N-(5-Chloro-1,3-benzodioxol-4-yl)-7-[2-(4-methylpiperazin-1-yl)ethoxy]-5-(tetrahydro-2*H*-pyran-4-yloxy)quinazolin-4-amine, a Novel, Highly Selective, Orally Available, Dual-Specific c-Src/Abl Kinase Inhibitor[†]

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Src family kinases (SFKs) are nonreceptor tyrosine kinases that are reported to be critical for cancer progression. We report here a novel subseries of C-5-substituted anilinoquinazolines that display high affinity and specificity for the tyrosine kinase domain of the c-Src and Abl enzymes. These compounds exhibit high selectivity for SFKs over a panel of recombinant protein kinases, excellent pharmacokinetics, and in vivo activity following oral dosing. *N*-(5-Chloro-1,3-benzodioxol-4-yl)-7-[2-(4-methylpiperazin-1-yl)ethoxy]-5-(tetrahydro-2*H*-pyran-4-yloxy)quinazolin-4-amine (AZD0530) inhibits c-Src and Abl enzymes at low nanomolar concentrations and is highly selective over a range of kinases. AZD0530 displays excellent pharmacokinetic parameters in animal preclinically and in man ($t_{1/2}$ = 40 h). AZD0530 is a potent inhibitor of tumor growth in a c-Src-transfected 3T3-fibroblast xenograft model in vivo and led to a significant increase in survival in a highly aggressive, orthotopic model of human pancreatic cancer when dosed orally once daily. AZD0530 is currently undergoing clinical evaluation in man.

Introduction

c-Src kinase is a nonreceptor tyrosine kinase that acts as a signal transduction inhibitor that is a critical component of multiple signaling pathways that control cell growth, proliferation, invasion, and apoptosis. While c-Src kinase is highly regulated and active only at low levels in most normal cells, studies have shown that c-Src kinase is upregulated in many human tumor types.[1,2] Recently emerging data support the hypothesis that the predominant consequence of increased c-Src activity in tumor cells is to reduce cell adhesion, facilitate motility, and thereby promote an invasive phenotype. Consequently, there is considerable interest in the inhibition of c-Src kinase as a treatment for cancer and in particular as an anti-invasion strategy. Tumor cell invasion is a feature common to all malignant tumors and is a process that occurs throughout the evolution of a cancer. There is evidence that c-Src kinase activity is an important component of the invasive phenotype in both early and advanced solid tumors. In early disease, c-Src kinase plays a key role in the epithelium to mesenchymal transition that marks the conversion of epithelial tumor cells to a more invasive phenotype.[3] Increased c-Src kinase activity has been linked with the disruption of E-cadherin-mediated cell−cell adhesion[4−6] and also impacts the assembly and turnover of focal adhesions, which are critical for cell migration.[7,8] Studies on colon tumors have found that the highest level of c-Src kinase activity occurs in metastatic tissue[9,10] and that increased c-Src kinase activity is an indicator of a poor prognosis.[11−13] Furthermore, emerging data suggest that c-Src kinase inhibition may enhance the antitumor efficacy of hormonal and cytotoxic agents in preclinical models.[14,15]

As well as having a role in solid tumors, c-Src family kinases might be involved in the progression of chronic myeloid and acute lymphoid leukemias (CMLs and ALLs) that are positive for the Philadelphia chromosome (Ph+). Studies have shown that c-Src kinases have a function in imatinib-resistant CML and ALL[16,17] and that inhibitors of c-Src and Bcr-Abl kinases have activity against both imatinib-sensitive and imatinib-resistant cell lines.[18] c-Src kinase activity is also implicated in metastatic bone disease, a characteristic of late-stage progression of many solid tumor types, for example, breast[19] and prostate,[20] and of leukemias.[21,22] In animal models, inhibition of c-Src kinase has been shown to limit invasion of bone metastases and destructive bone resorption.[23]

c-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 or for cancer by targeting tumor growth, cell adhesion, or motility.[24] However, several classes of molecules have been studied preclinically for their ability to inhibit c-Src and Abl kinases,[24−26] of which the three most advanced compounds (Figure 1) that are undergoing clinical evaluation are the anilinoquinazoline AZD0530 [*N*-(5-chloro-1,3-benzodioxol-4-yl)-7-[2-(4-methylpiperazin-1-yl)ethoxy]-5-(tetrahydro-2*H*-pyran-4-yloxy)quinazolin-4-amine,[27,28] the thiazolecarboxamide BMS-354825,[29] and the quinolinecarbonitrile SKI-606.[24]

AZD0530 (**33**) is a highly selective, orally available, dual-specific c-Src/Abl kinase inhibitor which is in clinical development for the treatment of a wide range of tumor types. On the basis of current knowledge of c-Src kinase activity, **33** is likely to have a therapeutic benefit as an anti-invasive agent, with potential for activity in early and advanced solid tumors, leukemia, and metastatic bone disease. In this paper, we describe

[†] The atomic coordinates and structure factors (PDB ID code 2H8H) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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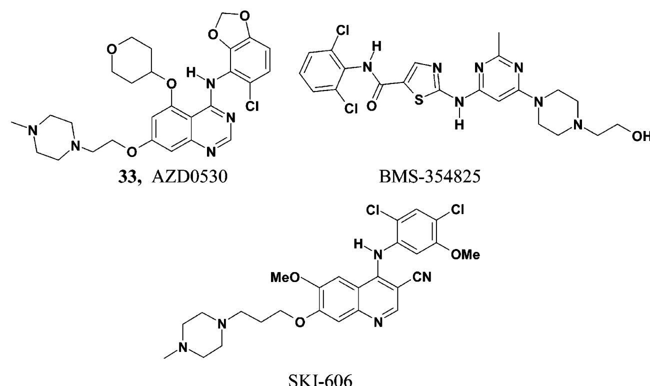


Figure 1. Structures of AZD0530, BMS-354825, and SKI606.

the rationale and structure−activity relationships (SARs) leading to the synthesis of **33**.

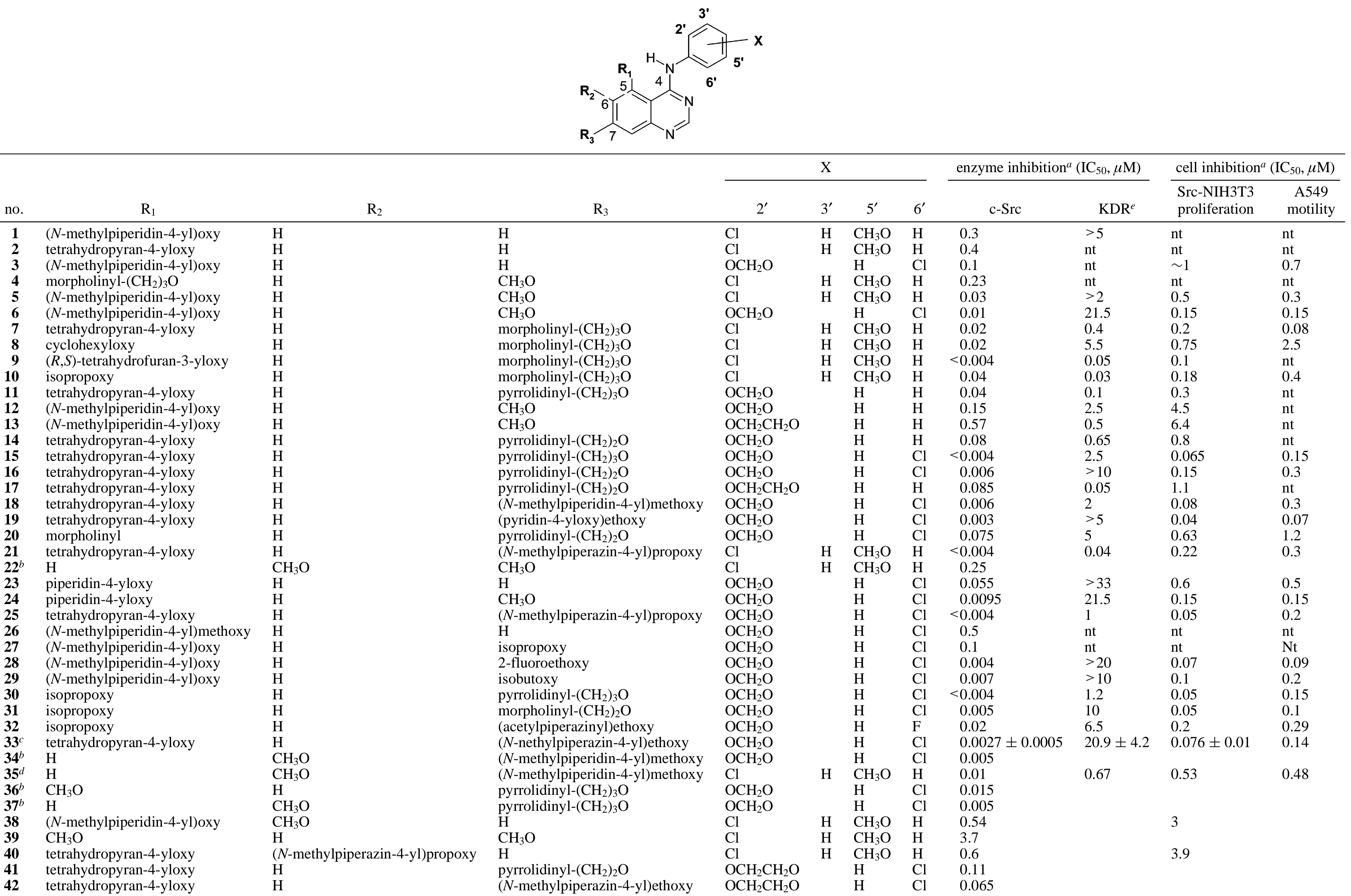
Chemistry

The C-5-substituted anilinoquinazolines described in Table 1 were usually prepared by one of the two main routes as described in Schemes 1−7. The general strategies to introduce the C-5 and C-7 substituents on the quinazoline core were based either on the selective dealkylation of the C-5 and/or C-7 methoxy of the (benzyloxy)quinazoline precursors **39**, **43**, **46,** **58**, **59**, **61**−**64**, **73**, **88**, **93**, **99**, and **113** or on the displacement by alkoxide anions of the C-5 and/or C-7 fluorine atom of the fluoroquinazoline intermediates **48**, **52**, **108**, and **109**. Displacement of the C-5 fluorine atom of the commercially available quinazolinone **48** by sodium methoxide led to **45** (Scheme 1). Subsequent activation of the C-4 position by $POCl_3$, followed by the nucleophilic displacement of the intermediate 4-chloroquinazoline with 2-chloro-5-methoxyaniline, led to **46**. Deprotection of the C-5-methoxy group was achieved by heating with pyridine/HCl to give **47**. When the same sequence of reactions was applied to the dimethoxy derivative **43**, the last step led to selective deprotection of the more reactive C-5 methoxy substituent over the less reactive C-7 methoxy to give rise to **44**. The phenols **44** and **47** were then treated under Mitsunobu conditions with a range of alcohols to give **1**, **2**, and **4**. Alternatively, as shown with the synthesis of **52**, the electron-withdrawing property of the C-5 fluorine atom was used to activate the C-4 position of the quinazoline **51** and facilitate the introduction of the 4-amino-5-chloro-1,3-benzodioxole[27] to give **52**. The C-5 fluorine atom, although less reactive in **52** than in **48**, was subsequently displaced by primary or secondary alkoxides in DMF at 80 °C to give **3** and **53**. Cleavage of the BOC protecting group of **53** under acidic conditions gave **54**, which was then methylated under reductive amination conditions to give **26** (Scheme 1). To achieve a mild and selective deprotection of the C-7 position after having introduced the desired C-5 alkoxy substituent, we prepared the 5,7-bis(benzyloxy)anilinoquinazoline **59** from the isatin precursor **55** (Scheme 2). Treatment of **59** with pyridine/HCl gave the phenol **60**, which was then reacted with a range of secondary alcohols under Mitsunobu conditions to give **61**−**64** with excellent yields. Deprotection of the C-7 benzyloxy protecting group with TFA led to **65**−**68**, and these were then reacted with either 4-(3-hydroxypropyl)morpholine[30] or 3-(4-methylpiperazin-1-yl)propan-1-ol to give **7**−**10** or **21**, respectively. The [(5-chloro-1,3-benzodioxolyl)amino]quinazolines were synthesized as described in Schemes 3−6. The dimethoxyquinazolinone **43** was selectively deprotected at the C-5 position using $MgBr_2$/pyridine to give **71**. The selectivity observed for the C-5 position over C-7, is most probably due to an internal coordination of the magnesium atom with the C-4 and C-5 oxygen atoms, favoring a concerted process. Selective protection of the N-3 position of **71** with the POM group led to **72**, which was then substituted at C-5 by the tetrahydropyran nucleus. The deprotection of the C-7 methoxy group of **73** required stronger conditions, namely, thiophenol and potassium carbonate in NMP at 195 °C to give the C-7 hydroxyquinazolinone **74**. Acylation of the C-7 position allowed subsequent versatile modification of either the C-4 or C-7 substituent to generate **11**, **14**−**16**, **18**, **19**, **25**, **33**, **41**, **42**, and **80** after conventional deprotection and Mitsunobu reactions. The 6′-chlorobenzodioxanamine[31] was prepared and coupled to the quinazolinone **75** (Scheme 3) after in situ activation, as described for the preparation of **77**. Compound **120** was then deprotected as described for **77** to give **121**, which was subsequently alkylated to give **41** and **42**. The synthesis of the C-5 piperidinyloxy derivatives is described in Scheme 4 and utilizes the *N*-protected quinazolinones **72** and **83**. Introduction of the *N*-methylpiperidinyl moiety or its *N*-Boc-protected equivalent at the C-5 position gave the key protected precursors **84**−**87**, which were subsequently modified at positions C-4 and C-7 to give **5**, **6**, **12**, **13**, **28**, **29**, **96**, and **97**. Deprotection under acidic conditions of the BOC protecting group of **97** gave **24**. Reductive amination of the secondary piperidine **96** using sodium borohydride triacetate and formaldehyde in acetic acid/methanol gave the corresponding *N*-methyl derivative **27**. The series of C-5 isopropoxy derivatives was obtained as shown in Scheme 5. The protected quinazolinone **83** was reacted with 2-propanol and subsequently deprotected using ammonia/methanol in a one-pot process to give **99**. Debenzylation followed by acylation at C-7, chlorination at C-4, and displacement of the chlorine by the 4-amino-5-fluoro-1,3-benzodioxole or 4-amino-5-chloro-1,3-benzodioxole gave **101** and **102**. Alkylation with 2-chloroethanol gave two chloroalkyl precursors, **103** and **104**. Nucleophilic displacement of the aliphatic chlorine atom of **103** and **104** by morpholine and *N*-acetylpiperazine led, respectively, to **31** and **32**. The difluoroquinazolinone **108**, prepared from the commercially available 3,5-difluoroaniline, also proved to be a valuable intermediate in our strategies (Scheme 6). Activation of the C-5 fluorine atom by the quinazoline carbonyl group, together with the second fluorine at the C-7 position allowed selective nucleophilic displacement of the C-5 fluorine by morpholine to give **109**. Subsequent displacement of the C-7 fluorine atom by the (2-hydroxyethyl)-pyrrolidine side chain required the formation of an intermediate alkoxide to give **110**. Chlorination followed by reaction with the aminobenzodioxole gave **20**. The C-5- and C-6-disubstituted derivatives were synthesized as shown in Scheme 7. The 5-hydroxy functionality of **111** was generated from the 5-(benzyloxy)-6-methoxybenzoquinazolone using TFA. Subsequently, the N-3 position of **111** was selectively protected to give **112**. Reaction with tetrahydropyran-4-ol or *N*-methylpiperidin-4-ol led to **113** and **114**, respectively. These key intermediate quinazolinones were subsequently processed through conventional functionalization of the C-6 and C-4 positions to give **38** and **40**.

Results and Discussion

For clarity of discussion, data on only a limited, representative set of compounds are used to describe the structure−activity relationships. Where trends are exemplified by a single pair of compounds, it is to be understood that more examples exist to support the SAR described.[32]

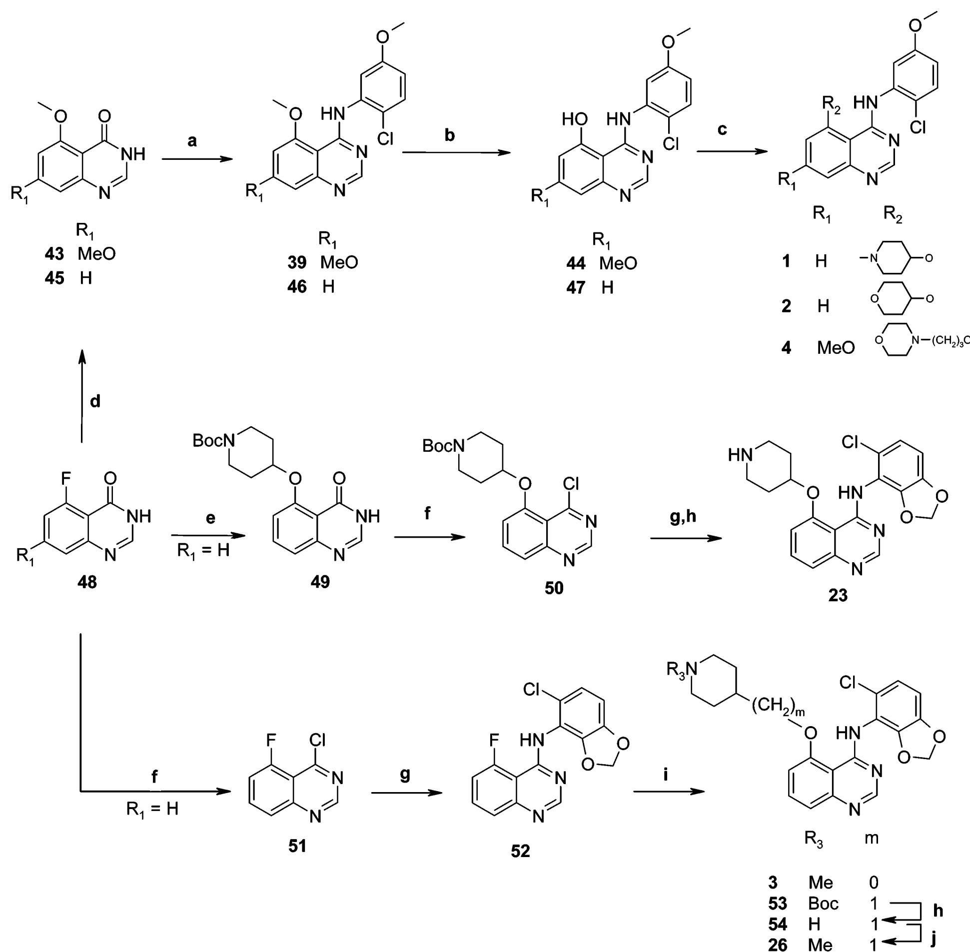
Table 1. Structure and in Vitro Activities of Compounds **1**−**42**



no.	R_1	R_2	R_3	X 2′	X 3′	X 5′	X 6′	enzyme inhibition[a] (IC_{50}, μM) c-Src	enzyme inhibition[a] (IC_{50}, μM) KDR[e]	cell inhibition[a] (IC_{50}, μM) Src-NIH3T3 proliferation	cell inhibition[a] (IC_{50}, μM) A549 motility
1	(*N*-methylpiperidin-4-yl)oxy	H	H	Cl	H	CH_3O	H	0.3	>5	nt	nt
2	tetrahydropyran-4-yloxy	H	H	Cl	H	CH_3O	H	0.4	nt	nt	nt
3	(*N*-methylpiperidin-4-yl)oxy	H	H	OCH_2O		H	Cl	0.1	nt	~1	0.7
4	morpholinyl-$(CH_2)_3O$	H	CH_3O	Cl	H	CH_3O	H	0.23	nt	nt	nt
5	(*N*-methylpiperidin-4-yl)oxy	H	CH_3O	Cl	H	CH_3O	H	0.03	>2	0.5	0.3
6	(*N*-methylpiperidin-4-yl)oxy	H	CH_3O	OCH_2O		H	Cl	0.01	21.5	0.15	0.15
7	tetrahydropyran-4-yloxy	H	morpholinyl-$(CH_2)_3O$	Cl	H	CH_3O	H	0.02	0.4	0.2	0.08
8	cyclohexyloxy	H	morpholinyl-$(CH_2)_3O$	Cl	H	CH_3O	H	0.02	5.5	0.75	2.5
9	(*R*,*S*)-tetrahydrofuran-3-yloxy	H	morpholinyl-$(CH_2)_3O$	Cl	H	CH_3O	H	<0.004	0.05	0.1	nt
10	isopropoxy	H	morpholinyl-$(CH_2)_3O$	Cl	H	CH_3O	H	0.04	0.03	0.18	0.4
11	tetrahydropyran-4-yloxy	H	pyrrolidinyl-$(CH_2)_3O$	OCH_2O		H	H	0.04	0.1	0.3	nt
12	(*N*-methylpiperidin-4-yl)oxy	H	CH_3O	OCH_2O		H	H	0.15	2.5	4.5	nt
13	(*N*-methylpiperidin-4-yl)oxy	H	CH_3O	OCH_2CH_2O		H	H	0.57	0.5	6.4	nt
14	tetrahydropyran-4-yloxy	H	pyrrolidinyl-$(CH_2)_2O$	OCH_2O		H	H	0.08	0.65	0.8	nt
15	tetrahydropyran-4-yloxy	H	pyrrolidinyl-$(CH_2)_3O$	OCH_2O		H	Cl	<0.004	2.5	0.065	0.15
16	tetrahydropyran-4-yloxy	H	pyrrolidinyl-$(CH_2)_2O$	OCH_2O		H	Cl	0.006	>10	0.15	0.3
17	tetrahydropyran-4-yloxy	H	pyrrolidinyl-$(CH_2)_2O$	OCH_2CH_2O		H	H	0.085	0.05	1.1	nt
18	tetrahydropyran-4-yloxy	H	(*N*-methylpiperidin-4-yl)methoxy	OCH_2O		H	Cl	0.006	2	0.08	0.3
19	tetrahydropyran-4-yloxy	H	(pyridin-4-yloxy)ethoxy	OCH_2O		H	Cl	0.003	>5	0.04	0.07
20	morpholinyl	H	pyrrolidinyl-$(CH_2)_2O$	OCH_2O		H	Cl	0.075	5	0.63	1.2
21	tetrahydropyran-4-yloxy	H	(*N*-methylpiperazin-4-yl)propoxy	Cl	H	CH_3O	H	<0.004	0.04	0.22	0.3
22[b]	H	CH_3O	CH_3O	Cl	H	CH_3O	H	0.25			
23	piperidin-4-yloxy	H	H	OCH_2O		H	Cl	0.055	>33	0.6	0.5
24	piperidin-4-yloxy	H	CH_3O	OCH_2O		H	Cl	0.0095	21.5	0.15	0.15
25	tetrahydropyran-4-yloxy	H	(*N*-methylpiperazin-4-yl)propoxy	OCH_2O		H	Cl	<0.004	1	0.05	0.2
26	(*N*-methylpiperidin-4-yl)methoxy	H	H	OCH_2O		H	Cl	0.5	nt	nt	nt
27	(*N*-methylpiperidin-4-yl)oxy	H	isopropoxy	OCH_2O		H	Cl	0.1	nt	nt	Nt
28	(*N*-methylpiperidin-4-yl)oxy	H	2-fluoroethoxy	OCH_2O		H	Cl	0.004	>20	0.07	0.09
29	(*N*-methylpiperidin-4-yl)oxy	H	isobutoxy	OCH_2O		H	Cl	0.007	>10	0.1	0.2
30	isopropoxy	H	pyrrolidinyl-$(CH_2)_3O$	OCH_2O		H	Cl	<0.004	1.2	0.05	0.15
31	isopropoxy	H	morpholinyl-$(CH_2)_2O$	OCH_2O		H	Cl	0.005	10	0.05	0.1
32	isopropoxy	H	(acetylpiperazinyl)ethoxy	OCH_2O		H	F	0.02	6.5	0.2	0.29
33[c]	tetrahydropyran-4-yloxy	H	(*N*-nethylpiperazin-4-yl)ethoxy	OCH_2O		H	Cl	0.0027 ± 0.0005	20.9 ± 4.2	0.076 ± 0.01	0.14
34[b]	H	CH_3O	(*N*-methylpiperidin-4-yl)methoxy	OCH_2O		H	Cl	0.005			
35[d]	H	CH_3O	(*N*-methylpiperidin-4-yl)methoxy	Cl	H	CH_3O	H	0.01	0.67	0.53	0.48
36[b]	CH_3O	H	pyrrolidinyl-$(CH_2)_3O$	OCH_2O		H	Cl	0.015			
37[b]	H	CH_3O	pyrrolidinyl-$(CH_2)_3O$	OCH_2O		H	Cl	0.005			
38	(*N*-methylpiperidin-4-yl)oxy	CH_3O	H	Cl	H	CH_3O	H	0.54		3	
39	CH_3O	H	CH_3O	Cl	H	CH_3O	H	3.7			
40	tetrahydropyran-4-yloxy	(*N*-methylpiperazin-4-yl)propoxy	H	Cl	H	CH_3O	H	0.6		3.9	
41	tetrahydropyran-4-yloxy	H	pyrrolidinyl-$(CH_2)_2O$	OCH_2CH_2O		H	Cl	0.11			
42	tetrahydropyran-4-yloxy	H	(*N*-methylpiperazin-4-yl)ethoxy	OCH_2CH_2O		H	Cl	0.065			

[a] Values are means from at least three independent dose−response curves. Variation was generally ±15% for Src and KDR enzymes and ±20% in 3T3 and A549 cells. [b] See ref 27. [c] Compound **33** is AZD0530. [d] Compound **35** is M475271. [e] VEGFR-2, also called KDR.

Scheme 1[a]



[a] Reagents and conditions: (a) (i) $POCl_3$, DIPEA, DCE, reflux; (ii) aniline, ^{i}PrOH, 80 °C; (b) pyridine hydrochloride; (c) R_2OH, PPh_3, DTAD, DCM; (d) MeONa, THF, reflux; (e) *tert*-butyl-4-hydroxypiperidine-1-carboxylate, NaH, DMF; (f) PPh_3, CCl_4, DCE; (g) aniline, HCl(cat.), ^{i}PrOH, 80 °C; (h) HCl, Et_2O; (i) ROH, NaH, DMF, 80 °C; (j) HCHO, AcOH, MeOH, $NaBH(OAc)_3$.

Kinases are important biological targets, and tremendous efforts have been made over the past few years to solve their three-dimensional structures complexed with different classes of inhibitors.[33] This has supplied key information, not only on the nature and topology of the ATP binding site, but also on the conformation of their active and inactive forms, revealing something of their complex and varied modes of activation.[34-39] Structural studies of kinase complexes have revealed the existence of a pattern of clustered residues and conserved hydrogen bond interactions surrounding the active site, providing a basis for the understanding of the binding of ATP or competitive inhibitors.[34,35] The adenine moiety of ATP itself is anchored in the active site by two such bonds as illustrated in Figure 2. This hydrogen bond network has been widely exploited to design purine-mimetic scaffolds and develop novel chemical series of ATP competitive kinase inhibitors[27,40-50] as shown in many kinase–inhibitor complexes.[45-47,51-54] Although hydrogen bonding is essential for this mode of inhibition, the number of protein–ligand hydrogen bonds is not correlated with potency. Structural studies have also revealed the presence of other pockets within the active site which play important roles in the potency and selectivity of small-molecule inhibitors. One feature frequently exploited in the design of kinase inhibitors is the presence of a deep hydrophobic pocket adjacent to the adenine binding site (Figure 2).[41,42,44,46,47,52,56,57] This pocket is made up of conserved and nonconserved residues and is not occupied by ATP itself. While its nature remains mainly hydrophobic,

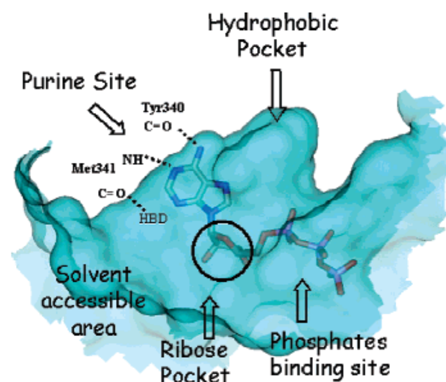


Figure 2. Schematic representation of ATP bound to the Src kinase domain.[36] The binding site is represented by its molecular surface in blue.[73] Molecules are illustrated in stick representation with colored elements. The adenine moiety is anchored by one donator and one acceptor H bond with Ty340 and Met341, respectively. Crystal structures of kinase–inhibitor complexes[43,50,51,55] have shown that another H bond could also be formed between an H bond donor (HBD) and the backbone carbonyl of a residue equivalent to Met341. The locations of the ATP binding site pockets exploited in drug design are also shown.

its size and shape vary noticeably between kinases and depend on the activation state of the enzyme.[36,37,39a,58-60] The residue present at the entrance of this pocket, also known as the

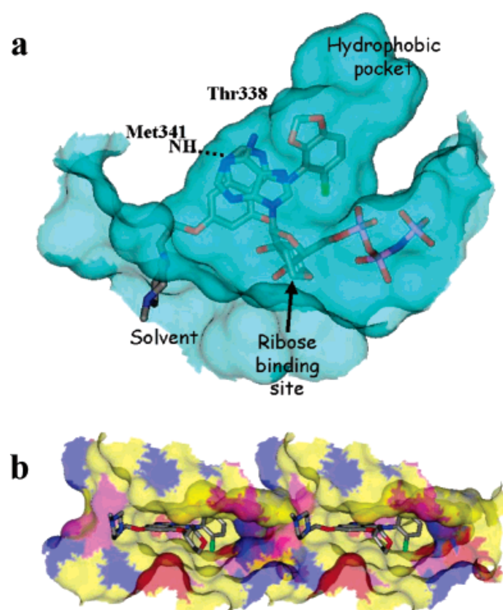


Figure 3. Compound **33** (AZD0530) in complex with c-Src (inactivated form). (a) Overlay of AZD0530 with ATP bound to c-Src. The tetrahydropyran ring fits closely to the ribose ring. The chlorobenzodioxole moiety of AZD0530 is buried in the hydrophobic pocket. Note that this pocket is not fully occupied by the benzodioxole, in agreement with the fact that this pocket is deeper in the inactivated (c-Src) than in the activated (Lck) form. (b) Stereoview of AZD0530 in the ATP binding site. The molecular surface of the binding site is colored according to the hydrophobicity of the surrounding residues. Yellow areas indicate hydrophobic regions, magenta hydrophilic, and blue and red, respectively, positively and negatively charged residues. The tetrahydropyran ring is seen to pack with a hydrophobic pocket in the ribose binding site. The interactions are predominantly formed with the glycine loop in the N-terminal domain (Leu273, Gly274) and with Leu393 and Ser345 in the C-terminal domain.

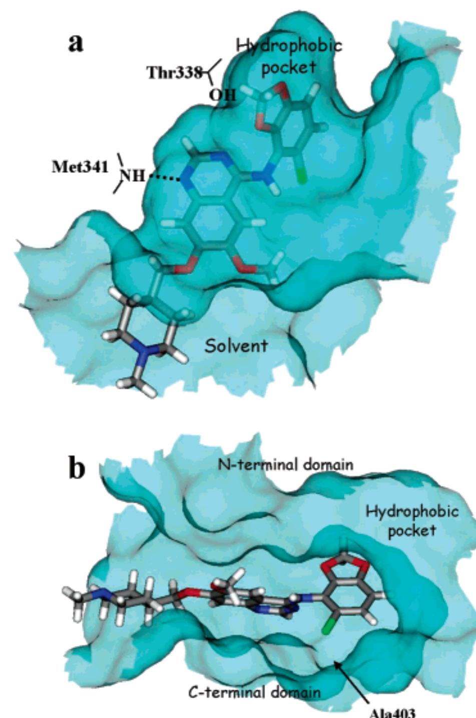


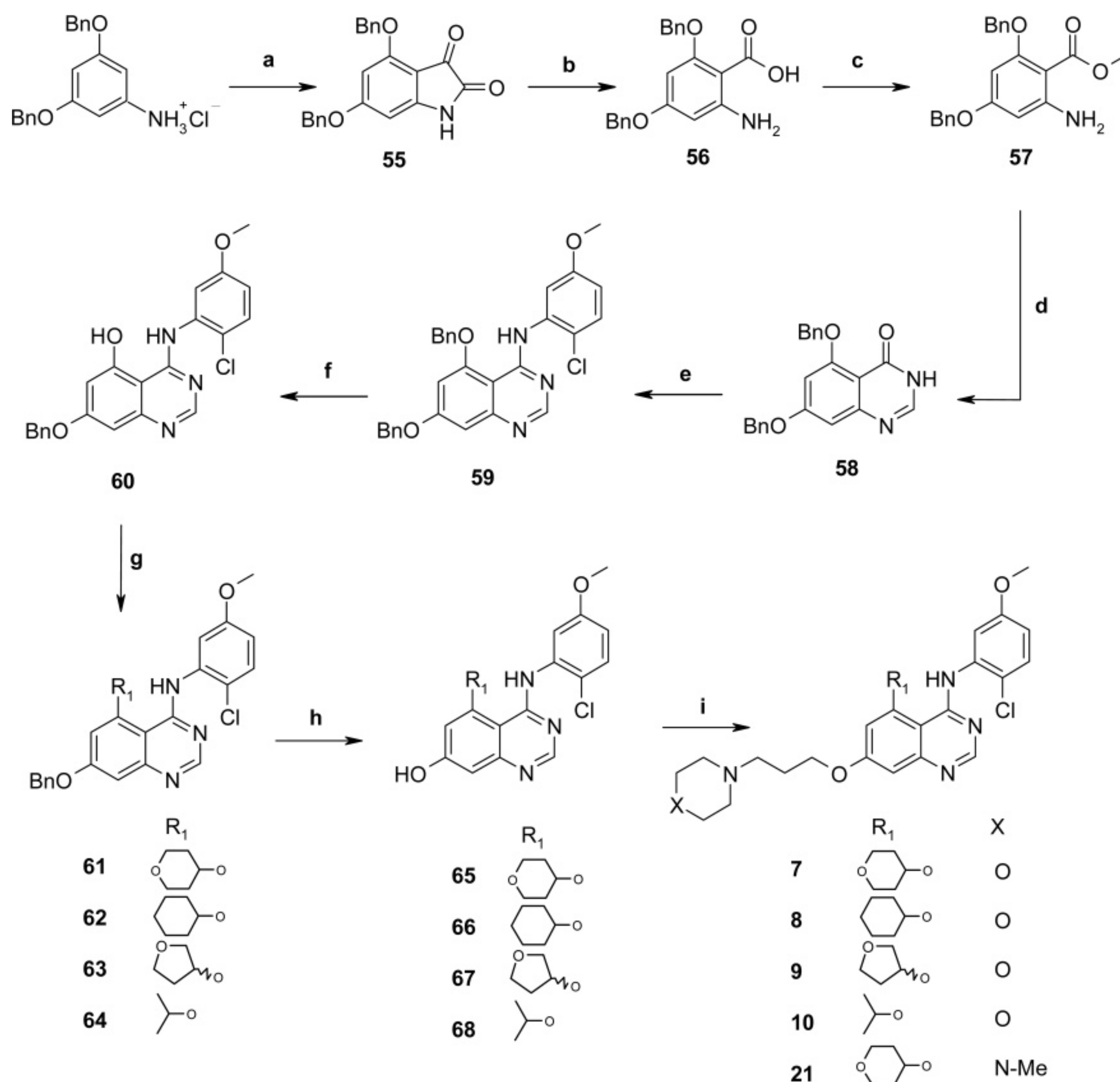
Figure 4. A [(5′-chlorobenzodioxolyl)amino]quinazoline derivative **34**[27] docked in the ATP binding site of the Lck 3D structure used as a model for activated c-Src.[74] For clarity, the residue numbering of c-Src has been used. (a) The quinazoline ring occupies the adenine binding site. The H bond interaction between the quinazoline N1 and the backbone NH of Met341 is represented by a black dotted line. The chlorobenzodioxole moiety is buried in the hydrophobic pocket, characterized by a Thr residue at its entry (Thr338). The C7 basic side chain lies in the solvent. (b) Edge-on view showing how well the chlorobenzodioxole fits into the hydrophobic pocket and is adequately designed to be selective for the c-Src kinase family.

gatekeeper, is another key determinant for selective inhibition within a kinase family.[44,61,62]

We have previously reported that 2′-chloro-5′-methoxyanilinoquinazolines substituted at the C-6 and C-7 positions of the quinazoline ring (Table 1) display good inhibition of the c-Src kinase enzyme.[27] Interestingly, these inhibitors do not occupy the ribose binding site. In this work we investigated a novel series of anilinoquinazolines substituted at the C-5 position of the quinazoline ring. The C-5 position allows substituents to access the ATP ribose binding site and could thus provide additional binding affinity for the enzyme. Unlike the selectivity pocket, the ribose pocket is partially open to the solvent and lined by a series of hydrophobic and hydrophilic residues (Figure 2). Modeling and docking of potential inhibitors into our 3D model of c-Src suggested to us that heterocycles linked to the position C-5 of the quinazoline would nicely fit the shape and the chemical nature of the ribose pocket. Other work on CDK1−2 (cyclin-dependent kinase) inhibitors had shown that the ribose pocket could be occupied by a broad range of substituents, including straight or branched alkyl chains, phenyl, or cyclic amines.[50a,b,63,64]

Despite extensive SAR studies aimed at modifying the quinazoline core, little information was known about the possible beneficial effects of C-5 substitution at the time of initiation of this work. A thorough investigation of the SAR around the substitution at the C-5 position of the quinazoline nucleus was thus made in both the monocyclic 2′-chloro-5′-methoxyanilinoquinazolines and the bicyclic [(chlorobenzodioxolyl)amino]-quinazoline series with the aim of finding suitable substituents that would optimally fit the ribose pocket and provide additional affinity for the enzyme active site. A wide range of substituents, flexible or rigid, linear or cyclic, neutral or basic, proved to be well tolerated at this C-5 position (Table 1). However, cyclic substituents generally led to more potent enzyme inhibitors than did acyclic, flexible substituents (Table 1; compare **4** and **5**), consistent with an accessible ribose pocket of limited dimensions. Oxygen- and nitrogen-containing heterocycles such as the tetrahydropyran-4-yloxy, tetrahydrofuran-3-yloxy, and the (*N*-methylpiperidin-4-yl)oxy rings proved overall to be preferred (Table 1, **5** and **7**−**10**). Comparison of compounds **5** and **22** shows that these C-5 cyclic substituents can improve enzyme affinity by ∼10-fold over a C-5 hydrogen. When we previously applied this C-5 substitution strategy to the design of novel anilinoquinazoline inhibitors of EGFR-TK (epidermal growth factor receptor tyrosine kinase), we observed that a basic group at C-5 was clearly beneficial to sustain good potency,[64] probably due to the presence of aspartic residues within the EGF kinase ribose pocket. Unlike that, in c-Src the presence of a basic group at C-5 does not affect potency (compare compounds **1**, **4**, and **2**) despite the fact that the aspartic residues thought to play a role in charge interactions with the EGFR-TK inhibitors are conserved in c-Src (Asp404 in the conserved DFG motif and Asp348). This suggests that the acidic side chains might be orientated differently in these two enzymes and have a different impact on the electrostatic surface surrounding the ribose pocket. From an in-house crystal structure of our best compound **33** complexed with c-Src, the tetrahydropyran ring fits tightly in the ribose pocket (Figure 3). The interactions with the protein are predominantly hydrophobic, and no H-bonding interaction

Scheme 2[a]

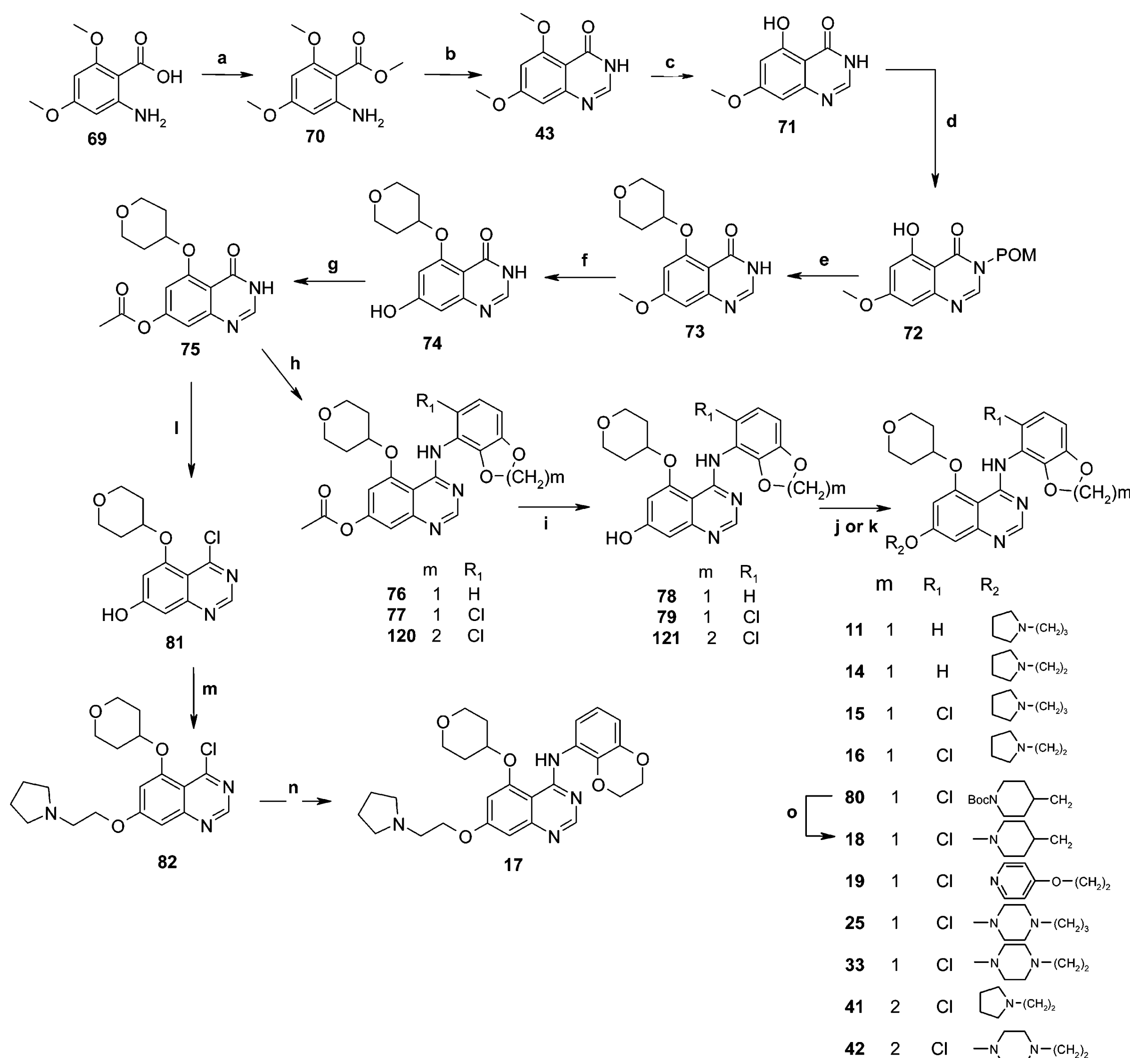


[a] Reagents and conditions: (a) $(ClCO)_2$, 170 °C, 1 h; (b) NaOH, H_2O_2, H_2O, 70 °C; (c) Diazald, EtOH, NaOH, DCM, 0 °C; (d) formamidine acetate, 2-methoxyethanol, reflux, 2 h; (e) (i) $POCl_3$, DIPEA, DCE, 80 °C, 2 h; (ii) 2-chloro-5-methoxyaniline, ^{i}PrOH, 80 °C, 30 min; (f) 2 equiv of HCl, pyridine, reflux, 9 h; (g) Ph_3P, DTAD, R_1H, DCM; (h) TFA, 80 °C, 5 h; (i) Ph_3P, DTAD, ROH, DCM.

is observed between the ring oxygen and the ribose pocket. Due to the presence of the bulky chlorobenzodioxole, the side chain of Asp404 is indeed deflected opposite the hydrophobic pocket. Increasing the degree of flexibility of the C-5 substituent as illustrated by the larger and more flexible (*N*-methylpiperidin-4-yl)methoxy substituent (compound **26**) or conversely reducing its flexibility as illustrated by the rigid and directly linked morpholinyl substituent (compound **20**) sustained a good level of enzyme inhibition, although up to 12-fold lower than that of the corresponding tetrahydropyran-4-yloxy or (*N*-methylpiperidin-4-yl)oxy equivalent (compare **26** and **3** and **20** and **16**). Branched alkyl chains, such as isopropoxy, gave potency equivalent to that of the broad range of tolerated cyclic nuclei, as shown by compounds **6**−**8**, **10**, **15**, **24**, **30**, and **36**. The improvement in affinity achieved with the C-5 tetrahydropyran is comparable in magnitude to that observed by interaction with the protein through the C-7 position of the quinazoline as shown by the comparison of **22** with **5** and **35**.

In the C-5 series, investigation of the substitution pattern of the aniline also proved extremely important to help improve the potency of our inhibitors. Bicyclic anilines such as the benzodioxole (compounds **11**, **12**, and **14**) were tolerated, although they were about 5-fold less potent than the monocyclic 2′-chloro-5′-methoxyanilines (compare **12** and **5**). Expansion of the benzodioxole ring size by one carbon atom as shown by the benzodioxane nucleus retained a good level of c-Src enzyme inhibition (comparison of compounds **12** and **13** and **14** and **17**). Interestingly, this result differs significantly from that observed in the C-6, C-7 quinazoline series where the benzodioxane was 26-fold less active than the benzodioxole equivalent.[27] This possibly suggests that the C-5 substituent slightly distorts the ATP site of the c-Src enzyme, thus slightly opening up the entrance of the hydrophobic pocket to accommodate the increased size of the benzodioxane ring. Further, introduction of a chlorine atom at the C-6′ position of the 2,3-(methylenedioxy)aniline ring (see Table 1) (compare compounds **6**, **11**, **12**, and **14**−**17**), as observed in our previous work,[27] also led to a significant improvement in potency of up to 15-fold. The chlorine atom is thought to add to the lipophilic interaction of the compound with an alanine residue within the c-Src kinase selectivity pocket (Ala403), thus increasing the binding affinity (Figure 4). The chlorine substituent can be replaced by bromine without loss in potency.[65] Replacement by the smaller halogen fluorine led to a slight but consistent reduction in potency

Scheme 3[a]



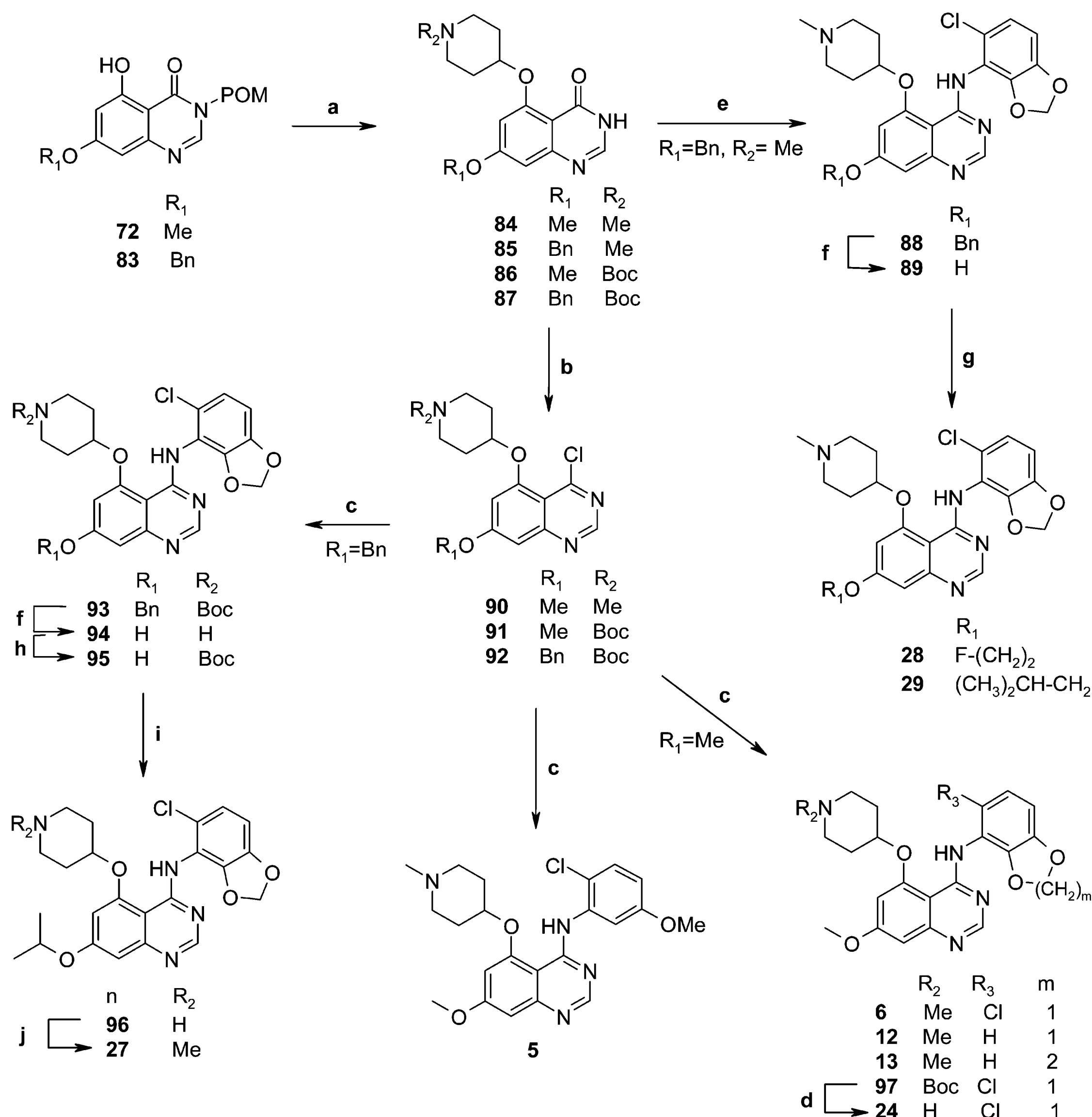
[a] Reagents and conditions: (a) Diazald, EtOH, NaOH, DCM, 0 °C; (b) formamidine acetate, 2-methoxyethanol, reflux, 2 h; (c) $MgBr_2$, pyridine, reflux; (d) NaH, ClPOM, 0 °C, DMF; (e) (i) Ph_3P, DTAD, 4-hydroxy-THP, DCM; (ii) MeOH, NH_3, overnight; (f) PhSH, K_2CO_3, NMP, 195 °C, 30 min; (g) Ac_2O, catalytic pyridine, 80 °C, 30 min; (h) (i) $POCl_3$, DIPEA, DCE, 80 °C, 2 h; (ii) aniline, ^{i}PrOH, 80 °C, 30 min; (i) MeOH, NH_3; (j) R_2Cl, K_2CO_3, DMF, 95 °C, 30 min; (k) Ph_3P, DTAD, R_2OH, DCM, 30 min; (l) (i) Ph_3P, CCl_4, DCE, 70 °C, 2 h; (ii) MeOH, NH_3, 2 h; (m) Ph_3P, DTAD, 2-pyrrolidin-1-ylethanol, DCM; (n) $ArNH_2$, ^{i}PrOH, reflux, 1.5 h; (o) HCHO, HCOOH, 100 °C.

(compare **31** and **32**). A subsequent in-house crystal structure of the benzodioxane derivative **17** in complex with c-Src has provided interesting information. Compound **17** binds similarly to the benzodioxole derivative **33** except that the benzodioxane ring, which occupies the hydrophobic pocket, is rotated by about 180° compared to the chlorobenzodioxole ring. This particular orientation may be necessary to relieve the steric bulk resulting from the larger benzodioxane ring. Interestingly, in this orientation the ethylenedioxy bridge is no longer in the vicinity of the gatekeeper residue (Thr338 in c-Src, Val916 in KDR), which may explain the good potency to KDR and the reduced selectivity.

We also noticed that the potency of the C-5-substituted series was again increased by up to 25-fold by the introduction of an electron-donating group (EDG) at the C-7 position of the quinazoline nucleus (compare **1** and **5**, **3** and **6**, **23** and **24**, and **3** and **28**). In this C-7 position a large diversity of side chains (basic, heterocyclic, heteroaromatic, neutral, etc.) were tolerated by the enzyme with compounds showing low nanomolar inhibition of c-Src (compounds **15**, **18**, **19**, **23**−**25**, and **27**−**33**). Moreover, this region of the molecule is exposed to the solvent and thus proved to be ideal to anchor flexible side chains bearing solubilizing groups to not only enhance the in vitro potency but also optimize the physicochemical properties of this series.[55,66] Interestingly, the improvement in potency achieved with the additional C-7 substituent proved to some extent to be dependent on its relative basicity (compare **5** and **1**, **7** and **2**, and **2** and **21**). Steric hindrance near the quinazoline was disfavored as indicated by the 10−25-fold reduction in potency observed with the isopropoxy derivative **27** compared with unbranched side chains, as illustrated by **6**, **28**, and **29**. If a second EDG is introduced at the C-6 position (*ortho* relative to that at C-5), the activity of the resulting compound is reduced by 17−100-fold compared with that of the C-5, C-7 isomer (compare **5** and **38** and **21** and **40**). This can probably be explained by a conformational effect in which the relative *peri* and *ortho* relations of the C-4, C-5, and C-6 substituents forces the C-5 substituent to be oriented slightly out of the plane of the quinazoline and thus induces unfavorable positioning or steric contacts within the ribose pocket.

Kinase Selectivity. Selectivity for c-Src over other tyrosine kinases was one of our major objectives to optimize the tolerance profile of our inhibitors. As shown by the 2′-chloro-5′-methoxyanilinoquinazoline **5**, the C-5 series proved very selec-

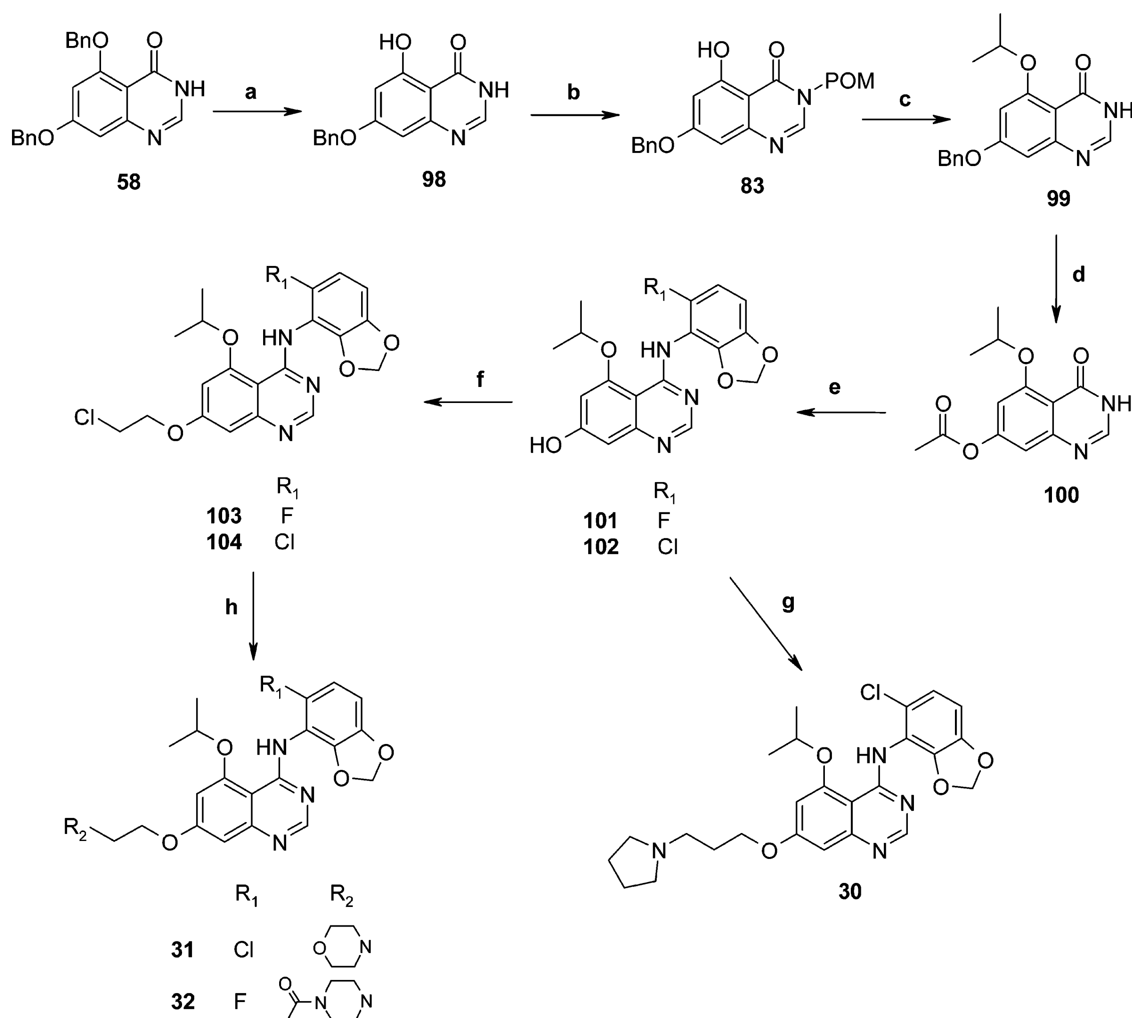
Scheme 4[a]



[a] Reagents and conditions: (a) (i) 1-R_2-piperidin-4-ol, PPh_3, DTAD, DCM; (ii) NaOH or NH_3, MeOH; (b) PPh_3, CCl_4, DCE, 70 °C, 2 h; (c) aniline, ^{i}PrOH, reflux; (d) HCl, Et_2O; (e) (i) PPh_3, CCl_4, DCE, reflux, 2 h; (ii) aniline, ^{i}PrOH, 80 °C, 1.5 h; (f) TFA, reflux, 6 h; (g) R_1OH, PPh_3, DTAD, DCM; (h) Boc_2O, DMF; (i) (i) ^{i}PrOH, PPh_3, DTAD, DCM; (ii) HCl, Et_2O; (j) $NaBH(OAc)_3$, HCHO, AcOH, MeOH.

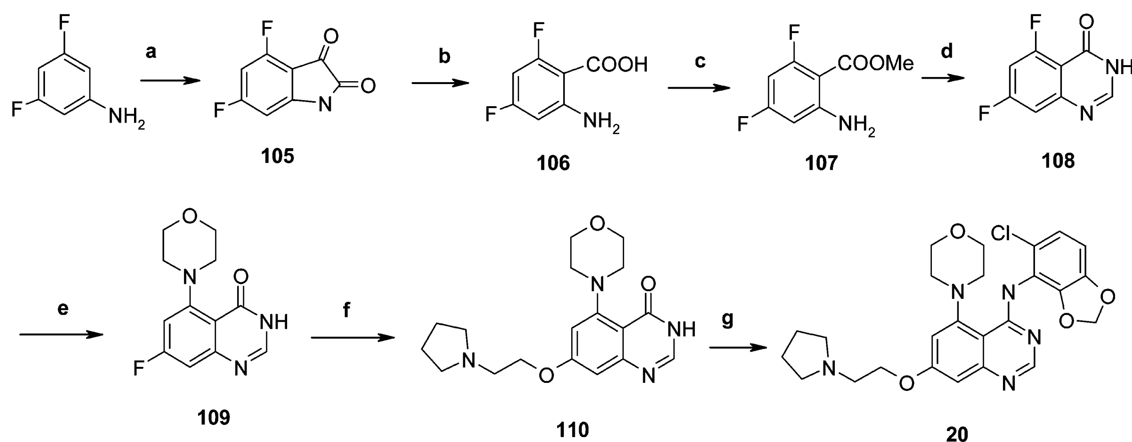
tive for c-Src over VEGFR-2 (vascular endothelial growth factor receptor 2, also known as KDR) (c-Src/KDR IC_{50} ratio >70-fold). We had previously reported that in the binding to KDR and c-Src of our C-6,C-7-disubstituted quinazoline-based inhibitors[27] the quinazoline ring is sandwiched between the N- and C-terminal domains of the kinase and forms a number of hydrophobic contacts (Figure 4). The exploitation of the hydrophobic pocket had led us to design and develop the selective (4-aminobenzodioxolyl)quinazoline series.[27] In the C-6,C-7-disubstituted quinazoline series, the 6′-*H*-benzodioxolamine fits particularly well into the hydrophobic pocket, providing an excellent increase in selectivity over that of the monocyclic 2′-chloro-5′-methoxyaniline, in particular versus KDR.[27] The increase in selectivity between c-Src and KDR observed in the C-6, C-7 series had been rationalized by the presence of a threonine at the entrance of the selectivity pocket in c-Src (Thr338), which gives more favorable contacts with the benzodioxole oxygens than the corresponding Val916 present in KDR.[27,67] In contrast to what we reported in the C-6, C-7 series,[27] the 6′-*H*-benzodioxole aniline unexpectedly led to a 5−10-fold reduction in selectivity for c-Src over KDR compared with the monocyclic aniline as shown by the comparison of **12** and **5** and **11** and **7**. In the C-5 series, as previously suggested for the c-Src enzyme, the size of this C-5 substituent probably distorts slightly the entrance of the hydrophobic/selectivity pocket of KDR, thus minimizing the differences between the two enzymes and leading to a reduced selectivity. As a direct consequence of the benzodioxane ring fitting KDR quite well in the C-5 quinazoline series, the selectivity of these benzodioxane derivatives for c-Src over KDR was reduced by 6−20-fold compared with that of the corresponding benzodioxole (compare **14** and **17** and **12** and **13**). An improvement in selectivity was achieved in the C-5-substituted benzodioxole series by the introduction of the 6′-chlorine atom as shown by the comparison of compounds **14** and **16** and **11** and **15** (Figure 4). The chlorine lies in a hydrophobic groove within the selectivity pocket, lined by an alanine residue in c-Src (Ala403), whereas this residue is a sterically and electronically less favorable cysteine in KDR. Overall this series proved to deliver excellent selectivity for c-Src inhibition over KDR inhibition as shown by the KDR/c-

Scheme 5[a]



[a] Reagents and conditions: (a) $MgBr_2$, pyridine, reflux, 2 h; (b) NaH, ClPOM, 0 °C, DMF; (c) (i) Ph_3P, DTAD, ^{i}PrOH, DCM; (ii) MeOH, NH_3, overnight; (d) (i) $NH_4 + CO_2^-$, 10% Pd/C, DMF; (ii) Ac_2O, catalytic pyridine, 80 °C, 30 min; (e) (i) $POCl_3$, DIPEA, DCE, 80 °C; (ii) aniline, ^{i}PrOH, 80 °C, 30 min; (iii) MeOH, NH_3, 2 h; (f) DCE, DMF, K_2CO_3, 80 °C, 24 h; (g) Ph_3P, DTAD, 3-pyrrolidin-1-ylpropan-1-ol, DCM; (h) amine, K_2CO_3, KI, DMF, 80 °C.

Scheme 6[a]

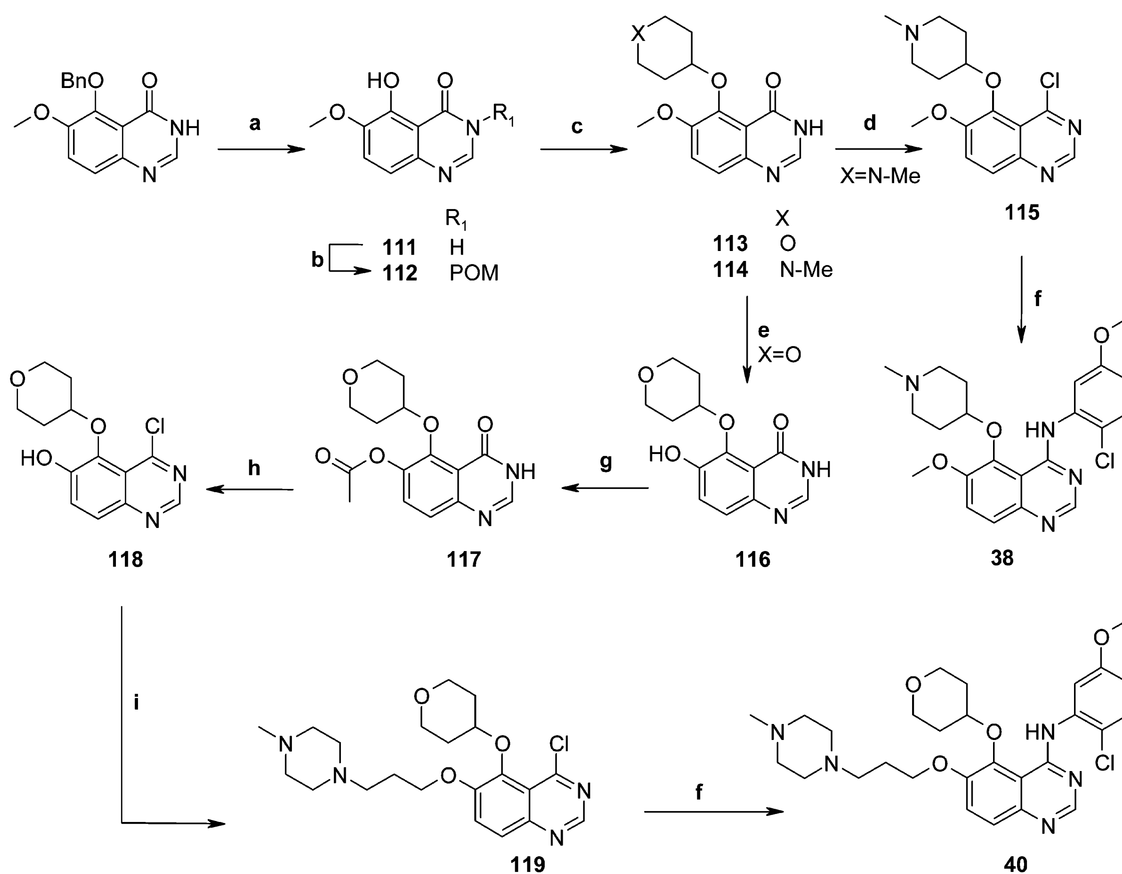


[a] Reagents and conditions: (a) (i) Chloral, NH_2OH, H_2O; (ii) H_2SO_4; (b) NaOH, H_2O_2; (c) Ph_3P, DEAD, MeOH, CH_2Cl_2; (d) formamidine acetate, methoxyethanol, reflux; (e) morpholine, DMF; (f) 2-pyrrolidin-1-ylethanol, NaH, DMF; (g) (i) $POCl_3$, DIPEA; (ii) $ArNH_2$, ^{i}PrOH, 80 °C.

Src IC_{50} ratios (62-fold to >7000-fold) of compounds **6**, **15**, **16**, **18**, **19**, **23**−**25**, and **28**−**33** (Table 1).

The excellent selectivity of these C-5-substituted anilinoquinazolines for c-Src reported in Table 1 is not limited to KDR. As shown in Table 4, this class of compounds proved to be very selective against a range of kinases including cell cycle kinases such as CDK2 and Aurora as well as antiproliferative receptor and nonreceptor kinases such as EGFR-TK and MEK. They also proved to be selective over Csk, the natural inactivator of c-Src as demonstrated by compounds **15** and **33** (Csk/c-Src IC_{50} ratios, respectively, >70-fold and 310-fold). However, this series of compounds proved to possess significant activity versus

Scheme 7[a]



[a] Reagents and conditions: (a) TFA; (b) NaH, ClPOM, 0 °C, DMF; (c) (i) Ph_3P, DTAD, ROH, DCM; (ii) MeOH, NH_3, overnght; (d) Ph_3P, CCl_4, DCE, 70 °C, 2 h; (e) PhSH, K_2CO_3, NMP, 195 °C, 30 min; (f) aniline, HCl(cat.), IPA, reflux; (g) Ac_2O, catalytic pyridine, 80 °C, 30 min; (h) (i) Ph_3P, CCl_4, DCE, 70 °C, 2 h; (ii) MeOH, NH_3, overnight; (i) Ph_3P, DTAD, ROH, DCM.

other c-Src kinase family members and c-Src-related kinases such as c-Yes and Lck as well as v-Abl (Table 4). The kinase selectivity profile of **33** is representative of this series of molecules and demonstrates potent dual-specific inhibition of c-Src and Abl kinases. The functional redundancy demonstrated in mouse gene knockout experiments among the ubiquitously expressed SFKs c-Src, Yes, and Fyn coupled with emerging evidence suggestive of similar mechanisms in cancer cells suggests a pan SFK selective agent, such as AZD0530, might be the profile required to ensure maximum inhibition of SFK activity in cancer cells and tissues. It is anticipated a pan SFK inhibitor could impact aspects of the immune cell function. Although we have not seen evidence of this in preclinical studies with AZD0530, active monitoring for immune cell and immune function effects of the drug is being carried out in man.

In Vitro Cellular Activity. Inhibition of c-Src activity in cells was evaluated in mouse NIH 3T3 cells transfected with constitutively active human c-Src. The C-5-substituted anilinoquinazoline series showed good permeability through cell membranes (**15**, $P_{A-B} > 1 \times 10^6$ cm/s; **33**, $P_{A-B} > 30 \times 10^6$ cm/s), and consequently, an inhibition of c-Src at concentrations below 10 nM in the enzyme assay translated into inhibition of the c-Src-transfected NIH 3T3 cell proliferation at concentrations below 100 nM. Inhibition of in vitro random cell motility (chemokinesis) was measured by the ability of compounds to prevent the migration of human lung tumor cells (A549 NSCLC) suspended in an agarose microdroplet. In this assay the activity of this series of molecules was consistently submicromolar and again mirrored the potency found in the enzyme assay. Compound **33** displayed IC_{50} values of 76 nM in the proliferation assay (c-Src-transfected NIH3T3 cells) and 140 nM in the migration assays (A549 cells). The inhibition of migration cannot be attributed to a direct antiproliferative effect on the A549 cells in view of the weak antiproliferative activity of **33** on these cells ($IC_{50} = 14 \pm 1.5$ μM ($n = 4$)). Cellular activity against Abl was evaluated in K562 cells (human line CML). **33** displayed in vitro antiproliferative IC_{50} values of 220 nM ($n = 3$) in K562 (WT Ph+) cells.

Paxillin is a direct substrate of c-Src and is an adaptor/scaffolding protein thought to be essential in linking newly formed focal adhesions to the actin cytoskeleton. This link helps provide the contractile forces required for cell motility. Measurement of paxillin phosphorylation in tumor cells provides a reliable in vitro mechanistic measure of c-Src activity, which is linked to the adhesion/motility signaling pathway. Compounds of this series displayed good activity in this assay,[68] and in particular **33** inhibited paxillin phosphorylation in vitro in A549 cells by 70% at a concentration of 1 μM (measured by Western blot).

Moreover, the excellent selectivity observed for these compounds at the kinase enzyme level was confirmed in cell assays against selected targets with representatives of the series.[68]

Physicochemical Properties and DMPK. As illustrated by compounds **19** and **31**, the solubility of almost neutral [(6′-chlorobenzodioxolyl)amino]quinazolines is moderate at physiological pH (Table 3). We had shown previously that the solubility of anilinoquinazolines could be improved by the introduction of a basic nitrogen.[66] Introduction of basic side chains at either the C-5 or the C-7 position of the quinazoline core led to a very significant (>500-fold) increase in solubility

Table 2. Pharmacokinetic Parameters of Compounds **15**, **18**, **33**, and **35**

	33			**15**		**18**		**35**
	male rat[a]	female rat[a]	male dog	rat[b]	male dog	female rat[b]	male dog	male dog
$t_{1/2}$ (h)	5−7	5−7	7−19	3.5	29		22	4.2
V_d (L/kg)	10	10	11.6 ± 2.5	15	38	23.4	38	43
Cl [(L/h)/kg]	1.2	1	0.7 ± 0.1	2.9	0.9	7.9	0.3	2.1
F (%)	79	92	>50	60	85	93	57	42

[a] Rat doses: po, 25 mg/kg; iv, 2 mg/kg. [b] Rat doses: po, 20 mg/kg; iv, 2 mg/kg.

Table 3. Physicochemical Properties of Compounds **6**, **15**, **19**, **31**, **33**, and **35**

	6	**15**	**19**	**31**	**33**	**35**
molecular weight	442	527	537	487	542	443
polar surface area	85	96	114	101	106	80
no. of donor and acceptor H bonds	9	10	11	10	11	8
no. of rotatable bonds	5	9	9	8	8	7
pK_a[a]	8.2	9.5	6.4	6.5	7.98	~9
log $D_{7.4}$	2.9	2.3	>3.1	3.7	2.9	2
solubility (μM) at pH 7.4	>1000	1000	1.5	2	240[b]	324
f_u[c] (%) in rat	25	16	1.7	2.5	13	7.5
f_u (%) in dog		17			14	
f_u (%) in man					9	

[a] pK_a of the basic side chain. [b] Measured at pH 7. [c] Fraction unbound to plasma protein.

Table 4. Kinase Selectivity Profile of Compounds **15** and **33**[a]

	15		**33**	
enzyme	IC_{50} (μM)	no. of tests	IC_{50} (μM)	no. of tests
c-Src	<0.004	4	0.0027 ± 0.0005	16
VEGFR-2	2.5 ± 0.8	4	20.9 ± 4.1	4
Csk	0.27 ± 0.3	3	0.84 ± 0.31	3
c-Yes	0.003 ± 0.001	3	0.004 ± 0.001	2
Lck	0.007 ± 0.001	3	<0.004	2
v-Abl	nt		0.03	2
c-Kit	nt		0.2	2
Flt-1	>33	2	>100	2
Flt-4	>10	2	>10	3
EGFR	0.9 ± 0.1	2	2.59	2
FGFR-1	37	1	>10	2
MEK	nt		14	
Aurora-3	8.9	1	>10	1
CDK-2	>10	2	10	1
PDGFR-β	nt		>5	3
PDGFR-α	nt		10	2
MAPKK	nt		14	1

[a] IC_{50} > 10 μM for the following enzymes: GSK3b, Chk, JNK, PDK, PKA, PKCa, PI3K.

when measured at pH 7.4 by virtue of protonation of the basic moiety. Even with moderately basic C-7 substituents such as (*N*-methylpiperazinyl)ethoxy (compound **33**, pK_a = 7.9), the solubility increased very significantly compared with that of their neutral counterparts (comparison of compounds **33** and **19**). Moreover, the basic function led to a 5−15-fold increase in the fraction unbound in the plasma of these derivatives as illustrated by compounds **6**, **15**, and **33** over **19** and **31**.

To design compounds to incorporate good oral bioavailability amenable for chronic oral administration, we paid particular attention to the molecular properties that have been reported to affect absorption such as the total polar surface area (PSA), molecular weight (MW), H-bond donor−acceptor (HBDA) properties, and number of rotatable bonds.[69] Our C-5 derivatives were designed to possess moderate PSA and a minimum number of rotatable bonds. This approach led us to design compounds with excellent exposure as shown by the total area under the curve ($AUC_{0-24\,h}$) in mice following oral administration (Table 5). However, all the C-5-substituted subseries are not equivalent

Table 5. Mouse Total Plasma Levels

compd	$AUC_{0-24\,h}$[a] [(μg·h)/mL]	compd	$AUC_{0-24\,h}$[a] [(μg·h)/mL]	compd	$AUC_{0-24\,h}$[a] [(μg·h)/mL]
6	0.05	**19**	6	**34**	38
15	13	**24**	0.1	**37**	13
16	9	**29**	0.6		
18	31	**33**	12		

[a] After administration of a 20 mg/kg oral dose.

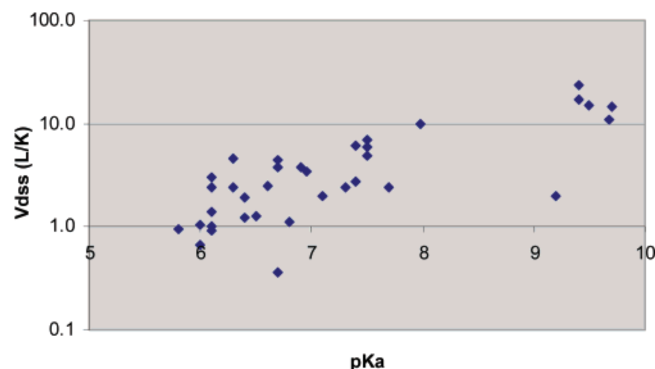


Figure 5. Correlation between volume of distribution V_d (L/kg) and pK_a.

with regard to their ADME properties. Of the two main subseries, the C-5 tetrahydropyranyl one (compounds **15** and **16**) always demonstrated significantly higher oral exposure than the C-5 piperidine one (compounds **6**, **24**, and **29**) despite the excellent Lipinski properties[69] and moderate pK_a of the latter (compound **6**, Table 3). The difference between the two C-5 subseries was suggested to be due to reduced permeability and increased efflux properties of the C-5 *N*-methylpiperidine series. In the C-5 tetrahydropyranyl series, absorption is good and excellent exposure is achieved even when basic side chains are present at C-7 as shown by compounds **15** (pK_a = 9.5) and **18** (pK_a = 9.4). The C-5-substituted series compares very favorably from an ADME properties point of view with the C-6,C-7-disubstituted series we previously reported (comparison of **15** and **18** and **34** and **37**). As shown in Table 2, anilinoquinazolines bearing basic functions can display large volumes of distribution (V_d) which tend to lead to long half-lives (**15**, **18**, **35**) when combined with moderate to low clearance.[66] Similarly to what was observed in the C6,C-7-disubstituted anilinoquinazolines, we have shown that in this C-5, C-7 series, the modulation of the pK_a of the side chain (at C-5 or C-7) correlated generally well with the V_d (Figure 5) for compounds displaying moderate clearance and quite similar unbound fractions in plasma.

This in turn permitted a relatively precise control of the $t_{1/2}$ of the final molecule. Moreover, we identified that the pK_a of the side chain was also playing an important role with regard to the affinity for the hERG receptor of our compounds. Very basic compounds proved to have more affinity for this receptor than less basic or neutral ones. With its moderate pK_a (7.9), compound **33** displayed very low affinity for the hERG channel.

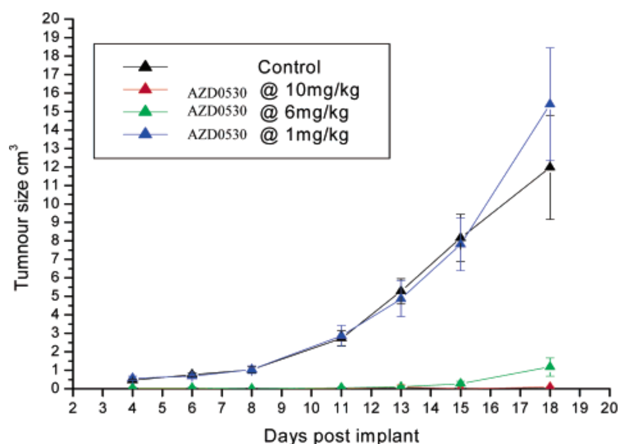


Figure 6. Inhibition of an Src-transfected 3T3 tumor xenograft following daily oral administration of AZD0530.

When tested in animal models over a range of doses, **33** did not display modification of the ECG nor prolongation of the QT interval of ECGs.[65] Compound **33** represented a good compromise among moderate pK_a, leading to excellent bioavailability and general ADME properties in preclinical species (Table 2), low affinity for hERG, and good physicochemical properties. The pharmacokinetic profiles of **33** observed in rat and dog (Table 2) were subsequently confirmed in man and proved consistent with once daily oral dosing with a mean terminal half-life at steady-state of 40 h (range 32−47) and low interpatient variability.[70]

In Vivo Activity. Mouse NIH 3T3 fibroblasts engineered to overexpress a deregulated, constitutively active form of human c-Src have a phenotype altered from that of the wild-type parental line. In vivo, the transfected cell line grows subcutaneously to form tumors in athymic rats and mice, while the wild-type 3T3 cells do not. Implanted subcutaneously in athymic rats, the transfected lines develop large tumors in approximately 3 weeks. Animals treated with **15** orally once daily at doses of 10 (mg/kg)/d for 18 days show 90% inhibition of tumor volume, while animals treated with **33** orally (Figure 6) once daily at doses of 6 (mg/kg)/d for 18 days show >95% inhibition of tumor volume and tumor weight at the end of the experiment.

The activity observed in this model is unlikely to be due to a direct inhibition of tumor angiogenesis. As expected from its very weak activity against VEGFR-2, **33** does not inhibit the in vitro VEGF- or FGF-stimulated growth of HUVECs (VEGF−HUVEC and FGF−HUVEC $IC_{50} > 5\ \mu M$ ($n = 4$)) and was shown to be inactive in in vivo angiogenesis assays.[65] Moreover, inhibition of paxillin phosphorylation at tyrosine Y31 as well as FAK (focal adhesion kinase) tyrosine phosphorylation demonstrated in vitro was confirmed in vivo. More than 80% inhibition of both paxillin and FAK phosphorylation has been demonstrated in Calu-6 xenograft tumors 6 h post last dose following oral administration of 20 (mg/kg)/d of **33**.[68]

In this xenograft model **33** is more active than our previously reported 2′-chloro,5′-methoxyanilinoquinazoline derivative **35** (M475271) and delivers complete inhibition of tumor growth at 1/10 of the dose required by **35** to achieve the same effect.[27]

c-Src is overexpressed in pancreatic adenocarcinomas, and we have previously reported that **35** prevented the formation of metastasis in an orthotopic L3.6pl human pancreatic tumor model. Untreated animals developed liver metastasis within a few weeks, whereas complete inhibition of liver metastasis formation was achieved with once daily oral administration of 25 (mg/kg)/d of **35**.[71] Combined with the superior activity of **33** in the NIH3T3 model, this suggested that **33** should also prevent formation of metastasis in vivo. We also demonstrated that AZD0530 prevents the formation of lymph node metastasis in a bladder model (NBT-II) and liver metastasis in an orthotopic L3.6 human pancreatic tumor model.[68,72] Moreover, in an orthotopic model of human pancreas (BxPC-3 cells), athymic mice treated with **33**, given orally once daily at doses of 25 (mg/kg)/d for more than 50 days, demonstrated a significant 38% ($p < 0.05$) increase in survival compared with control animals which may, in part, be attributable to an anti-invasive activity in this aggressive orthotopic tumor model.[85]

Conclusions

C-5-substituted (benzodioxolylamino)quinazolines are potent and dual-specific inhibitors of c-Src and Abl kinases. Their enzyme inhibition profiles translate very well into activity in cells in vitro in terms of inhibition of both proliferation and cell migration. The presence of a moderately basic side chain at the C-7 position confers excellent physicochemical properties, in particular good aqueous solubility and moderate binding to plasma proteins. The C-5 tetrahydropyranyl series possesses good pharmacokinetic properties and demonstrates appropriate exposure following oral administration to rodents and dogs. These properties, combined with good intrinsic potencies, led to good in vivo activity in a range of preclinical models. The full exploitation of our knowledge of the shape, size, and properties of the different binding sites of c-Src and KDR and of the best fit substituents led to the design of **33 (**AZD0530). **33** displays potent c-Src enzyme inhibition ($IC_{50} = 2.7$ nM) and excellent selectivity for c-Src over KDR (7740-fold). In view of its good physicochemical properties, selectivity profile, pharmacokinetics, and activity in preclinical models, compound **33** was selected for further development and is currently undergoing phase I clinical evaluation in patients with advanced cancers.

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 run isocratically with 60−70% MeOH + 0.2% CF_3COOH in water as eluent. Purification by preparative HPLC/MS was performed on a Waters LC/MS system using a Waters Symmetry column (C18, 5 μm, 19 mm diameter, 100 mm length) with a mixture of water (containing 1% acetic acid) and acetonitrile (gradient from 5% to 100%) as solvent. NMR spectra were obtained on a JEOL JNM EX 400 (400 MHz) spectrometer and Bruker Avance 500 (500 MHz) spectrometer. Chemical shifts are expressed in δ (ppm) units, and peak multiplicities are expressed as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; br s, broad singlet; m, multiplet. Mass spectrometry was carried out 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 were consistent with the proposed structures. For the microanalysis, all the adducts mentioned were measured: water was assayed by the Karl-Fisher method using a Mettler DL 18, the HCl content was determined on a Metrohm 686 by titration using silver nitrate solution, and organic adducts were measured by ^{1}H 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, (pivaloyloxy)methyl; TFA,

trifluoracetic acid; DCM, dichloromethane; THF, tetrahydrofuran; DIPEA, *N*,*N*-diisopropylethylamine; NMP, *N*-methylpyrrolidone.

***N*-(2-Chloro-5-methoxyphenyl)-5-[(1-methylpiperidin-4-yl)oxy]quinazolin-4-amine (1).** To a mixture of **47** (150 mg, 0.5 mmol), triphenylphosphine (210 mg, 0.8 mmol), and 1-methylpiperidin-4-ol (69 mg, 0.6 mmol) in dichloromethane (4 mL) at room temperature, DTAD (184 mg, 0.8 mmol) was added slowly. The mixture was stirred for 2 h at room temperature, the solvent evaporated, and the residue purified by flash chromatography using a mixture of dichloromethane/ethyl acetate (80:20) to remove the impurities and then using a mixture of dichloromethane/methanol (98:2). After evaporation of the solvent, **1** was dissolved in diethyl ether and treated with a 5 N solution of HCl gas in diethyl ether (100 μL) to give 100 mg of **1** as a hydrochloride (50%): ^{1}H NMR ($CDCl_3$) (free base) δ 2.0−2.1 (m, 2H), 2.1−2.2 (m, 2H), 2.25(s, 3H), 2.75−2.85 (m, 2H), 3.8 (s, 3H), 4.5−4.6 (m, 1H), 6.6 (dd, 1H), 6.9 (d, 1H), 7.25 (d, 1H), 7.4 (d, 1H), 7.6 (dd, 1H), 8.1 (d, 1H), 8.6 (s, 1H); MS-ESI *m*/*z* 399 and 401 [MH$^+$]. Anal. ($C_{21}H_{23}N_4O_2Cl{\cdot}H_2O{\cdot}2HCl$) C, H, N.

A similar procedure was used to prepare **2**.

***N*-(5-Chloro-1,3-benzodioxol-4-yl)-5-[(1-methylpiperidin-4-yl)oxy]quinazolin-4-amine (3).** To a suspension of 60% sodium hydride (100 mg, 2.4 mmol) in DMF (4 mL) was added 1-methylpiperidin-4-ol (50 mg, 0.42 mmol) under nitrogen at room temperature. The reaction mixture was stirred for 10 min, and then **52** (100 mg, 0.28 mmol) was added. The mixture was heated to 80 °C and stirred for 6 h. After cooling, the solution was poured dropwise into water (20 mL), extracted twice with ethyl acetate, dried over magnesium sulfate, and concentrated. The crude was purified by SiO_2 chromatography eluting with methanol/dichloromethane/ethyl acetate (2:48:50) and then with a solution of 7 N ammonia in methanol/dichloromethane/ethyl acetate (3:47:50) to give 54 mg of **3** (46%) after evaporation of the solvent: ^{1}H NMR ($CDCl_3$) δ 2.02 (m, 2H), 2.17 (m, 2H), 2.25−2.40 (m, 5H), 2.70 (m, 2H), 4.64 (m, 1H), 6.01 (s, 2H), 6.69 (d, 1H), 6.87 (d, 1H), 6.94 (d, 1H), 7.42 (d, 1H), 7.60 (t, 1H), 8.56 (s, 1H), 9.47 (s, 1H); MS-ESI *m*/*z* 413 and 415 [MH]$^+$. Anal. ($C_{21}H_{21}ClN_4O_3{\cdot}0.8H_2O$) C, H, N.

***N*-(2-Chloro-5-methoxyphenyl)-7-methoxy-5-(3-morpholin-4-ylpropoxy)quinazolin-4-amine (4).** To a mixture of **44** (200 mg, 0.6 mmol), triphenylphosphine (240 mg, 0.9 mmol), and 3-morpholin-4-ylpropan-1-ol (130 mg, 0.9 mmol) in dichloromethane (3 mL) was added portionwise DTAD (210 mg, 0.9 mmol) under nitrogen at room temperature. The solution was stirred for 1 h and then poured onto a column of silica gel eluting with pure dichloromethane to remove impurities and then with a solution of 7 N ammonia in methanol/dichloromethane (1:99). Evaporation of the solvent gave a solid which was triturated in diethyl ether, filtered off, and washed with diethyl ether to give 130 mg of **4** (48%): ^{1}H NMR (DMSO-d_6) δ 2.05 (m, 2H), 2.29 (m, 4H), 2.42 (t, 2H), 3.50 (t, 4H), 3.79 (s, 3H), 3.91 (s, 3H), 4.45 (t, 2H), 6.76 (dd, 1H), 6.82 (d, 1H), 6.85 (d, 1H), 7.46 (d, 1H), 8.30 (d, 1H), 8.52 (s, 1H), 10.09 (s, 1H); MS-ESI *m*/*z* 459 and 461 [MH]$^+$. Anal. ($C_{23}H_{27}ClN_4O_4$) C, H, N.

***N*-(2-Chloro-5-methoxyphenyl)-7-methoxy-5-[(1-methylpiperidin-4-yl)oxy]quinazolin-4-amine (5).** To a mixture of **90** (675 mg, 2.2 mmol), 2-chloro-5-methoxyaniline hydrochloride (510 mg, 2.6 mmol) in propan-2-ol (5 mL) was added a 5 N solution of HCl gas in propan-2-ol (36 μL, 0.2 mmol), and the resulting mixture was heated at 80 °C for 1.5 h. After cooling, the formed precipitate was filtered off and washed with propan-2-ol and then diethyl ether to give 1 g of **5** as a dihydrochloride salt (93%). ^{1}H NMR (DMSO-d_6) δ 2.15−2.35 (m, 2H), 2.40 (m, 2H), 2.76 (d, 3H), 3.12 (m, 2H), 3.55 (m, 2H), 3.80 (s, 3H), 3.98 (s, 3H), 5.08 (m, 0.75H), 5.21 (m, 0.25H), 6.95 (dd, 0.75H), 7.02 (m, 1H), 7.09 (d, 0.25H), 7.16 (d, 0.75H), 7.47 (d, 0.25H), 7.55 (m, 1H), 7.79 (d, 0.75H), 8.76 (s, 0.25H), 8.81 (s, 0.75H), 10.12 (br s, 0.25H), 10.29 (br s, 0.75H), 10.77 (br s, 0.75H), 11.08 (br s, 0.25H); MS-ESI *m*/*z* 429 [MH]$^+$. Anal. ($C_{22}H_{25}ClN_4O_3{\cdot}2.35HCl{\cdot}0.28C_3H_8O$) C, H, N.

The free base was generated by dissolving the dihydrochloride salt of the compound in a mixture of 7 N ammonia in methanol/dichloromethane (5:95); the resulting precipitate was eliminated by filtration, and the filtrate was evaporated down to give **5** as a free base: ^{1}H NMR ($CDCl_3$) (free base) δ 2.20−2.50 (m, 5H), 2.84 (m, 2H), 3.78 (s, 3H), 3.86 (s, 3H), 4.55 (m, 1H), 6.48 (d, 1H), 6.59 (dd, 1H), 6.80 (d, 1H), 7.25 (d, 1H), 8.51 (s, 1H), 9.70 (br s, 1H); MS-ESI *m*/*z* 443 and 445 [MH]$^+$.

***N*-(2-Chloro-5-methoxyphenyl)-7-(3-morpholin-4-ylpropoxy)-5-(tetrahydro-2*H*-pyran-4-yloxy)quinazolin-4-amine (7).** To a mixture of **65** (100 mg, 0.25 mmol), triphenylphosphine (105 mg, 0.4 mmol), and 3-morpholin-4-ylpropan-1-ol (44 mg, 0.3 mmol) in dichloromethane (5 mL) at 0 °C was slowly added DTAD (92 mg, 0.4 mmol). The mixture was stirred for 2 h at room temperature and was purified by SiO_2 chromatography using a mixture of dichloromethane/methanol (98:2) as eluent to remove the impurities and then a mixture of dichloromethane−methanol/ammonia (97:3) to give 60 mg of **7** (46%) as a white solid: ^{1}H NMR (DMSO-d_6 and CF_3CO_2D) δ 1.9−2.0 (m, 2H), 2.05−2.1 (m, 2H), 2.2−2.3 (m, 2H), 3.1−3.2 (m, 2H), 3.3−3.4 (m, 2H), 3.6−3.7 (m, 4H), 3.7−3.8 (m, 2H), 3.85 (s, 3H), 3.95−4.15 (m, 4H), 4.25−4.35 (m, 2H), 5.1−5.2 (m, 1H), 6.9 (d, 1H), 7.02 (dd, 1H), 7.1 (d, 1H), 7.53−7.55 (m, 2H), 8.87 (s, 1H); MS-ESI *m*/*z* 529 and 531 [MH]$^+$. Anal. ($C_{27}H_{33}ClN_4O_5$) C, H, N.

A similar procedure was used to prepare **8**, **9**, **10**, and **21**.

***N*-1,3-Benzodioxol-4-yl-7-(3-pyrrolidin-1-ylpropoxy)-5-(tetrahydro-2*H*-pyran-4-yloxy)quinazolin-4-amine (11).** To a mixture of **78** (114 mg, 0.3 mmol), triphenylphosphine (126 mg, 0.48 mmol), and 3-pyrrolidin-1-ylpropan-1-ol (47 mg, 3.6 mmol) in dichloromethane (5 mL) at 0 °C was slowly added DTAD (110 mg, 0.48 mmol). The mixture was stirred for 2 h at room temperature and was purified by SiO_2 chromatography using a mixture of 7 N ammonia solution in methanol/dichloromethane (4:96) as eluent to give 93 mg of **11** (63%): ^{1}H NMR (DMSO-d_6) δ 1.7−1.73 (m, 4H), 1.8−1.9 (m, 2H), 1.9−2.0 (m, 2H), 2.1−2.2 (m, 2H), 2.4−2.5 (m, 4H), 2.6 (t, 2H), 3.5−3.6 (m, 2H), 3.88−3.93 (m, 2H), 4.18 (t, 2H), 4.95−5.05 (m, 1H), 6.1(s, 2H), 6.72 (d, 1H), 6.8 (d, 1H), 6.85−6.95 (m, 2H), 8.08 (d, 1H), 8.48 (s, 1H), 9.83 (s, 1H); MS-ESI *m*/*z* 493 [MH]$^+$. Anal. ($C_{27}H_{32}N_4O_5{\cdot}0.1H_2O$) C, H, N.

A similar procedure was used to prepare **14**, **15**, **16**, **19**, **25**, and **33**.

***N*-(5-Chloro-1,3-benzodioxol-4-yl)-7-[2-(4-methylpiperazin-1-yl)ethoxy]-5-(t etrahydro-2*H*-pyran-4-yloxy)quinazolin-4-amine (33, AZD0530).** **79** (190 mg, 0.46 mmol) was reacted with 2-(4-methylpiperazin-1-yl)ethanol (79 mg, 0.55 mmol) to give 180 mg of **33** (AZD0530) (73%): ^{1}H NMR ($CDCl_3$) δ 1.9−2.05 (m, 2H), 2.2−2.3 (m, 2H), 2.31 (s, 3H), 2.4−2.7 (m, 8H), 2.87 (m, 2H), 3.6−3.7 (m, 2H), 3.95−4.05 (m, 2H), 4.25 (m, 2H), 4.7−4.8 (m, 1H), 6.05 (s, 2H), 6.55 (d, 1H), 6.72 (d, 1H), 6.83 (d, 1H), 6.97 (d, 1H), 8.52 (s, 1H), 9.26 (s, 1H); MS-ESI *m*/*z* 542 and 544 [MH]$^+$. Anal. ($C_{27}H_{32}N_5O_5Cl{\cdot}0.15H_2O$) C, H, N.

***N*-1,3-Benzodioxol-4-yl-7-methoxy-5-[(1-methylpiperidin-4-yl)oxy]quinazolin-4-amine (12).** A mixture of **90** (100 mg, 0.33 mmol), 1,3-benzodioxol-4-amine (50 mg, 0.36 mmol), and a 5 N solution of HCl gas in propan-2-ol (70 μL, 0.34 mmol) in propan-2-ol (2 mL) was heated at 80 °C for 1.5 h. After cooling, the formed precipitate was filtered off and washed with propan-2-ol and then diethyl ether to give 120 mg of **12** as a dihydrochloride salt (75%): ^{1}H NMR ($CDCl_3$) (free base) δ 2.05 (m, 2H), 2.26 (m, 2H), 2.33 (s, 3H), 2.84 (m, 2H), 3.92 (s, 3H), 4.55 (m, 1H), 6.02 (s, 2H), 6.50 (d, 1H), 6.66 (d, 1H), 6.84 (d, 1H), 6.90 (t, 1H), 8.00 (d, 1H), 8.58 (s, 1H), 9.72 (br s, 1H); MS-ESI *m*/*z* 409 [MH]$^+$. Anal. ($C_{22}H_{24}N_4O_4{\cdot}2.3HCl{\cdot}2.2H_2O$) C, H, N.

A similar procedure was used to prepare **6** and **13**.

***N*-(2,3-Dihydro-1,4-benzodioxin-5-yl)-7-(2-pyrrolidin-1-ylethoxy)-5-(tetrahydro-2*H*-pyran-4-yloxy)quinazolin-4-amine (17).** A mixture of **82** (95 mg, 0.25 mmol) and 2,3-dihydro-1,4-benzodioxin-5-amine hydrochloride (52 mg, 0.28 mmol) in 2-propanol (3 mL) was heated under reflux for 90 min. After cooling, the precipitate was filtered to give 84 mg of **17** (60%) as a hydrochloride: ^{1}H NMR (DMSO-d_6 and CF_3CO_2D) δ 1.9−2.0 (m, 4H), 2.0−2.1 (m, 2H), 2.1−2.2 (m, 2H), 3.1−3.2 (m, 2H), 3.5−

3.6 (m, 2H), 3.6−3.8 (m, 4H), 3.95−4.05 (m, 2H), 4.35−4.4 (m, 2H), 4.45−4.5 (m, 2H), 4.5−4.6 (m, 2H), 5.15−5.25 (m, 1H), 6.86 (dd, 1H), 6.95 (dd, 1H), 6.99 (d, 1H), 7.2 (d, 1H), 7.99 (d, 1H), 8.94 (d, 1H); MS-ESI *m/z* 493 $[MH]^+$. Anal. ($C_{27}H_{32}N_4O_5$·2HCl·2.7H_2O) C, H, N.

***N*-(5-Chloro-1,3-benzodioxol-4-yl)-7-[(1-methylpiperidin-4-yl-)methoxy]-5-(tetrahydro-2*H*-pyran-4-yloxy)quinazolin-4-amine (18).** A solution of **80** (250 mg, 0.4 mmol) in a mixture of formic acid (5 mL) and 37% aqueous formaldehyde solution (0.5 mL) was heated at 100 °C for 2 h. The volatiles were removed under vacuum, and the residue was made alkaline by addition of a 6 N solution of ammonia in methanol, dissolved in dichloromethane, and purified by SiO_2 chromatography using a mixture of 6 N ammonia in methanol/dichloromethane (4:96) as eluent to give 100 mg of **18** (50%): ^{1}H NMR ($CDCl_3$) δ 1.4−1.5 (m, 2H), 1.75−1.85 (m, 2H), 1.9−2.05 (m, 3H), 2.2−2.3 (m, 2H), 2.29 (s, 3H), 2.9−3.0 (m, 2H), 3.6−3.7 (m, 2H), 3.95 (d, 2H), 4.0−4.1 (m, 4H), 4.7−4.8 (m, 1H), 6.05 (s, 2H), 6.5 (d, 1H), 6.7 (d, 1H), 6.8 (d, 1H), 6.98 (d, 1H), 8.5 (s, 1H), 9.25 (s, 1H)). MS-ESI *m/z* 527 and 529 $[MH]^+$. Anal. ($C_{27}H_{31}N_4O_5$ Cl) C, H, N.

***N*-(5-Chloro-1,3-benzodioxol-4-yl)-5-morpholin-4-yl-7-(2-pyrrolidin-1-ylethoxy)quinazolin-4-amine (20).** A mixture of **110** (260 mg, 0.75 mmol), phosphorus oxychloride (84 μL, 0.9 mmol), and *N*,*N*-diisopropylethylamine (340 μL, 2 mmol) in 1,2-dichloroethane (20 mL) was heated to 75 °C for 2 h. The solvent was evaporated under vacuum, the crude intermediate was reacted with (5-chloro-1,3-benzodioxol-4-yl)amine (140 mg, 0.8 mmol) in 2-propanol (4 mL), and the reaction mixture was heated at 80 °C for 1 h. The volatiles were removed under vacuum, and the residue was made alkaline by addition of a solution of 6 N ammonia in methanol, filtered, and purified by SiO_2 chromatography using a mixture of 6 N ammonia in methanol/dichloromethane (3:97) as eluent to give after evaporation and trituration in diethyl ether 35 mg of **20** (10%): ^{1}H NMR ($CDCl_3$) δ 1.8−1.9 (m, 4H), 2.6−2.7 (m, 4H), 2.98 (t, 2H), 3.0−3.1 (m, 2H), 3.1−3.2 (m, 2H), 4.75−4.85 (m, 2H), 3.95−4.05 (m, 2H), 4.25 (t, 2H), 6.04 (s, 2H), 6.75 (d, 1H), 6.9−7.1 (3d, 3H), 8.52 (s, 1H), 11.4 (s, 1H); MS-ESI *m/z* 498 and 500 $[MH]^+$.

***N*-(5-Chloro-1,3-benzodioxol-4-yl)-7-methoxy-5-(piperidin-4-yloxy)quinazolin-4-amine (24).** **97** (170 mg, 0.32 mmol) was stirred in a 2 N solution of HCl gas in diethyl ether (15 mL) at room temperature for 1 h. The mixture was diluted with diethyl ether, and the precipitate was filtered off, washed with diethyl ether, and dried under vacuum to give 134 mg of **24** as a dihydrochloride salt (89%): ^{1}H NMR (DMSO-d_6) δ 2.2−2.4 (m, 4H), 3.12 (m, 2H), 3.34 (m, 2H), 4.0 (s, 3H), 5.17 (m, 1H), 6.17 (s, 2H), 7.05 (m, 2H), 7.15 (d, 1H), 7.18 (d, 1H), 8.82 (s, 1H), 9.11 (br s, 1H), 9.33 (br s, 1H), 10.09 (br s, 1H); MS-ESI *m/z* 429 and 431 $[MH]^+$. Anal. (free base) ($C_{21}H_{21}ClN_4O_4$·1.45H_2O) C, H, N.

A similar procedure was used to prepare **23**.

***N*-(5-Chloro-1,3-benzodioxol-4-yl)-7-isopropoxy-5-[(1-methylpiperidin-4-yl)oxy]quinazolin-4-amine (27).** To a mixture of **96** (0.125 g, 0.27 mmol), formaldehyde (42 μL, 0.55 mmol), and acetic acid (19 μL, 0.33 mmol) in a 2:5 mixture of methanol/dichloromethane (7 mL) was added portionwise sodium triacetoxyborohydride (0.09 g, 0.41 mmol). The reaction mixture was stirred for 1 h at room temperature and then concentrated. The residue was taken up in ethyl acetate and washed with a 1 N aqueous solution of sodium hydroxide. The aqueous phase was extracted with ethyl acetate, and the organics were combined, washed with brine, and dried ($MgSO_4$). Evaporation of the solvent gave 110 mg of **27** as a white foam (87%): ^{1}H NMR ($CDCl_3$) δ 1.42 (d, 6H), 2.03 (m, 2H), 2.25 (m, 2H), 2.35 (m, 5H), 2.75 (m, 2H), 4.66 (m, 1H), 4.72 (quint.), 6.05 (s, 2H), 6.48 (d, 1H), 6.72 (d, 1H), 6.82 (d, 1H), 6.97 (d, 1H), 8.52 (s, 1H), 9.27 (s, 1H); MS-ESI *m/z* 471 and 473 $[MH]^+$.

A similar procedure was used to prepare **26**.

***N*-(5-Chloro-1,3-benzodioxol-4-yl)-7-(2-fluoroethoxy)-5-[(1-methylpiperidin-4-yl)oxy]quinazolin-4-amine (28).** To a mixture of **89** (120 mg, 0.28 mmol), triphenylphosphine (147 mg, 0.56 mmol), and 2-fluoroethanol (25 μL, 0.42 mmol) in dichloromethane (2 mL) was added a solution of DTAD (130 mg, 0.56 mmol) in dichloromethane (1 mL) at room temperature, and the solution was stirred for 1 h. A 2 N solution of HCl gas in diethyl ether (3 mL) was added to the mixture, then the resulting mixture was stirred for 1.5 h. Then the mixture was diluted with diethyl ether (1 mL), and the precipitate was filtered and taken up in a mixture of a 7 N solution of ammonia in methanol/dichloromethane (1:9). The resulting solid was removed by filtration and the filtrate concentrated to give 74 mg of **28** (56%): ^{1}H NMR ($CDCl_3$) δ 2.05 (m, 2H), 2.21 (m, 2H), 2.3−2.5 (m, 5H), 2.74 (m, 2H), 4.34 (ddd, 2H), 4.63 (m, 1H), 4.83 (ddd, 2H), 6.06 (s, 2H), 6.59 (d, 1H), 6.73 (d, 1H), 6.81 (d, 1H), 6.98 (d, 1H), 8.53 (s, 1H), 9.28 (br s, 1H); MS-ESI *m/z* 475 and 477 $[MH]^+$. Anal. ($C_{23}H_{24}ClFN_4O_4$·0.6H_2O) C, H, N.

A similar procedure was used to prepare **29** and **96**.

***N*-(5-Chloro-1,3-benzodioxol-4-yl)-5-isopropoxy-7-(3-pyrrolidin-1-ylpropoxy)quinazolin-4-amine (30).** To a mixture of **102** (112 mg, 0.3 mmol), triphenylphosphine (126 mg, 0.48 mmol), and 3-pyrrolidin-1-ylpropan-1-ol (46 mg, 0.36 mmol) in dichloromethane (5 mL) at room temperature was added DTAD (110 mg, 0.48 mmol). After the resulting mixture was stirred for 2 h, the solvent was evaporated and the residue purified by preparative LC/MS using a gradient of ammonium carbonate and acetonitrile. After evaporation, the residue was dissolved in dichloromethane, dried ($MgSO_4$), and evaporated to give 120 mg of **30** (84%): ^{1}H NMR ($CDCl_3$) δ 1.5 (d, 2H), 1.8−1.9 (m, 4H), 2.0−2.1 (m, 2H), 2.5−2.6 (m, 4H), 2.6−2.7 (m, 2H), 4.12 (t, 2H), 4.8 (q, 1H), 6.0 (s, 2H), 6.5 (d, 1H), 6.7 (d, 1H), 6.8 (d, 1H), 6.95 (d, 1H), 8.5 (s, 1H), 9.4 (s, 1H); MS-ESI *m/z* 485 and 487 $[MH]^+$. Anal. ($C_{25}H_{29}ClN_4O_4$·2.3H_2O·2.3HCl) C, H, N.

7-[2-(4-Acetylpiperazin-1-yl)ethoxy]-*N*-(5-fluoro-1,3-benzodioxol-4-yl)-5-isopropoxyquinazolin-4-amine (32). A mixture of **103** (28 g, 67 mmol), potassium iodide (22 g, 133 mmol), and *N*-acetylpiperazine (25.7 g, 200 mmol) in DMF (120 mL) was heated at 95 °C under nitrogen for 5 h. After cooling, the reaction mixture was concentrated and the residue taken up in dichloromethane. Solids were removed by filtration, and a 7 N solution of ammonia in methanol was added. The solvent was evaporated off and the crude material purified by SiO_2 chromatography eluting with a gradient of methanol/dichloromethane (4:96 up to 10:90). After evaporation of the solvent, the solid was washed with diethyl ether and filtered off to give 28 g of **32** (83%): ^{1}H NMR ($CDCl_3$) δ 1.52 (d, 6H), 2.10 (s, 3H), 2.58 (quint, 4H), 2.88 (t, 2H), 3.51 (dd, 2H), 3.66 (dd, 2H), 4.23 (t, 2H), 4.81 (quint, 1H), 6.05 (s, 2H), 6.52 (d, 1H), 6.66 (m, 2H), 6.81 (d, 1H), 8.53 (s, 1H), 9.29 (br s, 1H); MS-ESI *m/z* 512 $[MH]^+$. Anal. ($C_{26}H_{30}FN_5O_5$) C, H, N.

A similar procedure was used to prepare **31**.

***N*-(2-Chloro-5-methoxyphenyl)-6-methoxy-5-[(1-methylpiperidin-4-yl)oxy]quinazolin-4-amine (38).** A mixture of **115** (65 mg, 0.21 mmol), (2-chloro-5-methoxyphenyl)amine hydrochloride (49 mg, 0.25 mmol), and a catalytic amount of HCl (from a 6 N HCl solution in propan-2-ol) in propan-2-ol (2 mL) was heated under reflux for 1 h. After cooling, the precipitate was filtered off and washed with propan-2-ol, ethyl acetate, and diethyl ether to give 69 mg of **38** (65%) as a dihydrochloride: ^{1}H NMR (free base) ($CDCl_3$) δ 1.9−2.2 (m, 6H), 2.25 (s, 3H), 2.8−3.0 (m, 2H), 3.85 (s, 3H), 3.99 (s, 3H), 4.40−4.55 (m, 1H), 6.65 (dd, 1H), 7.30 (d, 1H), 7.54 (d, 1H), 7.67 (d, 1H), 8.48 (d, 1H), 8.61 (s, 1H), 10.43 (s, 1H); MS-ESI *m/z* 429 and 431 $[MH]^+$.

***N*-(2-Chloro-5-methoxyphenyl)-5,7-dimethoxyquinazolin-4-amine (39).** A mixture of **43** (2 g, 9.7 mmol), phosphorus oxychloride (0.1 mL, 10.7 mmol), and DIPEA (4.2 mL, 27.7 mmol) in 1,2-dichloroethane (20 mL) was refluxed for 2 h under nitrogen. The dark solution was concentrated, and then propan-2-ol (20 mL) was added to the crude followed by 2-chloro-5-methoxyaniline (1.85 g, 11.7 mmol). The mixture was heated at 80 °C for 1 h and 30 min. After cooling, the precipitate was filtered off and washed with propan-2-ol and then diethyl ether to give 2.8 g of **39** as a hydrochloride salt (76%): ^{1}H NMR (DMSO-d_6) δ 3.85 (s, 3H), 4.00 (s, 3H), 4.16 (s, 3H), 7.00 (m, 3H), 7.56 (d, 1H), 7.60 (d, 1H), 8.8 (s, 1H), 10.90 (s, 1H); MS-ESI *m/z* 346 and 348 $[MH]^+$.

***N*-(2-Chloro-5-methoxyphenyl)-6-[3-(4-methylpiperazin-1-yl)-propoxy]-5-(tetrahydro-2*H*-pyran-4-yloxy)quinazolin-4-amine (40).** A mixture of **119** (90 mg, 0.21 mmol), (2-chloro-5-methoxyphenyl)amine hydrochloride (50 mg, 0.26 mmol), and a catalytic amount of HCl (from a 6 N HCl solution in propan-2-ol) in propan-2-ol (3 mL) was heated under reflux for 1 h. After cooling, the precipitate was filtered off and washed with propan-2-ol, ethyl acetate, and diethyl ether to give 90 mg of **40** (68%) as a dihydrochloride: ^{1}H NMR (free base) ($CDCl_3$) δ 1.9−2.2 (m, 6H), 2.49 (s, 3H), 2.6−2.9 (m, 10H), 3.3−3.4 (m, 2H), 3.90 (s, 3H), 4.0−4.1 (m, 2H), 4.23 (t, 2H), 4.5−4.6 (m, 1H), 6.65 (dd, 1H), 7.34 (d, 1H), 7.54 (d, 1H), 7.67 (d, 1H), 8.54 (d, 1H), 8.63 (s, 1H), 10.42 (s, 1H); MS-ESI *m/z* 542 and 544 $[MH]^+$.

5,7-Dimethoxyquinazolin-4(3*H*)-one (43). A mixture of **70** (16 g, 76 mmol) and formamidine acetate (24 g, 230 mmol) in 2-methoxyethanol (330 mL) was heated at reflux for 2 h. After cooling, the solution was concentrated, and the residue was taken up in water (100 mL). The formed white solid was filtered off, washed twice with water, and dried under vacuum at 50 °C in the presence of phosphorus pentoxide to give 14.5 g of **43** (94%): ^{1}H NMR (DMSO-d_6) δ 3.82 (s, 3H), 3.90 (s, 3H), 6.54 (d, 1H), 6.66 (d, 1H), 7.93 (s, 1H), 11.78 (br s, 1H); MS-ESI *m/z* 207 $[MH]^+$. Anal. ($C_{10}H_{10}N_2O_3$·$0.1H_2O$) C, H, N.

A similar procedure was used to prepare **58**.

4-[(2-Chloro-5-methoxyphenyl)amino]-7-methoxyquinazolin-5-ol (44). A mixture of **39** (2.5 g, 6.5 mmol) and pyridine hydrochloride (760 mg, 6.5 mmol) in pyridine (50 mL) was refluxed overnight. The solution was cooled and concentrated. The residue was taken up in water (50 mL) and made alkaline to pH 11 by addition of 30% NH_4OH. The solid was filtered off, washed with water, triturated in dichloromethane and then diethyl ether, and dried under vacuum at 50 °C in the presence of phosphorus pentoxide to give 2.14 g of **44** (98%), which was used in the next step without further purification: ^{1}H NMR (DMSO-d_6 and CF_3CO_2D) δ 3.84 (s, 3H), 3.95 (s, 3H), 6.81 (m, 2H), 6.98 (dd, 1H), 7.53 (d, 1H), 7.87 (d, 1H), 8.88 (s, 1H); MS-ESI *m/z* 332 and 334 $[MH]^+$.

5-Methoxyquinazolin-4(3*H*)-one (45). A mixture of **48** (1.64 g, 10 mmol) and sodium methoxide (1.2 g, 30 mmol) in THF (20 mL) was refluxed overnight. The solvent was evaporated under vacuum, and the residue was made acidic (pH 5) by addition of 2 N hydrochloric acid. The precipitate formed was filtered, washed thoroughly with water and diethyl ether, and dried to give 1.2 g of **45** (68%): ^{1}H NMR (DMSO-d_6) δ 3.85 (s, 3H), 7,0 (d, 1H), 7.15 (d, 1H), 7.7 (t, 1H), 7.95 (s,1H); MS-ESI *m/z* 177 $[MH^+]$. Anal. ($C_9H_8N_2O_2$) C, H, N.

***N*-(2-Chloro-5-methoxyphenyl)-5-methoxyquinazolin-4-amine (46).** A mixture of **45** (2.1 g, 12 mmol), phosphorus oxychloride (1.23 mL, 13.2 mmol), and DIPEA (5.2 mL, 30 mmol) in 1,2-dichloroethane (20 mL) was heated to 75 °C for 2 h. The solvent was evaporated under vacuum, the crude intermediate was reacted with 2-chloro-5-methoxyaniline (1.9 g, 12 mmol) in propan-2-ol (20 mL) containing a catalytic amount of a 5 N solution of HCl gas in 2-propanol (330 μL, 2 mmol), and the reaction mixture was heated at 80 °C for 30 min. After cooling, the precipitate formed was filtered, washed with propan-2-ol and diethyl ether, and dried to give 3.4 g of **46** as a hydrochloride (81%): ^{1}H NMR (DMSO-d_6) δ 3.8 (s, 3H), 4.17 (s, 3H), 7.02 (dd, 1H), 7.43 (d, 1H), 7.54−7.58 (m, 3H), 8.07 (t, 1H), 8.89 (s, 1H), 11.18 (br s, 1H); MS-ESI *m/z* 316 and 318 $[MH^+]$. Anal. ($C_{16}H_{14}N_3O_2Cl$·1.5HCl·$1.5H_2O$) C, H, N, Cl.

4-[(2-Chloro-5-methoxyphenyl)amino]quinazolin-5-ol (47). A mixture of **46** (3.3 g, 9.4 mmol) and pyridine hydrochloride (1.1 g, 10 mmol) in pyridine (30 mL) was heated under reflux overnight. Pyridine was evaporated, and the residue was made alkaline by addition of 30% NH_4OH. The precipitate formed was washed with water and diethyl ether and dried to give 1.4 g of **47** (50%): ^{1}H NMR (DMSO-d_6) δ 3.8 (s, 3H), 7.02 (dd, 1 H), 7.25 (dd, 1H), 7.30 (d, 1H), 7.57 (d, 1H), 7.82 (d, 1H), 7.92 (t, 1H), 8.94 (s, 1H); MS-ESI *m/z* 302 and 304 $[MH^+]$. Anal. ($C_{15}H_{12}N_3O_2Cl$·$0.3H_2O$) C, H, N.

***tert*-Butyl 4-[(4-Oxo-3,4-dihydroquinazolin-5-yl)oxy]piperidine-1-carboxylate (49).** To a suspension of NaH (60% dispersion in mineral oil) (110 mg, 2.7 mmol) in DMF (2 mL) was added *tert*-butyl 4-hydroxypiperidine-1-carboxylate (330 mg, 1.64 mmol). After the resulting mixture was stirred for 15 min at room temperature, **48** (180 mg, 1.1 mmol) was added to the mixture, which was stirred overnight at room temperature. To complete the reaction, NaH (60% dispersion in mineral oil) (66 mg, 1.6 mmol) and then *tert*-butyl 4-hydroxypiperidine-1-carboxylate (220 mg, 1.1 mmol) were added, and the reaction temperature was raised to 50 °C for 1 h. After cooling, the reaction mixture was poured into water (20 mL), and the pH was adjusted to 5 with acetic acid. The precipitate was filtered off, washed with water, and dried under vacuum at 50 °C in the presence of phosphorus pentoxide to give 300 mg of **49** as a white solid (79%): ^{1}H NMR ($CDCl_3$) δ 1.47 (s, 9H), 1.94 (m, 2H), 3.55 (m, 2H), 3.69 (m, 2H), 4.70 (quint, 1H), 6.96 (d, 1H), 7.33 (d, 1H), 7.65 (t, 1H), 7.95 (s, 1H); MS-ESI *m/z* 346 $[MH]^+$.

***N*-(5-Chloro-1,3-benzodioxol-4-yl)-5-fluoroquinazolin-4-amine (52).** A mixture of **51** (400 mg, 2.2 mmol), (5-chloro-1,3-benzodioxol-4-yl)amine (410 mg, 2.4 mmol), and a catalytic amount of a 5 N solution of HCl gas in propan-2-ol was heated in propan-2-ol (4 mL) at 80 °C for 1.5 h. After cooling, the formed precipitate was filtered off and washed with propan-2-ol and then diethyl ether to give 730 mg of **52** as a hydrochloride salt (94%): ^{1}H NMR (DMSO-d_6) δ 6.14 (s, 2H), 7.04 (d, 1H), 7.11 (d, 1H), 7.71 (dd, 1H), 7.84 (d, 1H), 8.11 (td, 1H), 8.86 (s, 1H), 10.3 (br s, 1H); MS-ESI *m/z* 318 and 320 $[MH]^+$.

***tert*-Butyl 4-[({4-[(5-Chloro-1,3-benzodioxol-4-yl)amino]quinazolin-5-yl}oxy)methyl]piperidine-1-carboxylate (53).** To a suspension of NaH (60% dispersion in oil) (100 mg, 2.4 mmol) in DMF (4 mL) was added *tert*-butyl 4-(hydroxymethyl)piperidine-1-carboxylate (90 mg, 0.42 mmol) under nitrogen at room temperature. The reaction mixture was stirred for 10 min, and then **52** (100 mg, 0.28 mmol) was added. The mixture was stirred for 4 h at 80 °C. After cooling, the solution was poured dropwise into water (20 mL), and the precipitate was filtered off, washed with water, and dried overnight at 50 °C under vacuum in the presence of phosphorus pentoxide to give 140 mg of **53** (97%), which was used in the next step without further purification: ^{1}H NMR ($CDCl_3$) δ 1.30 (m, 2H), 1.45 (s, 9H), 1.92 (m, 2H), 2.20 (m, 1H), 2.78 (m, 2H), 4.11 (d, 2H), 4.20 (m, 2H), 6.05 (s, 2H), 6.73 (d, 1H), 6.89 (d, 1H), 6.98 (d, 1H), 7.49 (d, 1H), 7.65 (t, 1H), 8.61 (s, 1H), 9.33 (s, 1H); MS-ESI *m/z* 513 and 515 $[MH]^+$.

***N*-(5-Chloro-1,3-benzodioxol-4-yl)-5-(piperidin-4-ylmethoxy)-quinazolin-4-amine (54).** **53** (140 mg, 0.27 mmol) was stirred in a 2 N solution of HCl gas in diethyl ether (20 mL) at room temperature for 3 h. The mixture was diluted with diethyl ether, and the precipitate was filtered off, washed with diethyl ether, and dried under vacuum to give 134 mg of **54** as a dihydrochloride salt (89%), which was used in the next step without further purification: ^{1}H NMR (DMSO-d_6) δ 1.53 (m, 2H), 1.96 (m, 2H), 2.39 (m, 1H), 2.88 (m, 2H), 3.35 (m, 2H), 4.42 (d, 2H), 6.15 (s, 2H), 7.07 (d, 1H), 7.15 (d, 1H), 7.53 (m, 2H), 8.07 (t, 1H), 8.80−9.00 (m, 3H), 10.57 (br s, 1H); MS-ESI *m/z* 513 $[MH]^+$.

4,6-Bis(benzyloxy)-1*H*-indole-2,3-dione (55). Using a procedure similar to that described by Newmam,[76] [3,5-bis(benzyloxy)phenyl]-amine[75] (10 g, 29 mmol) gave 8.8 g of **55** (84%): ^{1}H NMR (DMSO-d_6) δ 5.25 (s, 2H), 5.27 (s, 2H), 6.13 (s, 1H), 6.42 (s, 1H), 7.25−7.60 (m, 10H); MS-ESI *m/z* 382 $[MNa]^+$.

2-Amino-4,6-bis(benzyloxy)benzoic Acid (56). Using a procedure similar to that described by Newmam,[76] **55** (14.3 g, 40 mmol) gave 8 g of **56** (57%): ^{1}H NMR (DMSO-d_6) δ 5.06 (s, 2H), 5.14 (s, 2H), 6.01 (d, 1H), 6.05 (d, 1H), 7.25−7.60 (m, 10H); MS-ESI *m/z* 372 $[MNa]^+$.

Methyl 2-Amino-4,6-bis(benzyloxy)benzoate (57). **57** was prepared using the method described by Lombardi.[77] In a new capped flask was dissolved Diazald (11.5 g, 53 mmol) in ethanol (75 mL) in which nitrogen was bubbling. From the top of it Teflon tubing was connected to a second flask and plunged into a solution of **56** (8 g, 23 mmol) in dichloromethane (170 mL) at 0 °C to

produce bubbling. To the Diazald solution was cautiously added a concentrated aqueous solution of sodium hydroxide (30 mL) while an important nitrogen flow rate was maintained. At the end of addition, the dichloromethane solution was stirred for 30 min and then concentrated. The obtained red solid was washed with diethyl ether and filtered off, giving 7.7 g of **57** (91%) as a white solid: ^{1}H NMR (DMSO-d_6) δ 3.74 (s, 3H), 5.08 (s, 2H), 5.12 (s, 2H), 5.99 (d, 1H), 6.04 (d, 1H), 6.24 (br s, 2H), 7.25−7.55 (m, 10H); MS-ESI *m/z* 386 [MNa]$^+$.

5,7-Bis(benzyloxy)-*N*-(2-chloro-5-methoxyphenyl)quinazolin-4-amine (59). To a mixture of **58** (5.7 g, 16 mmol) and DIPEA (7.25 mL; 42 mmol) in 1,2-dichloroethane (120 mL) was added dropwise $POCl_3$ (1.8 mL, 20 mmol). The solution was then heated at 80 °C for 2 h. The solvent was evaporated, and the crude 4-chloroquinazoline was reacted with 2-chloro-5-methoxyaniline hydrochloride (3.1 g, 16 mmol) in propan-2-ol (50 mL). The mixture was heated at 80 °C for 30 min. After cooling, the precipitate was filtered off and dried under vacuum to give 6.34 g of **59** as a hydrochloride salt (71%): ^{1}H NMR (DMSO-d_6) δ 3.81 (s, 3H), 5.31 (s, 2H), 5.64 (s, 2H), 6.98 (dd, 1H), 7.01 (d, 1H), 7.12 (d, 1H), 7.4−7.6 (m, 9H), 7.58 (d, 2H), 7.68 (d, 1H), 8.8 (s, 1H); MS-ESI *m/z* 498 and 500 [MH]$^+$.

7-(Benzyloxy)-4-[(2-chloro-5-methoxyphenyl)amino]quinazolin-5-ol (60). A mixture of **59**·HCl (4.35 g, 8.1 mmol) and pyridine hydrochloride (941 mg, 8.1 mmol) in pyridine (90 mL) was heated at reflux for 9 h. After evaporation, the residue was taken up in water (90 mL) to give after filtration and drying 3.14 g of **60** (95%): ^{1}H NMR (DMSO-d_6 and CF_3CO_2D) δ 3.85 (s, 3H), 5.3 (s, 2H), 6.85 (s, 2H), 7.01 (dd, 1H), 7.4−7.6(m, 6H), 7.8 (d, 1H), 8.85 (s, 1H); MS-ESI *m/z* 407 and 409 [MH]$^+$.

7-(Benzyloxy)-*N*-(2-chloro-5-methoxyphenyl)-5-(tetrahydro-2*H*-pyran-4-yloxy)quinazolin-4-amine (61). To a mixture of **60** (1.22 g, 3 mmol), triphenylphosphine (1.26 g, 4.8 mmol), and tetrahydro-2*H*-pyran-4-ol (350 μL, 3.6 mmol) in dichloromethane (30 mL) cooled in an ice bath at 0 °C was slowly added DTAD (1.1 g, 4.8 mmol). The mixture was stirred for 2 h at room temperature. The crude was purified by SiO_2 chromatography and eluted with a mixture of dichloromethane/ethyl acetate (9:1 up to 8:2) to give 800 mg of **61** (55%) as a white solid: ^{1}H NMR (DMSO-d_6) δ 1.75−1.85 (m, 2H), 2.1−2.2 (m, 2H), 3.45−3.55 (m, 2H), 3.85−3.95 (m, 2H), 3.8 (s, 3H), 5.0−5.1 (m, 1H), 5.3 (s, 2H), 6.8 (dd, 1H), 6.95 (d, 1H), 7.02 (d, 1H), 7.3−7.6 (m, 6H), 8.1 (d, 1H), 8.5 (s, 1H), 9.86 (s, 1H); MS-ESI *m/z* 491 and 493[MH]$^+$.

A similar procedure was used to prepare **62**−**64**.

4-[(2-Chloro-5-methoxyphenyl)amino]-5-(tetrahydro-2*H*-pyran-4-yloxy)qui nazolin-7-ol (65). A solution of **61** (780 mg, 1.6 mmol) in trifluoroacetic acid (5 mL) was heated at 80 °C for 5 h. After evaporation the residue was made alkaline using a 7 N solution of ammonia in methanol, and dichloromethane was added. The solution was filtered and purified by SiO_2 chromatography using a mixture of dichloromethane/methanol (96:4) as eluent to give 470 mg of **65** (86%) as a white solid: ^{1}H NMR (DMSO-d_6) δ 1.75−1.85 (m, 2H), 2.1−2.2 (m, 2H), 3.45−3.55 (m, 2H), 3.8 (s, 3H), 3.85−3.95 (m, 2H), 4.9−5.0 (m, 1H), 6.7 (d, 1H), 6.81 (dd, 1H), 7.5 (d, 1H), 8.1 (d, 1H), 8.4 (s, 1H), 9.8 (s, 1H); MS-ESI *m/z* 401 and 403 [MH]$^+$.

A similar procedure was used to prepare **66**−**68**.

Methyl 2-Amino-4,6-dimethoxybenzoate (70). **70** was prepared using the method described by Lombardi.[77] **69**[78] (15.9 g, 81 mmol) was reacted with diazomethane generated from Diazald (31 g, 145 mmol) to give 16.2 g of **70** (95%): ^{1}H NMR (DMSO-d_6) δ 3.72 (m, 9H), 5.76 (d, 1H), 5.91 (d, 1H), 6.15 (br s, 2H); MS-ESI *m/z* 234 [MNa]$^+$.

5-Hydroxy-7-methoxyquinazolin-4(3*H*)-one (71). To a mixture of **43** (103 g, 500 mmol) in pyridine (1 L) was slowly added $MgBr_2$ (92 g, 0.5 mol) at room temperature. The reaction mixture was then heated at reflux temperature for 1.5 h. Pyridine was then evaporated, the residue taken up with a mixture of water (1 L) and acetic acid (200 mL), and the resulting mixture stirred for 15 min. The precipitate formed was filtered off, washed thoroughly with water and diethyl ether, and dried at 60 °C under vacuum to give 94 g of **71** (98%) as a white solid: ^{1}H NMR (DMSO-d_6) δ 3.86 (s, 3H), 6.45 (d, 1H), 6.64 (d, 1H), 8.08 (s, 1H); MS-ESI *m/z* 193 [MH]$^+$.

A similar procedure was used to prepare **98**.

***tert*-Butyl [(5-Hydroxy-7-methoxy-4-oxoquinazolin-3(4*H*)-yl)-methyl]carbonate (72).** To a suspension of NaH (60% suspension in oil) (44 g, 1.1 mol) in DMF (1 L) cooled at 0 °C was slowly added solid **71** (93 g, 480 mmol). The mixture was then stirred at room temperature for 1 h and cooled again in an ice bath before dropwise addition of chloromethyl pivalate (99 mL, 1.4 mol). After being stirred at 0 °C for 1 h, the reaction mixture was poured into a solution of acetic acid (230 mL) in water (3 L). The precipitate was filtered and taken up in dichloromethane. The solution was washed with brine, dried ($MgSO_4$), and evaporated to give 121 g of **72** (83%): ^{1}H NMR ($CDCl_3$) δ 1.2 (s, 9H), 3.85 (s, 3H), 5.85 (s, 2H), 6.48 (d, 1H), 6.65 (d, 1H), 8.12 (s, 1H); MS-ESI *m/z* 307 [MH]$^+$.

A similar procedure was used to prepare **83**.

7-Methoxy-5-(tetrahydro-2*H*-pyran-4-yloxy)quinazolin-4(3*H*)-one (73). To a mixture of **72** (120 g, 390 mmol), triphenylphosphine (164 g, 620 mmol), and tetrahydro-2*H*-pyran-4-ol (44 g, 430 mmol) in dichloromethane (1.5 L) at 0 °C was slowly added DTAD (143 g, 620 mmol). The mixture was then stirred at room temperature for 1 h and evaporated. The residue was taken up in a 6 N methanol/ammonia solution and the resulting mixture stirred at room temperature overnight. The precipitate was filtered, washed with methanol and dichloromethane, and dried under vacuum to give 94 g of **73** (88%) as a beige solid: ^{1}H NMR (DMSO-d_6) δ 1.6−1.7 (m, 2H), 1.9−2.0 (m, 2H), 3.5−3.6 (m, 2H), 3.86 (s, 3H), 3.85−3.95 (m, 2H), 4.7−4.8 (m, 1H), 6.63 (d, 1H), 6.68 (d, 1H), 7.92 (s, 1H); MS-ESI *m/z* 277 [MH]$^+$. Anal. ($C_{14}H_{16}N_2O_4 \cdot 0.4H_2O$) C, H, N.

7-Hydroxy-5-(tetrahydro-2*H*-pyran-4-yloxy)quinazolin-4(3*H*)-one (74). A mixture of **73** (94 g, 340 mmol), potassium carbonate (69 g, 500 mmol), and benzenethiol (51 mL, 500 mmol) in NMP (400 mL) was heated at 195 °C for 35 min. The solvent was evaporated and the residue acidified at pH 5 with 6 N hydrochloric acid. The precipitate formed was filtered, washed thoroughly with water and dichloromethane, and dried to give 98 g of **74** (100%): ^{1}H NMR (DMSO-d_6) δ 1.6−1.7 (m, 2H), 1.9−2.0 (m, 2H), 3.4−3.5 (m, 2H), 3.85−3.95 (m, 2H), 4.6−4.7 (m, 1H), 6.55 (d, 1H), 6.60 (d, 1H), 8.1 (s, 1H); MS-ESI *m/z* 263 [MH]$^+$. Anal. ($C_{13}H_{14}N_2O_4 \cdot 0.2H_2O$) C, H, N.

4-Oxo-5-(tetrahydro-2*H*-pyran-4-yloxy)-3,4-dihydroquinazolin-7-yl Acetate (75). A mixture of **74** (90 g, 340 mmol), acetic anhydride (600 mL), and pyridine (900 mL) was heated at 120 °C for 2 h. After evaporation the residue was taken up in a mixture of methanol (400 mL) and water (400 mL) and the resulting mixture stirred for 45 min at room temperature. After evaporation to dryness the crude material was purified by SiO_2 chromatography eluting with a mixture of dichloromethane/methanol (98:2 up to 96:4) as eluent to give 58.5 g of **75** (57%): ^{1}H NMR (DMSO-d_6) δ 1.6−1.7 (m, 2H), 1.9−2.0 (m, 2H), 2.29 (s, 3H), 3.45−3.55 (m, 2H), 3.85−3.95 (m, 2H), 4.65−4.75 (m, 1H), 6.91 (d, 1H), 6.93 (d, 1H), 7.97 (s, 1H); MS-ESI *m/z* 305 [MH]$^+$. Anal. ($C_{15}H_{16}N_2O_5 \cdot 0.2H_2O$) C, H, N.

4-(1,3-Benzodioxol-4-ylamino)-5-(tetrahydro-2*H*-pyran-4-yloxy)-quinazolin-7-yl Acetate (76). To a mixture of **75** (3.04 g, 10 mmol) and DIEA (4.34 mL, 25 mmol) in 1,2-dichloroethane (60 mL) was added dropwise $POCl_3$ (1.08 mL, 11 mmol). The solution was then heated at 80 °C for 2 h. The solvent was evaporated, the intermediate 4-chloroquinazoline was reacted immediately with 2,3-(methylenedioxy)aniline (1.5 g, 11 mmol) in propan-2-ol (20 mL), and the reaction mixture was heated at 80 °C for 1 h. After cooling, the precipitate was washed with 2-propanol and diethyl ether, filtered, and dried under vacuum to give 3.6 g of **76** as a hydrochloride (78%): ^{1}H NMR (DMSO-d_6 and CF_3CO_2D) δ 1.9−2.0 (m, 2H), 2.1−2.2 (m, 2H), 2.38 (s, 3H), 3.5−3.6 (m, 2H), 3.9−4.0 (m, 2H), 5.05−5.15 (m, 1H), 6.17 (s, 2H), 6.9−7.0 (m, 2H), 7.32 (d, 1H), 7.50 (d, 1H), 7.60 (dd, 1H), 8.99 (s, 1H); MS-ESI *m/z* 424 [MH]$^+$. Anal. (free base) ($C_{22}H_{21}N_3O_6$) C, H, N.

A similar procedure was used to prepare **77**.

4-(1,3-Benzodioxol-4-ylamino)-5-(tetrahydro-2*H*-pyran-4-yloxy)-quinazolin-7-ol (78). A mixture of **76**·HCl (4 g, 7.6 mmol) in a 6 N methanol/ammonia solution (50 mL) was stirred at room temperature for 6 h. The solvent was evaporated and the residue thoroughly washed with water, dissolved in dichloromethane and methanol, and rapidly purified by SiO_2 chromatography using a mixture of dichloromethane/methanol (95:5) as eluent to give 2.3 g of **78** (80%): ^{1}H NMR (DMSO-d_6) δ 1.8−1.88 (m, 2H), 2.1−2.2 (m, 2H), 3.5−3.6 (m, 2H), 3.9−3.95 (m, 2H), 4.9−5.0 (m, 1H), 6.11 (s, 2H), 6.66 (d, 1H), 6.71−6.76 (m, 2H), 6.87 (t, 1H), 8.04 (d, 1H), 8.4 (s, 1H), 9.8 (s, 1H); MS-ESI *m/z* 382 [MH]$^+$.

A similar procedure was used to prepare **79**.

***tert*-Butyl 4-({[4-[(5-Chloro-1,3-benzodioxol-4-yl)amino]-5-(tetrahydro-2*H*-pyran-4-yloxy)quinazolin-7-yl]oxy}methyl)-piperidine-1-carboxylate (80).** A mixture of **79** (410 mg, 1 mmol), *tert*-butyl 4-((((4-methylphenyl)sulfonyl)oxy)methyl)piperidine-1-carboxylate (410 mg, 3.3 mmol) and cesium fluoride (460 mg, 3 mmol) in DMF (5 mL) was heated at 70 °C for 4 h. The volatiles were removed under vacuum, and the residue was diluted with water and then extracted with ethyl acetate The organic layer was washed with saturated aqueous sodium hydrogen carbonate solution, water, and brine, dried ($MgSO_4$), evaporated. After evaporation of the solvents, the residue was purified by SiO_2 chromatography using a mixture of dichloromethane/methanol (98:2) as eluent to give 500 mg of **80** (81%): ^{1}H NMR ($CDCl_3$) δ 1.3−1.4 (m, 2H), 1.4 (m, 9H), 1.75−1.85 (m, 2H), 1.9−2.05 (m, 3H), 2.2−2.3 (m, 2H), 2.7−2.8 (m, 2H), 3.6−3.7 (m, 2H), 3.95 (d, 2H), 4.0−4.1 (m, 2H), 4.1−4.25 (m, 2H), 4.7−4.8 (m, 1H), 6.05 (s, 2H), 6.5 (d, 1H), 6.7 (d, 1H), 6.8 (d, 1H), 6.98 (d, 1H), 8.5 (s, 1H), 9.28 (s, 1H); MS-ESI *m/z* 613 and 615 [MH]$^+$.

4-Chloro-5-(tetrahydro-2*H*-pyran-4-yloxy)quinazolin-7-ol (81). A mixture of **75** (1.92 g, 6.3 mmol), triphenylphosphine (3.3 g, 13 mmol), and carbon tetrachloride (1.83 mL, 19 mmol) in 1,2-dichloroethane (150 mL) was heated at 70 °C for 2 h. The solvent was evaporated and the residue purified by SiO_2 chromatography using a mixture of dichloromethane/ethyl acetate (90:10) for elution to give 2 g of 4-chloro-5-(tetrahydro-2*H*-pyran-4-yloxy)quinazolin-7-yl acetate (66%). This intermediate was taken up in a 6 N solution of ammonia in methanol and the resulting mixture stirred at room temperature for 2 h to give a precipitate that was filtered and dried under vacuum to give 860 mg of **81** (78%): ^{1}H NMR (DMSO-d_6) δ 1.7−1.8 (m, 2H), 2.0−2.1 (m, 2H), 3.55−3.65 (m, 2H), 3.85−3.95 (m, 2H), 4.8−4.9 (m, 1H), 6.83 (2d, 2H), 8.71 (s, 1H); MS-ESI *m/z* 281 and 283 [MH]$^+$.

4-Chloro-7-(2-pyrrolidin-1-ylethoxy)-5-(tetrahydro-2*H*-pyran-4-yloxy)quinazoline (82). To a mixture **81** (750 mg, 2.7 mmol), triphenylphosphine (1.14 g, 4.3 mmol), and 2-pyrrolidin-1-ylethanol (372 mg, 3.3 mmol) in dichloromethane (20 mL) at room temperature was added DTAD (990 mg, 4.3 mmol). After the resulting mixture was stirred for 30 min, the solvent was evaporated and the residue purified by SiO_2 chromatography using a mixture of dichloromethane−6 N methanol/ammonia solution (97:3) as eluent to give 900 mg of **82** (88%): ^{1}H NMR ($CDCl_3$) δ 1.8−1.9 (m, 4H), 1.9−2.0 (m, 2H), 2.1−2.2 (m, 2H), 2.6−2.7 (m, 4H), 3.0 (t, 2H), 3.65−3.75 (m, 2H), 4.0−4.1 (m, 2H), 4.25 (t, 2H), 4.7−4.8 (m, 1H), 6.7 (d, 1H), 6.96 (d, 1H), 8.81 (s, 1H); MS-ESI *m/z* 378 and 380 [MH]$^+$.

7-Methoxy-5-[(1-methylpiperidin-4-yl)oxy]quinazolin-4(3*H*)-one (84). To a mixture of **72** (1.5 g, 4.9 mmol), triphenylphosphine (1.9 g, 7.3 mmol), and 1-methylpiperidin-4-ol (675 mg, 5.9 mmol) in dichloromethane (20 mL) was added dropwise a solution of DTAD (1.7 g, 7.3 mmol) in dichloromethane (5 mL) under nitrogen at 0 °C. The solution was stirred for 1 h at room temperature and then poured onto a column of silica gel eluting with methanol/dichloromethane/ethyl acetate (5:45:50) to remove impurities and then with a 7 N solution of ammonia in methanol/dichloromethane/ethyl acetate (5:45:50). Evaporation of the solvent gave 1.75 g of [7-methoxy-5-[(1-methylpiperidin-4-yl)oxy]-4-oxoquinazolin-3(4*H*)-yl]methyl pivalate (89%): ^{1}H NMR ($CDCl_3$) δ 1.20 (s, 9H), 2.04 (m, 4H), 2.25−2.55 (m, 5H), 3.86 (m, 2H), 3.89 (s, 3H), 4.46 (m, 1H), 5.89 (s, 2H), 6.50 (d, 1H), 6.71 (d, 1H), 8.18 (s, 1H); MS-ESI *m/z* 404 [MH]$^+$.

[7-Methoxy-5-[(1-methylpiperidin-4-yl)oxy]-4-oxoquinazolin-3(4*H*)-yl]methyl pivalate (1.75 g, 4.3 mmol) was stirred in a 7 N solution of ammonia in methanol (100 mL) at room temperature overnight. The mixture was concentrated and taken up in diethyl ether. The formed precipitate was filtered off, washed with diethyl ether, and dried under vacuum at 50 °C to give 930 mg of **84** (75%): ^{1}H NMR (DMSO-d_6) δ 1.70 (m, 2H), 1.90 (m, 2H), 2.18 (s, 3H), 2.20 (m, 2H), 2.65 (m, 2H), 3.82 (s, 3H), 4.48 (m, 1H), 6.57 (d, 1H), 6.66 (d, 1H), 7.89 (s, 1H); MS-ESI *m/z* 290 [MH]$^+$.

7-(Benzyloxy)-5-[(1-methylpiperidin-4-yl)oxy]quinazolin-4(3*H*)-one (85). To a mixture of **83** (6 g, 15.7 mmol), triphenylphosphine (6.2 g, 23.5 mmol), and 1-methylpiperidin-4-ol (2.2 g, 19 mmol) in dichloromethane (100 mL) was added dropwise a solution of DTAD (5.4 g, 23.5 mmol) in dichloromethane (20 mL) under nitrogen at 0 °C. The solution was stirred for 1 h at room temperature and then poured onto a column of silica gel eluting with methanol/dichloromethane/ethyl acetate (5:45:50) to remove impurities and then with a solution of 7 N ammonia in methanol/dichloromethane/ethyl acetate (5:45:50). Evaporation of the solvent gave the intermediate, which was dissolved and stirred overnight in a 7 N solution of ammonia in methanol (240 mL) at room temperature. The mixture was concentrated and taken up in diethyl ether. The precipitate was filtered off, washed with diethyl ether, and dried under vacuum at 50 °C to give 3.7 g of **85** (65%): ^{1}H NMR ($CDCl_3$) δ 2.00 (m, 4H), 2.29 (s, 3H), 2.35 (m, 2H), 2.73 (m, 2H), 4.46 (m, 1H), 5.13 (s, 1H), 6.56 (d, 1H), 6.82 (d, 1H), 7.3−7.55 (m, 5H), 7.91 (s, 1H), 11.2 (br s, 1H); MS-ESI *m/z* 366 [MH]$^+$.

***tert*-Butyl 4-{[7-(Benzyloxy)-4-oxo-3,4-dihydroquinazolin-5-yl]oxy}piperidine-1-carboxylate (87).** To a mixture of **83** (1.95 g, 5.1 mmol), triphenylphosphine (2 g, 7.6 mmol), and *tert*-butyl 4-hydroxypiperidine-1-carboxylate (1.23 g, 6.1 mmol) in dichloromethane (15 mL) was added portionwise DTAD (0.88 g, 7.6 mmol) under nitrogen at 15 °C. The solution was stirred for 1 h at room temperature and then concentrated. The crude was taken up in methanol (25 mL), and NaOH pellets (360 mg, 8.9 mmol) were added to the solution. The reaction mixture was stirred for 30 min until the NaOH pellets dissolved. The solvent was evaporated, and the crude product was purified on silica gel eluting with methanol/dichloromethane/ethyl acetate (1:49:50 up to 5:45:50). Evaporation of the solvent gave 1.4 g of **87** (62%): ^{1}H NMR ($CDCl_3$) δ 1.48 (s, 9H), 1.93 (m, 4H), 3.54 (m, 2H), 3.71 (m, 2H), 4.65 (m, 1H), 5.17 (s, 2H), 6.59 (d, 1H), 6.87 (d, 1H), 7.35−7.55 (m, 5H), 7.92 (s, 1H), 10.56 (br s, 1H); MS-ESI *m/z* 452 [MH]$^+$.

7-(Benzyloxy)-*N*-(5-chloro-1,3-benzodioxol-4-yl)-5-[(1-methylpiperidin-4-yl)oxy]quinazolin-4-amine (88). A mixture of **85** (3.7 g, 10 mmol), triphenylphosphine (5.3 g, 20 mmol), and carbon tetrachloride (9.65 mL, 100 mmol) in 1,2-dichloroethane (100 mL) was heated at 70 °C for 2 h under nitrogen. The dark solution was concentrated, and then propan-2-ol (20 mL) was added to the crude followed by 6-chloro-2,3-(methylenedioxy)aniline (1.9 g, 11 mmol) and a 5 N solution of HCl gas in propan-2-ol (2.1 mL, 10.5 mmol). The mixture was heated at 80 °C for 30 min. The solution was concentrated, taken up in a mixture of 7 N solution of ammonia in methanol/dichloromethane (5:95). The resulting precipitate was eliminated by filtration, and the filtrate was evaporated down and purified by SiO_2 chromatography eluting with methanol/ethyl acetate/dichloromethane (3:50:47) to give 4.2 g of **88** (81%): ^{1}H NMR ($CDCl_3$) δ 2.04 (m, 2H), 2.19 (m, 2H), 2.31 (m, 5H), 2.74 (m, 2H), 4.59 (m, 1H), 5.18 (s, 2H), 6.06 (s, 2H), 6.60 (d, 1H), 6.73 (d, 1H), 6.94 (d, 1H), 6.98 (d, 1H), 7.32−7.55 (m, 5H), 8.53 (s, 1H), 9.28 (s, 1H); MS-ESI *m/z* 519 and 521 [MH]$^+$. Anal. ($C_{28}H_{27}ClN_4O_4$) C, H, N.

4-[(5-Chloro-1,3-benzodioxol-4-yl)amino]-5-[(1-methylpiperidin-4-yl)oxy]quinazolin-7-ol (89). 88 (1.5 g, 2.9 mmol) was heated under reflux in TFA (15 mL) for 6 h. After cooling, the solvent was evaporated off, and water was added to the residue. The solution was made alkaline by adding portionwise sodium bicarbonate powder until pH 9 and extracted three times with ethyl acetate.

The combined organic phases were washed with brine, dried over magnesium sulfate, and concentrated. The crude material was purified by SiO_2 chromatography eluting with methanol/ethyl acetate/dichloromethane (5:47.5:47.5 up to 10:45:45) to give 800 mg of **89** (64%): ^{1}H NMR ($CDCl_3$) δ 1.98 (m, 2H), 2.11 (m, 2H), 2.2−2.4 (m, 5H), 2.69 (m, 2H), 4.47 (m, 1H), 6.06 (s, 2H), 6.49 (d, 1H), 6.70 (d, 1H), 6.84 (d, 1H), 6.94 (d, 1H), 8.42 (s, 1H), 9.35 (s, 1H); MS-ESI *m/z* 429 and 431 [MH]$^+$. Anal. ($C_{21}H_{21}ClN_4O_4$·$0.6H_2O$·$0.1C_4H_8O_2$·$0.05CH_2Cl_2$) C, H, N.

4-Chloro-7-methoxy-5-[(1-methylpiperazin-4-yl)oxy]quinazoline (90). A mixture of **84** (7 g, 24 mmol), triphenylphosphine (12.7 g, 48 mmol), and carbon tetrachloride (7 mL, 72 mmol) in 1,2-dichloroethane (100 mL) was heated at 70 °C for 2 h under nitrogen. After cooling, the solvent was evaporated off, and the crude was purified by SiO_2 chromatography eluting with methanol/dichloromethane/ethyl acetate (5:45:50) to remove impurities and then with a 7 N solution of ammonia in methanol/dichloromethane/ethyl acetate (5:45:50) to give 5.3 g of **90** (71%): ^{1}H NMR ($CDCl_3$) δ 2.09 (m, 4H), 2.34 (s, 3H), 2.44 (m, 2H), 2.73 (m, 2H), 3.95 (s, 3H), 4.58 (m, 1H), 6.60 (d, 1H), 6.94 (d, 1H), 8.81 (s, 1H); MS-ESI *m/z* 308 and 310 [MH]$^+$. Anal. ($C_{15}H_{18}ClN_3O_2$·$0.55H_2O$·$0.01C_4H_{10}O$·$0.04CH_2Cl_2$) C, H, N.

A similar procedure was used to prepare **50**, **51**, **86**, **91**, and **92**.

***tert*-Butyl 4-({7-(Benzyloxy)-4-[(5-chloro-1,3-benzodioxol-4-yl)amino]quinazolin-5-yl}oxy)piperidine-1-carboxylate (93).** A mixture of **87** (1.9 g, 4.1 mmol), (5-chloro-1,3-benzodioxol-4-yl)-amine (77 mg, 4.5 mmol), and a 5 N HCl solution in propan-2-ol (36 μL, 0.2 mmol) in propan-2-ol (20 mL) was heated at 50 °C for 30 min. After cooling, the precipitate formed was filtered off and washed with propan-2-ol and then diethyl ether to give 2.4 g of **93** as a hydrochloride salt (92%): ^{1}H NMR (DMSO-d_6) δ 1.4 (s, 9H), 1.9 (m, 2H), 2.0 (m, 2H), 3.1 (m, 2H), 3.9 (m, 2H), 5.1 (m, 1H), 5.35 (s, 2H), 6.1 (s, 2H), 7.0 (m, 2H), 7.1 (d, 1H), 7.2 (s, 1H), 7.3−7.6 (m, 5H), 8.75 (s, 1H), 10.1 (br s, 1H); MS-ESI *m/z* 605 and 607 [MH]$^+$. Anal. (free base) ($C_{32}H_{33}ClN_4O_6$) C, H, N.

4-[(5-Chloro-1,3-benzodioxol-4-yl)amino]-5-(piperidin-4-yloxy)quinazolin-7-ol (94). A solution of **93** (2.8 g, 4.3 mmol) in TFA (28 mL) was refluxed under nitrogen for 6 h. After cooling, the solvent was evaporated off, and the crude was taken up in water. The pH was adjusted to 10 by adding a 1 N solution of NaOH. The mixture was then stirred at room temperature for 1 h, and the precipitate was filtered off, washed with water, and dried under vacuum at 50 °C in the presence of phosphorus pentoxide to give 1.4 g of **94** (78%), which was used in the next step without further purification: ^{1}H NMR (DMSO-d_6) δ 1.71 (m, 2H), 2.06 (m, 2H), 2.69 (m, 2H), 2.95 (m, 2H), 4.80 (m, 1H), 6.08 (s, 2H), 6.63 (d, 1H), 6.69 (d, 1H), 6.91 (d, 1H), 7.05 (d, 1H), 8.26 (s, 1H), 9.22 (s, 1H); MS-ESI *m/z* 415 and 417 [MH]$^+$.

***tert*-Butyl 4-({4-[(5-Chloro-1,3-benzodioxol-4-yl)amino]-7-hydroxyquinazolin-5-yl}oxy)piperidine-1-carboxylate (95).** A mixture of **94** (1.4 g, 3.4 mmol) and Boc_2O (74 mg, 3.4 mmol) in DMF (14 mL) was stirred at room temperature under nitrogen for 2 h, then the solvent was removed under vacuum, and the residue was dissolved in ethyl acetate, washed with water and brine, and dried over magnesium sulfate. The crude was purified by SiO_2 chromatography eluting with a methanol/dichloromethane mixture (0:100 up to 4:96) to give 1.2 g of **95** (68%): ^{1}H NMR ($CDCl_3$) δ 1.47 (s, 9H), 1.78 (m, 2H), 2.09 (m, 2H), 3.06 (m, 2H), 3.86 (m, 2H), 4.59 (m, 1H), 6.03 (s, 2H), 6.54 (s, 1H), 6.72 (d, 1H), 6.96 (m, 2H), 8.42 (s, 1H), 9.38 (s, 1H); MS-ESI *m/z* 415 and 417 [MH]$^+$. Anal. ($C_{25}H_{27}ClN_4O_6$·$0.1H_2O$) C, H, N.

***tert*-Butyl 4-({4-[(5-Chloro-1,3-benzodioxol-4-yl)amino]-7-methoxyquinazolin-5-yl}oxy)piperidine-1-carboxylate (97).** A mixture of **91** (140 mg, 0.33 mmol), (5-chloro-1,3-benzodioxol-4-yl)amine (60 mg, 0.36 mmol), and a catalytic amount of a 5 N solution of HCl gas in propan-2-ol was heated in propan-2-ol (2 mL) at 80 °C for 1.5 h. After cooling, the solution was concentrated to give a residue which was treated with diethyl ether, and the resulting precipitate was filtered and washed with ethyl acetate and then diethyl ether to give 170 mg of **97** as a hydrochloride salt (92%), which was used in the next step without further purification: ^{1}H NMR (DMSO-d_6) δ 1.42 (s, 9H), 1.95 (m, 2H), 2.10 (m, 2H), 3.08 (m, 2H), 3.88 (m, 2H), 4.00 (s, 3H), 5.09 (m, 1H), 6.14 (s, 2H), 6.97 (d, 1H), 7.05 (d, 1H), 7.13 (d, 1H), 7.16 (d, 1H), 8.78 (s, 1H), 10.11 (br s, 1H); MS-ESI *m/z* 529 and 531 [MH]$^+$.

7-(Benzyloxy)-5-isopropoxyquinazolin-4(3*H*)-one (99). To a mixture of **83** (30 g, 78 mmol), triphenylphosphine (33 g, 125 mmol), and 2-propanol (7.3 mL, 94 mmol) in dichloromethane (350 mL) was added portionwise DTAD (29 g, 125 mmol) over 30 min under nitrogen at 0 °C. The solution was stirred for 1 h at room temperature and then concentrated. The residue was dissolved and stirred overnight in a 7 N solution of ammonia in methanol (450 mL) at room temperature, then concentrated, and treated with diethyl ether. The resulting precipitate was filtered, washed with diethyl ether, and purified by chromatography using methanol/dichloromethane as eluent (2:98 up to 5:95) to give 24 g of **99** (97%): ^{1}H NMR (DMSO-d_6) δ 1.29 (d, 6H), 4.66 (quint, 1H), 5.23 (s, 2H), 6.62 (d, 1H), 6.75 (d, 1H), 7.3−7.6 (m, 5H), 7.89 (s, 1H); MS-ESI *m/z* 311 [MH]$^+$.

5-Isopropoxy-4-oxo-3,4-dihydroquinazolin-7-yl Acetate (100). To a solution of **99** (24 g, 77 mmol) in DMF (300 mL) were added successively Pd/C (10%) (2.8 g) and ammonium formate (48 g, 768 mmol). The mixture was stirred for 2 h at room temperature. The solution was passed through a pad of Celite, and the filtrate was concentrated. The solid was taken up in water (250 mL), filtered off, washed twice with water and diethyl ether, and dried under vacuum at 50 °C in the presence of phosphorus pentoxide to give the intermediate 7-hydroxy-5-isopropoxyquinazolin-4(3*H*)-one as a white solid (16 g). A mixture of 7-hydroxy-5-isopropoxyquinazolin-4(3*H*)-one (15.9 g, 72 mmol), acetic anhydride (34 mL), and pyridine (0.62 mL, 7.2 mmol) was heated at 70 °C for 30 min. After evaporation the residue was taken up in water (200 mL) and the resulting mixture stirred for 20 min at 80 °C. The precipitate was filtered and dried on phosphorus pentoxide under vacuum to give 17.8 g of **100** (94%): ^{1}H NMR (DMSO-d_6) δ 1.32 (d, 6H), 2.31 (s, 3H), 4.66 (quint., 1H), 6.86 (d, 1H), 6.91 (d, 1H), 7.93 (s, 1H); MS-ESI *m/z* 263 [MH]$^+$.

4-[(5-Chloro-1,3-benzodioxol-4-yl)amino]-5-isopropoxyquinazolin-7-ol (102). To a mixture of **100** (22 g, 84 mmol) and DIPEA (38 mL, 218 mmol) in 1,2-dichloroethane (600 mL) was added dropwise $POCl_3$ (9.4 mL, 101 mmol). The solution was then heated at 80 °C for 2 h. The solvent was evaporated, the intermediate 4-chloroquinazoline was reacted immediately with (5-chloro-1,3-benzodioxol-4-yl)amine (15.2 g, 85 mmol) in propan-2-ol (200 mL), and the reaction mixture was heated at 80 °C for 1.5 h. After cooling, propan-2-ol was evaporated, and the residue was stirred in a 6 N solution of ammonia in methanol (25 mL) for 30 min at room temperature. Methanol was removed under vacuum, and after addition of dichloromethane the organic phase was filtered and purified by SiO_2 chromatography using a mixture of dichloromethane−6 N methanol/ammonia (99:1 to 93:7) as eluent to give 29 g of **102** (92%): ^{1}H NMR (DMSO-d_6) δ 1.44 (d, 6H), 4.91 (quint., 1H), 6.07 (s, 2H), 6.64 (m, 2H), 6.91 (d, 1H), 7.05 (d, 1H), 8.26 (d, 1H), 9.26 (s, 1H); MS-ESI *m/z* 374 and 376 [MH]$^+$.

A similar procedure was used to prepare **101**.

***N*-(5-Fluoro-1,3-benzodioxol-4-yl)-7-(2-chloroethoxy)-5-isopropoxyquinazolin-4-amine (103).** A mixture of **101** (32 g, 90 mmol) and potassium carbonate (22 g, 161 mmol) in 1,2-dichloroethane (450 mL) and DMF (225 mL) was heated at 85 °C under nitrogen for 24 h. After cooling, the solid was removed by filtration and after concentration of the filtrate. The crude was purified by SiO_2 chromatography eluting with methanol/dichloromethane (0:100 up to 2:98) to give 29 g of **103** (78%): ^{1}H NMR ($CDCl_3$) δ 1.52 (d, 6H), 3.89 (t, 2H), 4.36 (t, 2H), 4.82 (quint, 1H), 6.05 (s, 2H), 6.55 (d, 1H), 6.66 (m, 2H), 6.79 (d, 1H), 8.54 (s, 1H), 9.30 (br s, 1H); MS-ESI *m/z* 420 and 422 [MH]$^+$.

A similar procedure was used to prepare **104**.

4,6-Difluoro-2,3-indolinedione (105). To a reactor were introduced Na_2SO_4·$10H_2O$ (3.5 kg), water (3.5 L), and chloral (132 mL, 1.36 mol). In another flask, 3,5-difluoroaniline (160 g, 1.24 mol) in water (1.3 L) was converted into its hydrochloride by adding concentrated HCl (104 mL, 1.24 mol), and the solution obtained

was added to the reactor. Then an aqueous solution of $NH_2OH \cdot HCl$ (258.5 g, 3.72 mol) in water (1.25 L) was added rapidly. The mixture was heated at 120 °C for 2 h. After cooling, the precipitate was filtered, washed with water, and dried. To a flask containing concentrated H_2SO_4 (700 mL) was added portionwise the previous intermediate (177 g) under stirring. The reaction mixture was heated at 80 °C for 10 min. After cooling, it was poured on a mixture of water (4 L)/ice (3 kg). The solid was filtered, washed with water and diethyl ether, and dried to give 157 g of **105** (72%): ^{1}H NMR (DMSO-d_6) δ 6.6 (dd, 1H), 6.9 (dd, 1H); MS-ESI m/z 182 [M − H]. Anal. ($C_8H_3NO_2F_2$) C, H, N.

2-Amino-4,6-difluorobenzoic Acid (106). To a large beaker containing a solution of NaOH pellets (424 g, 10.6 mol) in water (1.3 L) was added **105** (157 g, 0.856 mol). The mixture was heated at 60−70 °C, and 30% H_2O_2 (260 mL) was added dropwise with stirring. After strong gas evolution occurred, the mixture was heated under stirring for 30 min. The mixture was cooled to 0 °C and acidified at pH 4 by addition of 12 N HCl (770 mL). The mixture was extracted with ethyl acetate. The organic phase was washed, dried ($MgSO_4$), and evaporated to give 118 g of **106** as a solid (80%): ^{1}H NMR (DMSO-d_6) δ 6.25 (dd, 1H), 6.35 (dd, 1H); MS-ESI m/z 172 [M − H]. Anal. ($C_7H_5NO_2F_2$) C, H, N.

Methyl 2-Amino-4,6-difluorobenzoate (107). To a mixture of **104** (118 g, 0.68 mol), triphenylphosphine (196 g, 0.75 mol), and methanol (39 mL, 0.95 mol) in dichloromethane (1.5 L) cooled in ice was added dropwise DEAD (118 mL, 0.75 mol). The mixture was stirred for 1 h at room temperature. The organic phase was purified by chromatography on SiO_2 and eluted with dichloromethane to give after evaporation 111 g of **107** (87%) as a white solid: ^{1}H NMR (DMSO-d_6) δ 3.9 (s, 3H), 5.9 (m, 2H), 6.1(m, 2H); MS-ESI m/z 188 [MH$^+$]. Anal. ($C_8H_7NO_2F_2$) C, H, N.

5,7-Difluoroquinazolin-4(3*H*)-one (108). A mixture of **107** (82 g, 440 mmol) and formamidine acetate (137 g, 1320 mmol) in 2-methoxyethanol (1.5 L) was heated at reflux temperature for 10 h. The solvent was evaporated and the residue taken up in water. The precipitate was filtered, washed with water and Et_2O, purified by SiO_2 chromatography, and eluted using a mixture of dichloromethane/methanol (95:5) to give 61 g of **108** (76%): ^{1}H NMR (DMSO-d_6) δ 7.3 (m, 2H), 8.12 (s,1H); MS-ESI m/z 183 [MH$^+$]. Anal. ($C_8H_4N_2OF_2$) C, H, N.

7-Fluoro-5-morpholin-4-ylquinazolin-4(3*H*)-one (109). A mixture of **108** (910 mg, 5 mmol) and morpholine (900 μL) in DMF (20 mL) was heated at 100 °C for 1 h. The volatiles were removed under vacuum, and the residue was made alkaline by addition of 6 N methanol/ammonia solution and again evaporated. The solid formed was washed with water and diethyl ether to give 850 mg of **109** (69%): ^{1}H NMR (DMSO-d_6) δ 2.95−3.05 (m, 4H), 3.7−3.8 (m, 4H), 6.8 (dd, 1H), 6.9 (dd, 1H), 8.0 (s, 1H); MS-ESI m/z 250 [MH]$^+$.

5-Morpholin-4-yl-7-(2-pyrrolidin-1-ylethoxy)quinazolin-4(3*H*)-one (110). To an ice-cold solution of 3-pyrrolidin-1-ylpropan-1-ol (0.7 mL, 6 mmol) in DMF (15 mL) was added under stirring NaH (60% suspension in oil) (600 mg, 1.5 mmol). The mixture was stirred at room temperature for 15 min, and **109** (750 mg, 3 mmol) was added as a solid. The mixture was heated at 90 °C for 4 h. The volatiles were removed under vacuum, after addition of acetic acid (900 μL, 15 mmol)) the residue was dissolved in a mixture of dichloromethane/methanol and filtered to remove impurities, and the organic phase was purified by SiO_2 chromatography using a mixture of dichloromethane−6 N methanol/ammonia (95:5) as eluent to give after evaporation 500 mg of **110** (50%): ^{1}H NMR (DMSO-d_6) δ 1.6−1.7 (m, 4H), 2.8−2.9 (m, 2H), 2.95−3.05 (m, 4H), 3.7−3.8 (m, 4H), 4.2 (t, 2H), 6.45 (d, 1H), 6.7 (d, 1H), 8.0 (s, 1H), 11.6 (s, 1H); MS-ESI m/z 345 [MH]$^+$.

5-Hydroxy-6-methoxyquinazolin-4(3*H*)-one (111). A suspension of 5-(benzyloxy)-6-methoxyquinazolin-4(3*H*)-one[78] (5 g, 17.7 mmol) in a mixture of TFA (50 mL) and water (100 mL) was stirred at room temperature for 30 min, and then the obtained solution was concentrated off. The crude was taken up in water (100 mL), and the pH was adjusted to 8 by adding solid sodium bicarbonate. The formed precipitate was filtered off, washed with water, and dried under vacuum at 50 °C in the presence of phosphorus pentoxide, giving 3.6 g of **111** as a white solid (quantitative), which was used in the next step without further purification: ^{1}H NMR (DMSO-d_6) δ 3.85 (s, 3H), 7.13 (d, 1H), 7.53 (d, 1H), 7.99 (s, 1H), 11.89 (br s, 1H)), 12.2 (br s, 1H); MS-ESI m/z 193 [MH]$^+$.

(5-Hydroxy-6-methoxy-4-oxoquinazolin-3(4*H*)-yl)methyl Pivalate (112). Solid **111** (3.6 g, 19 mmol) was slowly added to a suspension of NaH (60% suspension in oil) (1.6 g, 40 mmol) in DMF (36 mL) cooled at 0 °C and under a nitrogen atmosphere. The mixture was then stirred at room temperature for 1 h and cooled again in an ice bath before dropwise addition of chloromethyl pivalate (4.1 mL, 28.5 mmol). After being stirred at room temperature for 1.5 h, the reaction mixture was poured into a solution of acetic acid (2 mL) in water (180 mL). The precipitate was filtered off and taken up in dichloromethane; the solution was dried ($MgSO_4$) and evaporated to give 4.6 g of **112** (79%): ^{1}H NMR ($CDCl_3$) δ 1.2 (s, 9H), 3.97 (s, 3H), 5.90 (s, 2H), 7.21 (d, 1H), 7.36 (d, 1H), 8.06 (s, 1H), 11.49 (s, 1H); MS-ESI m/z 307 [MH]$^+$.

6-Methoxy-5-(tetrahydro-2*H*-pyran-4-yloxy)quinazolin-4(3*H*)-one (113). To a mixture of **112** (3 g, 10 mmol), triphenylphosphine (4.2 g, 16 mmol), and tetrahydro-2H-pyran-4-ol (1.2 mL, 13 mmol) in dichloromethane (50 mL) cooled at 0 °C was added portionwise DTAD (3.6 g, 16 mmol). The mixture was then stirred at room temperature for 1 h and evaporated. The residue was taken up in a 7 N solution of ammonia in methanol and the resulting mixture stirred at room temperature for 7 h. The mixture was concentrated and purified by SiO_2 chromatography using a mixture of ethyl acetate/dichloromethane/methanol (50:48:2 up to 50:45:5) as eluent to give 2.3 g of **113** (83%): ^{1}H NMR (DMSO-d_6) δ 1.6−1.8 (m, 2H), 1.8−2.0 (m, 2H), 3.5−3.6 (m, 2H), 3.85−4.0 (m, 5H), 4.29 (m, 1H), 7.41 (d, 1H), 7.59 (d, 1H), 7.88 (s, 1H); MS-ESI m/z 277 [MH]$^+$.

6-Methoxy-5-[(1-methylpiperidin-4-yl)oxy]quinazolin-4(3*H*)-one (114). To a mixture of **112** (1.55 g, 5 mmol), triphenylphosphine (2 g, 7.6 mmol), and 1-methylpiperidin-4-ol (757 mg, 6.6 mmol) in dichloromethane (15 mL) cooled at 10 °C was added portionwise DTAD (1.75 g, 7.6 mmol). The mixture was then stirred at room temperature for 1 h and then poured onto a SiO_2 column. Purification was performed using a mixture of ethyl acetate/dichloromethane/methanol (50:50:0 up to 50:45:5) and then with a 7 N solution of ammonia in methanol/ethyl acetate/dichloromethane (5:50:45) to give the intermediate [6-methoxy-5-[(1-methylpiperidin-4-yl)oxy]-4-oxoquinazolin-3(4*H*)-yl]methyl pivalate, which was taken up in a 7 N solution of ammonia in methanol, and the resulting mixture was stirred at room temperature over the weekend. The mixture was concentrated and then triturated in diethyl ether. The formed precipitate was filtered off to give 920 mg of **114** (63%): ^{1}H NMR (DMSO-d_6) δ 1.7−1.9 (m, 4H), 1.9−2.0 (m, 2H), 2.15 (s, 3H), 2.65−2.75 (m, 2H), 3.85 (s, 3H), 4.08 (m, 1H), 7.39 (d, 1H), 7.57 (d, 1H), 7.87 (s, 1H); MS-ESI m/z 290 [MH]$^+$.

4-Chloro-6-methoxy-5-[(1-methylpiperidin-4-yl)oxy]quinazoline (115). A mixture of **112** (300 mg, 1 mmol), triphenylphosphine (544 mg, 2 mmol), and carbon tetrachloride (300 μL, 3 mmol) in 1,2-dichloroethane (13 mL) was heated at 70 °C for 2.5 h. The solution was allowed to cool to room temperature and then poured onto a SiO_2 column. Purification was performed using a mixture of ethyl acetate/dichloromethane/methanol (50:50:0 up to 50:45:5) as eluent and then with a 7 N solution of ammonia in methanol/ethyl acetate/dichloromethane (5:50:45) to give 216 mg of **115** (68%): ^{1}H NMR ($CDCl_3$) δ 1.8−2.1 (m, 6H), 2.25 (s, 3H), 2.8−2.95 (m, 2H), 3.97 (s, 3H), 4.38 (m, 1H), 7.67 (d, 1H), 7.81 (d, 1H), 8.81 (s, 1H); MS-ESI m/z 308 and 310 [MH]$^+$.

6-Hydroxy-5-(tetrahydro-2*H*-pyran-4-yloxy)quinazolin-4(3*H*)-one (116). A mixture of **113** (1.9 g, 6.9 mmol), potassium carbonate (1.4 g, 10 mmol), and benzenethiol (1 mL, 10 mmol) in NMP (20 mL) was heated at 200 °C for 30 min. The solvent was evaporated, and the residue was dissolved in a mixture of dichloromethane (25 mL), methanol (1 mL), and acetic acid (2 mL) and then poured onto a SiO_2 column. The crude was purified using a mixture of ethyl acetate/dichloromethane/methanol (50:48:2 up to 50:45:5) as

eluent to give 1.65 g of **116** (91%): ^{1}H NMR (DMSO-d_6) δ 1.7−1.9 (m, 4H), 3.2−3.4 (m, 2H), 3.85−3.95 (m, 2H), 4.30 (m, 1H), 7.29 (d, 1H), 7.35 (d, 1H), 7.83 (s, 1H), 9.2−9.6 (br s, 1H), 11.5−12.0 (br s, 1H); MS-ESI m/z 263 [MH]$^+$.

4-Oxo-5-(tetrahydro-2*H*-pyran-4-yloxy)-3,4-dihydroquinazolin-6-yl Acetate (117). A mixture of **116** (700 mg, 2.7 mmol), acetic anhydride (10 mL), and pyridine (700 μL) was heated at 100 °C for 1 h. After evaporation the residue was taken up in a mixture of methanol (9 mL) and water (9 mL) and the resulting mixture stirred for 1 h at room temperature. The formed precipitate was filtered off, and methanol evaporation gave a second crop after filtration. Both precipitates were combined to give 540 mg of **117** (65%): ^{1}H NMR (DMSO-d_6) δ 1.55−1.75 (m, 2H), 1.8−1.9 (m, 2H), 2.31 (s, 3H), 3.2−3.4 (m, 2H), 3.75−3.85 (m, 2H), 4.25 (m, 1H), 7.41 (d, 1H), 7.57 (d, 1H), 8.01 (s, 1H), 11.9−12−5 (br s, 1H); MS-ESI m/z 305 [MH]$^+$.

4-Chloro-5-(tetrahydro-2*H*-pyran-4-yloxy)quinazolin-6-ol (118). A mixture of **117** (540 mg, 1.8 mmol), triphenylphosphine (930 mg, 3.5 mmol), and carbon tetrachloride (515 μL, 5.3 mmol) in 1,2-dichloroethane (24 mL) was heated at 70 °C for 2.5 h. The solvent was evaporated, and the residue was taken up in a 7 N solution of ammonia in methanol (20 mL). The solution was stirred at room temperature for 1 h, and then the solvent was evaporated off. The crude was purified by SiO_2 chromatography using a mixture of ethyl acetate/dichloromethane (0:100 up to 50:50) as eluent to give 1.12 g of a mixture of **118** and 2 equiv of triphenylphosphine oxide (corrected 74%). **118** was used in the next step without further purification: ^{1}H NMR ($CDCl_3$) δ 1.85−2.05 (m, 4H), 3.30−3.45 (m, 2H), 3.95−4.10 (m, 2H), 4.4−4.5 (m, 1H), 7.75 (d, 1H), 7.80 (d, 1H), 7.96 (s, 1H), 8.88 (s, 1H); MS-ESI m/z 281 and 283 [MH]$^+$.

4-Chloro-6-[3-(4-methylpiperazin-1-yl)propoxy]-5-(tetrahydro-2*H*-pyran-4-yloxy)quinazoline (119). To a mixture of impure **118** (1.12 g), triphenylphosphine (527 mg, 2 mmol), and 3-(4-methylpiperazin-1-yl)propan-1-ol (254 mg, 1.6 mmol) in dichloromethane (10 mL) was added DTAD (462 mg, 2 mmol) at room temperature. After being stirred for 30 min, the solution was poured onto a SiO_2 column. Chromatography was performed using a mixture of ethyl acetate/dichloromethane/methanol (50:50:0 up to 50:45:5) as eluent and then with a 7 N solution of ammonia in methanol/ethyl acetate/dichloromethane (5:50:45) to give 510 mg of **119** containing 30 mol % 3-(4-methylpiperazin-1-yl)propan-1-ol (corrected 81%). **119** was used in the next step without further purification: ^{1}H NMR ($CDCl_3$) δ 1.8−2.2 (m, 6H), 2.31 (s, 3H), 2.4−2.7 (m, 10H), 3.3−3.4 (m, 2H), 4.0−4.1 (m, 2H), 4.24 (t, 2H), 4.6−4.7 (m, 1H), 7.74 (d, 1H), 7.85 (d, 1H), 8.86 (s, 1H); MS-ESI m/z 421 and 423 [MH]$^+$.

Biological Evaluation. IC_{50} values reported are means of at least three to five measurements.

(i) In vitro Src Kinase Inhibition Test. This assay determines the ability of test compounds to inhibit c-Src kinase activity. The method is as reported previously.[27]

(ii) In vitro Abl Kinase Inhibition Test. This assay determines the ability of test compounds to inhibit Abl kinase activity. A poly-(Glu, Ala, Tyr) 6:3:1 random copolymer (Sigma-Aldrich, Poole, U.K.) was used as the tyrosine-containing substrate. It was stored as a 2 mg/mL stock solution in PBS at −20 °C and diluted 1:1000 with PBS to coat 96-well plates (100 μL/well). The 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 4 times the final required reaction concentrations. Solutions of 12 μM ATP in 80 mM $MgCl_2$ and 80 mM $MgCl_2$ alone (for −ve controls) were prepared. Abl protein tyrosine kinase (PTK) is a truncated form (45 kDa) of v-Abl PTK and is identical to the normal c-Abl PTK. Abl isolated from a strain of *E. coli* cells carrying the Abl kinase catalytic domain encoded by the Abelson murine leukemia virus under the control of a T7 expression system (New England BioLabs, Inc., Ipswich, MA) was diluted to 40 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 were 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 of Abl kinase in dilution buffer to start the reaction. The final reaction concentrations were 20 U/mL Abl kinase, 20 mM $MgCl_2$, and 3 μM ATP (determined as the K_m for ATP). The reaction time allowed was 22 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 the 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., California); this was diluted 1:5000 in PBST/B/O (PBST + 0.5% BSA + 0.1 mM sodium orthovanadate), added at 100 μL/well, and incubated for 1 h. The plates were again washed (7×). 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 gave an $A_{450\,nm}$ 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.

(iii) In Vitro KDR Kinase Inhibition Test. This assay determines the ability of test compounds to inhibit KDR kinase activity. The method is as reported previously.[48]

(iv) Other in Vitro Kinase Inhibition Test. Flt-4 tyrosine kinase was obtained from ProQinase GmbH (Freiburg, Germany) and c-Kit tyrosine kinase from Upstate Biotechnology, Inc. (Lake Placid, NY). Each additional kinase used was generated as a cell lysate, following infection of insect cells with recombinant baculoviruses containing kinase domains. All enzyme assays were run at, or just below, the respective K_m for ATP (0.2−30 μmol/L). The inhibitory activity of the compounds was determined against a range of recombinant tyrosine kinases [CSK, c-Yes, LCK, Flt-1, Flt-4, c-Kit, PDGFR-α, PDGFR-β, FGFR1, Abl, epidermal EGFR, and Aur-B] using ELISA methodology described previously.[48] Selectivity versus CDK2 serine/threonine kinase was examined using scintillation proximity assays with a retinoblastoma substrate and [γ-^{33}P]ATP.[66b] Activity versus the dual-specificity kinase MAPK kinase (MEK) was determined with a MAPK substrate, [γ-^{33}P]ATP, and paper capture/scintillation counting.[66b] Microcal Origin software (v. 3.78, Microcal Software, Inc., Northhampton, MA) was used to interpolate IC_{50} values by nonlinear regression.

(v) In Vitro c-Src 3T3 Proliferation Assay. This assay determines the ability of test compounds to inhibit the proliferation in culture of mouse NIH3T3 fibroblast cells transfected to over-express active c-Src kinase. The method is as reported previously.[27]

(vi) In Vitro K562 Proliferation Assay. These assays determine the ability of test compounds to inhibit the proliferation of cells in culture. The cells used were K-562, a human chronic myelogenous leukemia (CML) cell line, ATCC No. CCl-243. The K562 suspension cell line was maintained at between 10^5 and 10^6 cells/mL in phenol red free RPMI 1640 medium (Invitrogen) supplemented with 1% L-glutamine, 10% fetal bovine serum (Sigma), and 50 mM HEPES.

For assay the cells were plated into 96-well plates at 5000 cells/90 μL of the indicated growth medium per well. One extra plate designated the "predose plate" was also made at this time. After 2 h the cells were dosed with 10 μL/well of compound solution. (The compounds were stored in stock concentrations of 10mM in 100% DMSO.) The dilutions ensure that the final concentration of DMSO

in each assay well is 0.1% and that the final compound concentration in the assay wells ranges between 10 and 0.00 15μM. MTS reagent (20 μL) (no. G1111/2, Promega Corp., Madison, WI) was then added to the predose plate, and after a 2 h incubation the plate was read (as indicated below). After a further 72 h 20 μL of MTS reagent was added to all the remaining wells. After a 2 h incubation, 25 μL of 10% SDS was added to the wells. Then the plates were read at $A_{490\ nm}$. Only the inner 60 wells of the 96-well plates were used in this assay to minimize edge effects. The outer wells were filled with sterile PBS during the course of the assay to help prevent any evaporation. The average absorbance of the predose plate was subtracted from the later plate readings in determining the results.

(vii) In Vitro A549 Microdroplet Migration (Chemokinesis) Assay. This assay determines the ability of test compounds to inhibit the random motility (chemokinesis) of A549 cells (human epithelial lung carcinoma cells, ATCC CCL 185). The method is as reported previously.[27]

(viii) Pharmacokinetics. Pharmacokinetics were determined in mouse, rat, and dog following single intravenous or oral doses (doses described in Tables 2 and 6) of the compound. For the iv studies, the compounds were formulated in a mixture of 25% (w/v) (hydroxypropyl)-β-cyclodextrin/Sorenson's phosphate buffer (pH 5.5). For the oral studies, the compounds were formulated as a solution in either 1% polysorbate or 0.1 M citrate buffer (pH 3).

(ix) Rat Xenografts. This assay determines the ability of the test compound to inhibit the growth of c-Src-transfected 3T3 cells implanted subcutaneously following once daily oral administration. The method is as reported previously.[27]

(x) BxPC-3 Orthotopic Model. Female athymic nude NMRI *ν/ν* mice (Bioservices, The Netherlands) were orthotopically inoculated with human pancreatic tumor cells (BxPC-3) at day 0. The mice were treated by gavage (25 mg/kg of AZD0530 daily). The first oral treatment was administered 1 h prior to inoculation of the human pancreatic (BxPC-3) tumor cells and treatment continued thereafter until sacrifice. The primary outcome for AZD0530 activity was prolongation of survival, evaluated by two separate means. First, the percentage T/C (survival) was calculated by dividing the median day of sacrifice in the treated group "T" by the median day of sacrifice in the control group "C" and multiplying that number by 100. In this model, a T/C value >130% indicates a significant prolongation of survival when compared with the survival of the vehicle-treated control group. Second, the effect of AZD0530 was evaluated by conducting a Kaplan−Meier analysis with the cutoff level of significance (log-rank statistics) set at a p value of <0.05.

Solubility Measurements. The thermodynamic solubility of a research compound is measured under standard conditions. A known amount of compound is stirred in 0.1 M pH 7.4 phosphate buffer at constant temperature (25 °C) for 24 h. The supernatant is then separated from undissolved material by double centrifugation and subsequently analyzed and quantified against a standard of known concentration in DMSO using generic HPLC−UV methodology coupled with mass spectral peak identification.

Molecular Modeling. To be consistent with our enzymatic assay, which targets the activated form of c-Src kinase, initial modeling and docking studies of the kinase domain were performed using the crystal structure of activated Lck as a surrogate for Src.[74] Indeed, our in-house crystal structures of c-Src contain the SH2, SH3, and kinase domains in an inactivated form, as do published structures of c-Src. They differ from the closely related kinase Lck, which is published as the isolated and activated kinase domain.[79] Notable structural differences have been observed in the size of the hydrophobic pocket, which is deeper in the inactive form than in the active one,[36] although the shape of the sugar pocket is not significantly different. These observations supported the choice of the Lck structure as a model for activated c-Src. Our inhibitors were built using Quanta, and the charges were assigned by the Quanta charge template method.[80,81] These inhibitors were 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.[82]

Crystallography. Protein and crystals were prepared following Xu et al.[79] with minor modifications in crystallization. Crystals were grown by sitting drop vapor diffusion at 15 °C, combining 1.5 μL of reservoir solution (50 mM PIPES (pH 6.5), 10 mM DTT, 100 mM sodium chloride, and 4−9% PEG4000 (w/v)) with 1.5 μL of protein−inhibitor solution (made by mixing 10 μL of 10 mg/mL protein with 1.4 μL of 20 mM inhibitor dissolved in 10 mM PIPES (pH 6.5) and 20% DMSO, adjusted so that pH > 4.0). Crystals appeared within 48 h and were cryoprotected by being dipped into 50 mM PIPES (pH 6.5), 20% (w/v) PEG4000, 0.1 M sodium chloride, 10 mM DTT, and 22.5% glycerol before being cooled to 100 K. The crystals belong to space group $P2_12_12_1$ with unit cell dimensions $a = 49.81$ Å, $b = 72.47$ Å, and $c = 171.57$ Å, with one molecule in the asymmetric unit. Diffraction data were recorded at the ESRF (Grenoble), beamline ID14-4, on an ADSC Quantum4R CCD detector. Data were integrated with the program MOSFLM.[83] CCP4 programs[84] were used for subsequent data analysis. The structure was determined by molecular replacement using the published structure of c-Src.[79] Model building used QUANTA2000 (Accelrys), and refinement was to $R = 20.5\%$ ($R_{free} = 27.6\%$). The final statistics are given in Table 6. Thr523 and Glu524 were modeled as Ala because of poor side chain density. The coordinates are deposited in the Protein Data Bank with accession code 2H8H.

Table 6. Data Collection and Refinement Statistics

space group	$P2_12_12_1$
cell constants (a, b, c, Å)	49.81, 72.47, 171.57
resolution range (Å)	50.00−2.20
completeness (%)	95.49
no. of unique reflns	29365
multiplicity	3.4
$R_{merge}{}^a$ (%)	8.3
R^b (%)	20.4
$R_{free}{}^c$ (%)	27.2
rms deviation from ideal values	
bond lengths (Å)	0.013
bond angles (deg)	1.4
average B value (Å^2)	
protein main chain atoms	36.9
all protein atoms	37.7
ligand	36.0
solvent	38.8

^{a} $R_{merge} = \sum_{hkl}[(\sum_i|I_i - \langle I\rangle|)/\sum_i I_i]$. ^{b} $R = \sum_{hkl}||F_o| - |F_c||/\sum_{hkl}|F_o|$. ^{c} R_{free} is the cross-validatifon R factor computed for a test set of 5% of the unique reflections.

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Supporting Information Available: Microanalysis data, additional experimental details, and spectroscopic data for compounds **2**, **6**, **8**−**10**, **13**−**16**, **19**, **21**, **23**, **25**, **26**, **29**, **31**, **50**, **51**, **58**, **62**−**64**, **66**, **68**, **77**, **79**, **83**, **86**, **91**, **92**, **96**, **98**, **101**, and **104**. This material is available free of charge via the Internet at http://pubs.acs.org.

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