylene chloride, ethyl acetate, and methanol (from 6:3:1 to 5:3:2 to 75:0:25) as eluent. Evaporation of the solvents gave 2.03 g of **89** (49%). ^{1}H NMR (DMSO-d_6 and CF_3CO_2D): δ 2.2−2.3 (m, 2H), 2.7 (s, 3H), 3.05−3.25 (m, 2H), 3.35 (t, 2H), 3.55 (d, 2H), 3.7 (t, 2H), 4.05 (d, 2H), 4.32 (t, 2H), 7.38 (d, 1H), 7.4 (s, 1H), 8.1 (d, 1H), 9.05 (d, 1H). MS-ESI *m*/*z* 320 $[MH]^+$.

***N*-(5-Chloro-1,3-benzodioxol-4-yl)-7-(3-morpholin-4-ylpropoxy)quinazolin-4-amine (25).** 5-Chloro-1,3-benzodioxol-4-amine **71** (0.295 g, 1.72 mmol) was reacted with 4-methylthio-

7-(3-morpholinopropoxy)quinazoline **89** (0.5 g, 1.57 mmol) using a procedure similar to the one described for **11**. The crude product was purified by flash chromatography using increasingly polar solvent mixtures of dichloromethane:methanol (95:5 up to 90:10). Evaporation of the solvent gave 0.38 g of **25** (55%). ^{1}H NMR ($CDCl_3$): δ 1.80 (m, 2H), 2.35 (m, 4H), 2.45 (t, 2H), 3.6 (m, 4H), 4.05 (t, 2H), 5.90 (s, 2H), 6.60 (d, 1H, J = 8.4 Hz), 6.85 (d, 1H, J = 8.4 Hz), 6.90 (s, 1H), 7.05 (dd, 1H, J_1 = 9.4, J_2 = 2.6 Hz), 7.10 (d, 1H, J = 2.6 Hz), 7.70 (d, 1H, J = 9.4 Hz), 8.50 (s, 1H). MS-ESI m/z 443 and 445 $[MH]^+$. Anal. ($C_{22}H_{23}ClN_4O_4$) C, H, N.

7-Methoxy-6-(3-morpholinopropoxy)-3,4-dihydroquinazolin-4-one (91). A mixture of 4-(3-chloro-4-fluoroanilino)-7-methoxy-6-(3-morpholinopropoxy)quinazoline **90**[20] (6 g, 13 mmol) and 6 N HCl (120 mL) was stirred and heated to reflux for 6 h. The mixture was cooled to 0 °C and was carefully neutralized by the addition of concentrated ammonium hydroxide. The resultant precipitate was isolated, washed in turn with dilute ammonium hydroxide and water, and dried under vacuum to give 4.2 g of **91** (98%). ^{1}H NMR (DMSO-d_6): δ 2.4 (m, 6H), 3.59 (t, 4H), 3.75 (t, 2H), 3.9 (s, 3H), 4.12 (t, 2H), 7.12 (s, 1H), 7.43 (s, 1H), 7.98 (s, 1H), 12.0 (br s, 1H). MS-ESI m/z 320 $[MH]^+$.

4-Chloro-7-methoxy-6-(3-morpholinopropoxy)quinazoline (92). A solution of 7-methoxy-6-(3-morpholinopropoxy)-3,4-dihydroquinazolin-4-one **91** (0.990 g, 3.1 mmol) in thionyl chloride (10 mL) and DMF (0.1 mL) was heated at 80 °C for 1.5 h. The mixture was allowed to cool, toluene was added, and the solvent was removed by evaporation. The residue was partitioned between ethyl acetate and water and the aqueous layer was adjusted to pH 7.5 with 2 M NaOH. The organic layer was separated, washed with brine, dried over magnesium sulfate, and the solvent was removed by evaporation. The residue was purified by flash chromatography eluting with methylene chloride:methanol (95:5). The solid was triturated with hexane, collected by filtration, and washed with ether to give 0.614 g of **92** (58%). ^{1}H NMR ($CDCl_3$): δ 2.12 (m, 2H), 2.50 (br s, 4H), 2.59 (t, 2H), 3.73 (t, 4H), 4.05 (s, 3H), 4.27 (t, 2H), 7.33 (s, 1H), 7.40 (s, 1H), 8.86 (s, 1H).

***N*-(5-Chloro-1,3-benzodioxol-4-yl)-7-methoxy-6-(3-morpholin-4-ylpropoxy)quinazolin-4-amine (26)** was prepared as described for the synthesis of **1**. 4-Chloro-7-methoxy-6-(3-morpholinopropoxy)quinazoline **92** (0.25 g, 0.67 mmol) was reacted with 5-chloro-1,3-benzodioxol-4-amine **71** (0.14 g, 0.8 mmol) to give 0.08 g of **26** (25%). ^{1}H NMR (DMSO-d_6): δ 2.0 (m, 2H), 2.40 (m, 4H), 2.50 (t, 2H), 3.60 (m, 4H), 3.95 (s, 3H), 4.15 (t, 2H), 6.10 (s, 2H), 6.95 (d, 1H, J = 8.4 Hz), 7.05 (d, 1H, J = 8.4 Hz), 7.20 (s, 1H), 7.85 (s, 1H), 8.30 (s, 1H), 9.50 (s, 1H). MS-ESI m/z 473 and 475 $[MH]^+$. Anal. ($C_{23}H_{25}ClN_4O_5 \cdot 0.45H_2O$) C, H, N.

***N*-(5-Chloro-1,3-benzodioxol-4-yl)-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinazolin-4-amine (27).** 4-Chloro-6-methoxy-7-((1-methylpiperidin-4-yl)methoxy)quinazoline **36**[15] (0.8 g, 2.5 mmol) was reacted with 5-chloro-1,3-benzodioxol-4-amine **71** (0.47 g, 2.7 mmol) using a procedure similar to the one described for **2**. The crude product was purified by flash chromatography using increasingly polar solvent mixtures starting with dichloromethane:methanol (9:1) and ending with dichloromethane:methanol:methanol saturated with ammonia (9:8:2). Evaporation of the solvent gave 0.746 g of **27** (66%). ^{1}H NMR (DMSO-d_6 and CD_3CO_2D): δ 1.60 (m, 2H), 2.0 (m, 2H), 2.1 (m, 1H), 2.70 (s, 3H), 2.85 (m, 2H), 3.35 (m, 2H), 3.95 (s, 3H), 4.05 (d, 2H), 6.1 (s, 2H), 6.95 (d, 1H, J = 8.4 Hz), 7.05 (d, 1H, J = 8.4 Hz), 7.25 (s, 1H), 7.85 (s, 1H), 8.35 (d, 1H). MS-ESI m/z 455 and 457 $[M - H]^-$. Anal. ($C_{23}H_{25}ClN_4O_4$) C, H, N.

[6-Methoxy-4-oxo-7-(3-piperidin-1-ylpropoxy)quinazolin-3(4*H*)-yl]methyl Pivalate (93). 7-Hydroxy-6-methoxy-3-((pivaloyloxy)methyl)-3,4-dihydroquinazolin-4-one **31**[14] (2 g, 6.5 mmol) was dissolved in DMF (16 mL), and potassium carbonate (1.26 g, 9.1 mmol), and 1-(3-chloropropyl)piperidine[21] (1.26 g, 7.8 mmol) were added. The mixture was purged from oxygen with argon and heated at 90 °C for 1.5 h. The excess potassium carbonate was removed by filtration, the DMF evaporated by rotary evaporation, and the obtained oil placed under vacuum overnight to give 2.8 g of **93** (100%). ^{1}H NMR (DMSO-d_6 and CD_3CO_2D): δ 1.10 (s, 9H), 1.40 (m, 2H), 1.50 (m, 4H), 1.95 (m, 2H), 2.35 (m, 4H), 2.40 (m, 2H), 3.90 (s, 3H), 4.20 (t, 2H), 5.95 (s, 2H), 7.15 (s, 1H), 7.50 (s, 1H), 8.40 (s, 1H).

6-Methoxy-7-(3-piperidin-1-ylpropoxy)quinazolin-4(3*H*)-one (94). [6-Methoxy-4-oxo-7-(3-piperidin-1-ylpropoxy)quinazolin-3(4*H*)-yl]methyl pivalate **93** (2.8 g, 6.5 mmol) and a 7 N solution of ammonia in methanol (50 mL) were stirred for 16 h. The volatiles were evaporated, and the residue was triturated under diethyl ether. The resultant solid was isolated, washed in turn with diethyl ether and a 9:1 mixture of diethyl ether and methylene chloride, and dried under vacuum to give 2.06 g of **94** (100%). ^{1}H NMR (DMSO-d_6): δ 1.40 (m, 2H), 1.50 (m, 4H), 1.90 (m, 2H), 2.35 (m, 4H), 2.4 (t, 2H), 3.9 (s, 3H), 4.15 (t, 2H), 7.11 (s, 1H), 7.44 (s, 1H), 7.9 (s, 1H).

4-Chloro-6-methoxy-7-(3-piperidin-1-ylpropoxy)quinazoline (95). Several batches of 6-methoxy-7-(3-piperidin-1-ylpropoxy)quinazolin-4(3*H*)-one **94** (76.3 g, 240 mmol) were pooled together and suspended in thionyl chloride (750 mL) under anhydrous conditions. DMF (5 mL) was added and the reaction mixture was heated to reflux for 2 h and then allowed to cool overnight. The volatiles were evaporated, the residue was triturated with toluene, and the mixture was evaporated. The solid was dissolved in dichloromethane, and ice and sodium bicarbonate were added alternatively under stirring at 0 °C until the solution reached pH 6−7. Sodium hydroxide 2 N (∼400 mL) was then added until pH 9−10 was reached. The aqueous phase was extracted with dichloromethane, the organic phase was washed with water and brine, dried over magnesium sulfate, and filtered, and the solvent evaporated. The solid was triturated with petroleum ether:ether, collected by filtration, and dried under vacuum to give 64.2 g of **95** (80%). ^{1}H NMR (DMSO-d_6): δ 1.40 (m, 2H), 1.55 (m, 4H), 2.0 (m, 2H), 2.40 (m, 4H), 2.45 (t, 2H), 4.0 (s, 3H), 4.29 (t, 2H), 7.41 (s, 1H), 7.46 (s, 1H), 8.9 (s, 1H).

***N*-(5-Chloro-1,3-benzodioxol-4-yl)-6-methoxy-7-(3-piperidin-1-ylpropoxy)quinazolin-4-amine (28).** 5-Chloro-1,3-benzodioxol-4-amine **71** (1.07 g, 6.25 mmol) was reacted with NaHMDS (6.25 mL, 6.25 mmol, 1 M in THF) in the presence of 4-chloro-6-methoxy-7-(3-piperidin-1-ylpropoxy)-quinazoline **95** (1.0 g, 2.98 mmol) using a procedure similar to the one described for the synthesis of **11** and gave 1.4 g of **28** (75%). ^{1}H NMR (DMSO-d_6 and CD_3CO_2D): δ 1.5−1.64 (m, 2H), 1.66−1.84 (m, 4H), 2.18−2.32 (m, 2H), 3.11−3.39 (m, 6H), 3.95 (s, 3H), 4.25 (t, 2H), 6.07 (s, 2H), 6.93 (d, 1H, J = 8.4 Hz), 7.05 (d, 1H, J = 8.4 Hz), 7.26 (s, 1H), 7.89 (s, 1H), 8.33 (s, 1H). MS-ESI m/z 469 and 471 $[MH]^+$. mp: 163−165 °C. Anal. ($C_{24}H_{27}ClN_4O_4$ $0.2H_2O$) C, H, N.

4-(Hydroxymethyl)pyridine-2-carbonitrile (97). 4-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)pyridine-2-carbonitrile[22] (3.37 g, 15 mmol) was dissolved in THF, *tert*-butyl ammonium fluoride (24 mL, 1 M THF solution) was added under argon, and the mixture was stirred for 1 h. The solvent was evaporated, the residue was diluted with ethyl acetate, washed with a saturated solution of ammonium chloride, water, and brine, dried over magnesium sulfate, and filtered, and the solvent was evaporated. The residue was purified by flash chromatography using increasingly polar solvent mixtures starting with petroleum ether:ethyl acetate (6:4) and ending with ethyl acetate. Evaporation of the solvent gave 1.37 g of **97** (68%). ^{1}H NMR ($CDCl_3$): δ 2.25 (br s, 1H), 4.85 (s, 2H), 7.55 (d, 1H, J = 5.1 Hz), 7.75 (s, 1H, J = 5.1 Hz), 8.7 (d, 1H).

7-(Benzyloxy)-*N*-(5-chloro-1,3-benzodioxol-4-yl)-6-methoxyquinazolin-4-amine (99) was prepared using conditions similar to those described for the synthesis of **5**. 7-Benzyloxy-4-chloro-6-methoxyquinazoline **98** (7 g, 23 mmol) was reacted with 5-chloro-1,3-benzodioxol-4-amine **71** (4.4 g, 25 mmol) to give 9.1 g of **99** (84%) as a hydrochloride. ^{1}H NMR (DMSO-d_6): δ 4.0 (s, 3H), 5.35 (s, 2H), 6.15 (s, 2H), 7.05 (d, 1H, J = 8.4 Hz), 7.15 (d, 1H, J = 8.4 Hz), 7.45 (m, 4H), 7.55 (d, 2H, J = 7.0 Hz), 8.25 (s, 1H), 8.8 (s, 1H).

4-[(5-Chloro-1,3-benzodioxol-4-yl)amino]-6-methoxyquinazolin-7-ol (100). 7-(Benzyloxy)-*N*-(5-chloro-1,3-benzo-

dioxol-4-yl)-6-methoxyquinazolin-4-amine **99** (9.1 g, 19 mmol) was dissolved in TFA (150 mL) and heated to reflux for 4 h under anhydrous conditions. The solvent was evaporated, the residue was diluted with water and methanol, and the pH adjusted to 7.5 with a saturated solution of sodium carbonate. The methanol was evaporated and the solid collected by filtration, washed with water, and dried over magnesium sulfate to give 6 g of **100** (91%). ^{1}H NMR (DMSO-d_6): δ 3.95 (s, 3H), 6.1 (s, 2H), 6.95 (d, 1H), 7.1 (m, 2H), 7.90 (s, 1H), 8.3 (s, 1H), 9.60 (br s, 1H), 10.5 (br s, 1H). MS-ESI *m*/*z* 346 and 348 $[MH]^+$.

4-[({4-[(5-Chloro-1,3-benzodioxol-4-yl)amino]-6-methoxyquinazolin-7-yl}oxy)methyl]pyridine-2-carbonitrile (29). 4-[(5-Chloro-1,3-benzodioxol-4-yl)amino]-6-methoxyquinazolin-7-ol **100** (0.1 g, 0.28 mmol) was suspended in dichloromethane (3 mL) under argon, and triphenylphosphine (0.151 g, 0.58 mmol) and 4-(hydroxymethyl)pyridine-2-carbonitrile **97** (0.042 g, 0.35 mmol) were added followed by dropwise addition of DEAD (0.091 mL, 0.58 mmol). The solvent was removed by evaporation and the residue purified by flash chromatography using dichloromethane:ethyl acetate:methanol (60:35:5). Evaporation of the solvent gave 0.084 g of **29** (65%). ^{1}H NMR (DMSO-d_6 and CF_3CO_2D): δ 4.05 (s, 3H), 5.55 (s, 2H), 6.15 (s, 2H), 7.05 (d, 1H, J = 8.7 Hz), 7.15 (d, 1H, J = 8.7 Hz), 7.35 (s, 1H), 7.85 (d, 1H, J_1 = 1.6, J_2 = 5.0 Hz), 8.14 (dd, 1H, J_1 = 0.8, J_2 = 1.6 Hz), 8.18 (s, 1H), 8.85 (d, 1H, J_1 = 0.8, J_2 = 5.0 Hz), 8.9 (s, 1H). MS-ESI *m*/*z* 462 and 464 $[MH]^+$.

Molecular Modeling. Modeling and docking studies have been performed using the published crystal structure of activated Lck as a surrogate for Src.[26] Indeed, the 3D structure of Src was also available at that time, but the kinase was in an inactivated form.[33] Owing to the high sequence homology between the two kinases, particularly within the ATP binding site, and in order to be consistent with the enzymatic assay we used, we believed that the activated Lck structure was a better tool for our modeling studies. Our inhibitors were built in Quanta and the charges were assigned by the Quanta charge template method.[34,35] These inhibitors have been docked manually into the ATP binding site, and the most relevant solutions were then energy minimized with the CHARMm force field to relieve possible unfavorable contacts.[36]

Biological Evaluation. IC_{50} values reported are the mean of three to five measurements.

(i) In Vitro Src Kinase Inhibition Test. This assay determines the ability of test compounds to inhibit Src kinase activity. A poly(Glu,Tyr) 4:1 random copolymer (Sigma-Aldrich, Poole, UK) was used as the tyrosine-containing substrate. This is stored as a 10 mg/mL stock solution in PBS at −20 °C and diluted 1:200 with PBS to coat 96-well plates (100 μL/well). Substrate was plated the day before an assay, and the plates were covered with adhesive seals and stored overnight at 4 °C. On the day of the assay, the substrate solution was discarded, and the plates were then incubated with 120 μL/well of 5% BSA in PBS/A for 10 min. The plates were then washed once with PBST (PBS containing 0.05% v/v Tween 20) and then incubated with 50 mM HEPES pH 7.4 at 100 μL/well until the next stage. Test compounds were dissolved in DMSO at 10 mM. A dilution series was then made in doubly distilled H_2O to give solutions at four times the final required reaction concentrations. Solutions of 40 μM ATP in 80 mM $MgCl_2$ and 80 mM $MgCl_2$ alone (for -ve controls) were prepared. Src kinase, expressed in sf9 insect cells by recombinant baculovirus containing the human *c-src* gene (Upstate Biotechnology, Lake Placid, NY), was diluted to 0.3 U/mL in enzyme dilution buffer (100 mM HEPES, 2 mM DTT, 0.2 mM sodium orthovanadate, 0.02% BSA). The HEPES was discarded from the substrate plates and the following additions made in order: 25 μL/well compound dilution (water in the case of +ve and -ve controls), 25 μL/well of ATP/$MgCl_2$ or $MgCl_2$ (-ve controls) alone, and finally, 50 μL/well Src kinase in dilution buffer to start the reaction. The final reaction concentrations were 0.15 U/mL Src kinase, 20 mM $MgCl_2$, and 10 μM ATP (determined as the K_m for ATP). The reaction time allowed was 15 min at room temperature on a plate shaker. The assay was stopped by washing the plates four times with PBST (150 μL/well). Detection of resultant tyrosine phosphorylation was facilitated by the addition of an anti-phosphotyrosine monoclonal antibody conjugated to alkaline phosphatase (anti-pY/HRP, Santa Cruz Biotechnology Inc., Santa Cruz, CA); this was diluted 1:5000 in PBST/B/O (PBST + 0.5% BSA + 0.1 mM sodium orthovanadate) and added at 100 μL/well and incubated for 1 h. The plates were again washed (6×). One tablet of the HRP substrate 3,3′,5,5′-tetramethylbenzidine (TMB, Sigma-Aldrich) was dissolved in 100 μL of DMSO and added per 10 mL of phosphate−citrate buffer with sodium perborate (supplied as soluble capsules, Sigma-Aldrich). TMB substrate solution (100 μL/well) was added. After 5 min of color development, the reaction was stopped by the addition of 50 μL/well of 0.8 M H_2SO_4, and the positive control wells now gave an A_{450nm} of ca. 1.2−1.5. Control and blank wells were included on all plates, containing compound diluent and $MgCl_2$ solution with and without ATP, respectively, to determine the dynamic range of the assay. The curves were plotted and the IC_{50} values for compound enzyme inhibition were interpolated using KC3 Kineticalc software (Bio-Tek Instruments) following subtraction of the blank values.

(ii) In Vitro KDR Kinase Inhibition Test. This assay determines the ability of test compounds to inhibit KDR kinase activity and has been used as a selectivity screen. The method is as reported previously.[14]

(iii) In Vitro c-src3T3 Proliferation Assay. This assay determines the ability of test compounds to inhibit the proliferation of cells in culture. A mouse NIH3T3 fibroblast cell line transfected to overexpress active Src kinase (c-Src3T3) was made and provided by Sara Courtneidge and Sydonia Rayter. This line has been shown to grow in DMEM (Gibco, Invitrogen Corp., Paisley, Scotland) + 0.5% serum, conditions in which the parental NIH3T3 cells do not survive. Under these low-serum conditions the transfected cells are driven to proliferate through their expression of active Src kinase, and consequently, Src kinase inhibition should revert them to the parental phenotype. c-Src3T3 cells are routinely cultured in DMEM medium + 5% FCS. For assay they were harvested with trypsin and plated to 96-well plates at 1.5 × 10^4 cells per well. The outer wells of the plate had media added but are not used for the assay to avoid the possibility of edge effects. The following day a dilution series was made from the test compounds in neat DMSO. These were further diluted into DMEM + 5% FCS, and 100 μL of these dilutions was added per well to the cell plates. The final DMSO concentration per well was 0.5% in all wells. The plates were then incubated for a further 24 h. A colorimetric 5-bromo-2′-deoxyuridine (BrdU) Cell Proliferation ELISA kit (Roche Diagnostics GmbH) was then used to assess proliferation according to the manufacturer's instructions. Briefly, the cells were pulse labeled with BrdU for 2 h and fixed, and the cellular DNA was denatured with the provided solution and then incubated with Anti-BrdU-POD for 90 min. The plates were then washed (3×) with PBS and patted dry, the TMB substrate solution was added, and the plates were incubated on a plate shaker for 10−30 min until the positive control absorbance at 690 nm was ca. 1.5. Positive control and blank wells were included on all plates, containing cells + compound diluent and no plated cells + compound diluent, respectively, to determine the dynamic range of the assay. The curves were plotted and the IC_{50} values for compound inhibition of cell proliferation were interpolated using Microcalc Origin following subtraction of the blank values.

(iv) In Vitro A549 Microdroplet Migration (Chemokinesis) Assay. This assay is a development from the polymorph microdroplet migration assay.[37] The assay determines the ability of test compounds to inhibit the random motility (chemokinesis) of A549 cells (human epithelial lung carcinoma cells, ATCC CCL 185) in 96-well plates. Difco Nobel Agar (Difco Laboratories, Detroit, MI) was weighed, PBS/A was added, and the mixture was autoclaved to give a 2% sterile stock solution. While still hot this was transferred to a 42 °C waterbath to prevent setting. A549 cells, routinely cultured

in DMEM + 10% FCS, were harvested by trypsinization, counted by hemocytometer or Coulter counter, centrifuged (Heraeus Multifuge 3 L−R, 1000 rpm, 5 min), and resuspended at 2×10^7 cells/mL in medium plus 0.3% agarose at 37 °C. Microdroplets (2 μL) of the cell agarose suspension were carefully pipetted centrally to the wells of nontissue culture treated 96-well plates (Bibby Sterilin, Stone, Staffordshire, UK). The completed plates were then placed briefly on ice to assist gelling of the agarose. When set, 90 μL of chilled (4 °C) RPMI 1640 medium (Gibco) + 10% FCS was slowly added per well, and compound dilutions in medium were added as a further 10 μL per well. The plates were transferred to a tissue culture incubator and incubated for 72 h to allow migration to occur. The distance of the migration front from the cell source (agarose microdroplet) was measured as the mean of four perpendicular axis per well assessed by microscopy using a calibrated eyepiece graticule. Positive controls were included on all plates containing cells + compound diluent. The curves were plotted and the IC_{50} values for compound inhibition of cell motility were interpolated using a purpose-designed Excel spreadsheet. The addition of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, Sigma-Aldrich) for 1 h at the end of the experiment allowed a parallel assessment of the toxicity of the compounds. MTT is converted to purple/black formazan crystals by the mitochondria of viable cells, thus allowing a visual assessment of compound toxicity.

(v) Rat Pharmacokinetics. Pharmacokinetics was determined in the mouse and rat following single intravenous (2 mg/kg) or oral administrations (20 or 50 mg/kg) of the compound. For the iv study, the compounds were formulated in a mixture of 25% (w/v) hydroxypropyl-β-cyclodextrin/Sorrenson's phosphate buffer (pH 5.5). For the oral study, the compounds were formulated as a solution in either 1% polysorbate or 0.1 M citrate buffer (pH 3).

(vi) Rat Xenografts. Athymic rats (Hsd Han:RNUrnu, 6−8 weeks, male) were dosed po with test compound or vehicle (1% polysorbate 80) 1 h prior to cell inoculation and once daily thereafter. The cell inoculation, made on the left flank of each animal, consisted of (1×10^6 cells/0.2 mL) *c-src*-transfected 3T3 cells in PBS. A total of 10 rats were used per group. Tumor size was measured three times weekly with callipers and tumor volume estimated using the formula {[(square root of length × width) × (length × width)] × 0.5236}. Studies were terminated once the control tumors showed signs of ulceration or hemorrhage or had reached 10% of the body weight of the host animal (typically at day 15).

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