Optimization of 4-Phenylamino-3-quinolinecarbonitriles as Potent Inhibitors of Src Kinase Activity

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Subsequent to the discovery of 4-[(2,4-dichlorophenyl)amino]-6,7-dimethoxy-3-quinolinecarbonitrile (**1a**) as an inhibitor of Src kinase activity (IC$_{50}$ = 30 nM), several additional analogues were prepared. Optimization of the C-4 anilino group of **1a** led to **1c**, which contains a 2,4-dichloro-5-methoxy-substituted aniline. Replacement of the methoxy group at C-7 of **1c** with a 3-(morpholin-4-yl)propoxy group provided **2c**, resulting in increased inhibition of both Src kinase activity and Src-mediated cell proliferation. Analogues of **2c** with other trisubstituted anilines at C-4 were also potent Src inhibitors, and the propoxy group of **2c** was preferred over ethoxy, butoxy, or pentoxy. Replacement of the morpholine group of **2c** with a 4-methylpiperazine group provided **31a**, which had an IC$_{50}$ of 1.2 nM in the Src enzymatic assay, an IC$_{50}$ of 100 nM for the inhibition of Src-dependent cell proliferation and was selective for Src over non-Src family kinases. Compound **31a**, which had higher 1 and 4 h plasma levels than **2c**, effectively inhibited tumor growth in xenograft models.

Introduction

Tyrosine kinases (TKs) are enzymes that catalyze the specific phosphorylation of tyrosine residues on proteins. The TK Src is the prototype member of the Src family of kinases (SFKs), which share a common structural organization and are highly homologous in their ATP-binding regions.[1−4] Since Src is involved in several cell signaling pathways, inhibition of Src activity could be efficacious for the treatment of various diseases, including cancer.[5,6] Src is overexpressed in several types of human tumors, and increased Src activity is observed in metastatic tumors.[7−10] In addition, since Src has been implicated in VEGF (vascular endothelial growth factor) signaling in endothelial cells, Src inhibitors might inhibit tumor growth via an anti-angiogenesis mechanism.[11] Src inhibitors may also prevent secondary injury that results from VEGF-mediated increase in vascular permeability, including the brain damage that often follows stroke.[12] Lastly, since osteoclastic bone resorption requires Src activity, Src inhibitors could prove effective in the treatment of osteoporosis.[13−15]

We recently reported that a high-throughput yeast-based assay identified 4-[(2,4-dichlorophenyl)amino]-6,7-dimethoxy-3-quinolinecarbonitrile (**1a**) as a Src inhibitor.[16] Further studies showed that this compound inhibited the kinase activity of Src with an IC$_{50}$ of 30 nM. This earlier work demonstrated that the aniline group at C-4, the carbonitrile group at C-3, and the alkoxy groups at C-6 and C-7 of the quinoline were all crucial for optimal activity. In addition, while replace-

ment of the C-7 methoxy group with a 3-(morpholin-4-yl)-propoxy group to provide **2a** resulted in enhanced inhibition of both Src enzymatic and cell activities, this substitution at C-6 was detrimental. It was also reported that **3a** and **4a**, the corresponding quinazoline and quinoline analogues of **1a**, were less potent Src inhibitors.

1a

2a

3a: X = N
4a: X = CH
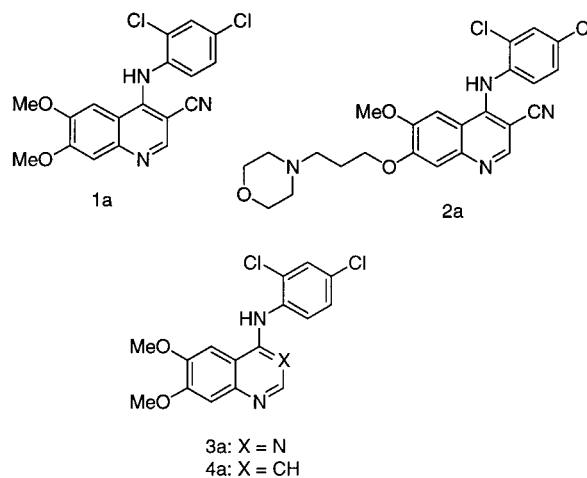
Other reports from our laboratories demonstrated that altering the substituents on the aniline group at C-4 of the 3-quinolinecarbonitrile resulted in compounds with selectivity for other kinases, including EGFr (epidermal growth factor receptor)[17] and MAPKK (mitogen-activated protein kinase kinase).[18] In the hope of improving the activity and selectivity for Src, we investigated other aniline groups at C-4.[19] To this end, several commercially available 2-chloroanilines were added to 4-chloro-6,7-dimethoxy-3-quinolinecarbonitrile (**5**).[17] When compared to **1a**, increased inhibition of Src
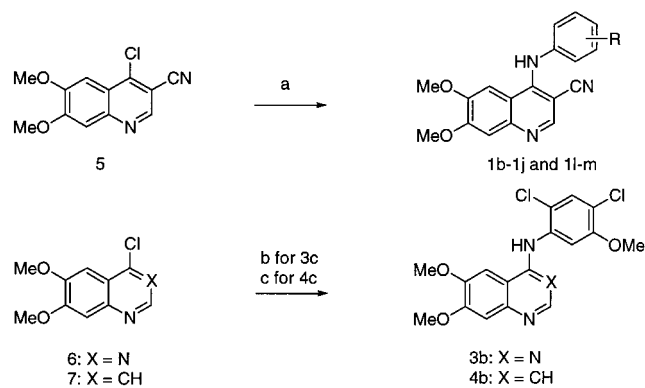
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Scheme 1[a]

5

1b-1j and 1l-m

6: X = N
7: X = CH

b for 3c
c for 4c

3b: X = N
4b: X = CH

[a] Reagents and conditions: (a) aniline, pyridine hydrochloride, 2-ethoxyethanol, reflux, or aniline, sodium hydride, tetrahydrofuran or N,N-dimethylformamide, reflux; (b) 2,4-dichloro-5-methoxyaniline, ethanol, reflux; (c) 2,4-dichloro-5-methoxyaniline, tris-(dibenzylidene)acetonedipalladium, 2-(cyclohexylphosphino)-2′-(N,N-dimethylamino)biphenyl, potassium phosphate, ethylene glycol dimethyl ether, reflux.
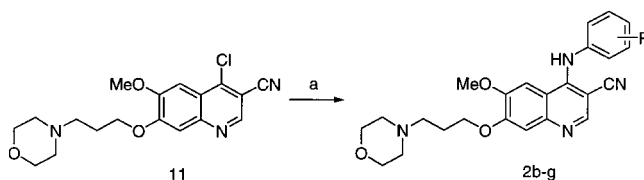
Scheme 2[a]

8a: R = Et
8b: R = n-Pr

8c: R = H

9

10
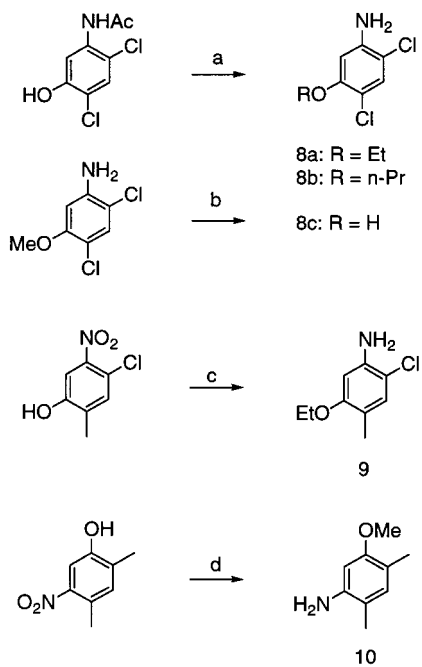
[a] Reagents and conditions: (a) (1) ethyl iodide or n-propyl bromide, potassium carbonate, acetone, reflux; (2) 5.0 N sodium hydroxide, aqueous ethanol, reflux; (b) boron tribromide, dichloromethane, −78 °C to room temperature; (c) (1) ethyl iodide, potassium carbonate, acetone, reflux; (2) iron, ethanol, aqueous hydrochloric acid, reflux; (d) (1) methyl iodide, potassium carbonate, acetone, reflux; (2) palladium on carbon, methanol, hydrogen at 45 psi.

kinase activity was observed with **1b** (IC_{50} = 10 nM), which contains a 2-chloro-5-methoxyaniline group at C-4. Combination of the groups on **1a** and **1b** provided **1c**, which had an IC_{50} of 4.3 nM. We present here a full structure−activity relationship (SAR) study of analogues of **1c** as Src kinase inhibitors.[20,21]

Chemistry

As depicted in Scheme 1, compounds **1b**−**1m** were prepared by treatment of **5**[17] with the corresponding aniline. Two protocols were used, either pyridine hy-

Scheme 3[a]

11

2b-g

[a] Reagents and conditions: (a) aniline, pyridine hydrochloride, 2-ethoxyethanol, reflux, or aniline, sodium hydride, tetrahydrofuran, reflux.

Scheme 4[a]

12: R = H
13: R = NO₂
14: R = NH₂

17: R = Cl
18: R = morpholine

15: R = OH
16: R = Cl
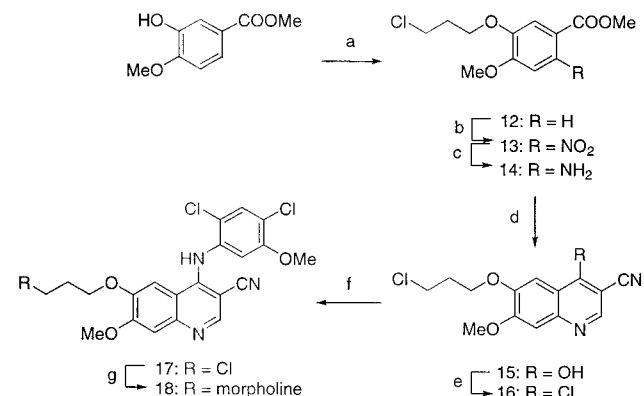
[a] Reagents and conditions: (a) 3-chloropropyl p-toluenesulfonate, potassium carbonate, tricaprylmethylammonium chloride, acetone, reflux; (b) 70% nitric acid, acetic acid, reflux; (c) iron, ammonium chloride, aqueous methanol, reflux; (d) (1) dimethylformamide dimethylacetal, reflux; (2) n-butyllithium, acetonitrile, tetrahydrofuran, −78 °C to room temperature; (e) phosphorous oxychloride, reflux; (f) 2,4-dichloro-5-methoxyaniline, pyridine hydrochloride, 2-ethoxyethanol, reflux; (g) morpholine, sodium iodide, 90 °C.

drochloride in 2-ethoxyethanol or sodium hydride in tetrahydrofuran. The quinazoline **3b** was obtained by reaction of 4-chloro-6,7-dimethoxyquinazoline (**6**)[22] with 2,4-dichloro-5-methoxyaniline[23] in ethanol. All of these conditions were ineffective in the reaction of 4-chloro-6,7-dimethoxyquinoline (**7**)[24] with this aniline, and it was necessary to use a palladium-mediated coupling condition reported by Buchwald[25] to prepare **4b**.

None of the trisubstituted anilines used to prepare **1c**−**1m** were commercially available. Those anilines not previously reported in the literature were prepared as shown in Scheme 2. Reaction of 2,4-dichloro-5-hydroxyacetanilide[26] with ethyl iodide followed by removal of the acetyl group provided **8a**. Similar reaction conditions employing n-propyl bromide as the alkylating agent led to **8b**. The corresponding hydroxy derivative **8c** was obtained by treatment of 2,4-dichloro-5-methoxyaniline[23] with boron tribromide. Alkylation of 2-chloro-4-methyl-5-nitrophenol[23] with ethyl iodide followed by reduction of the nitro group led to **9**. An analogous sequence was used to convert 2,4-dimethyl-5-nitrophenol[27] to **10**.

As shown in Scheme 3, several of these trisubstituted anilines were added to the 4-chloro group of **11**[28] to provide compounds **2c**−**2g**, which have a 3-(morpholin-4-yl)propoxy group at C-7. The isomer of **2c**, with the 3-(morpholin-4-yl)propoxy group at C-6, namely, **18**, was prepared as shown in Scheme 4. Alkylation of methyl 3-hydroxy-4-methoxybenzoate with 3-chloropropyl p-toluenesulfonate[29] provided **12**. Nitration of **12** gave **13**,

Scheme 5[a]
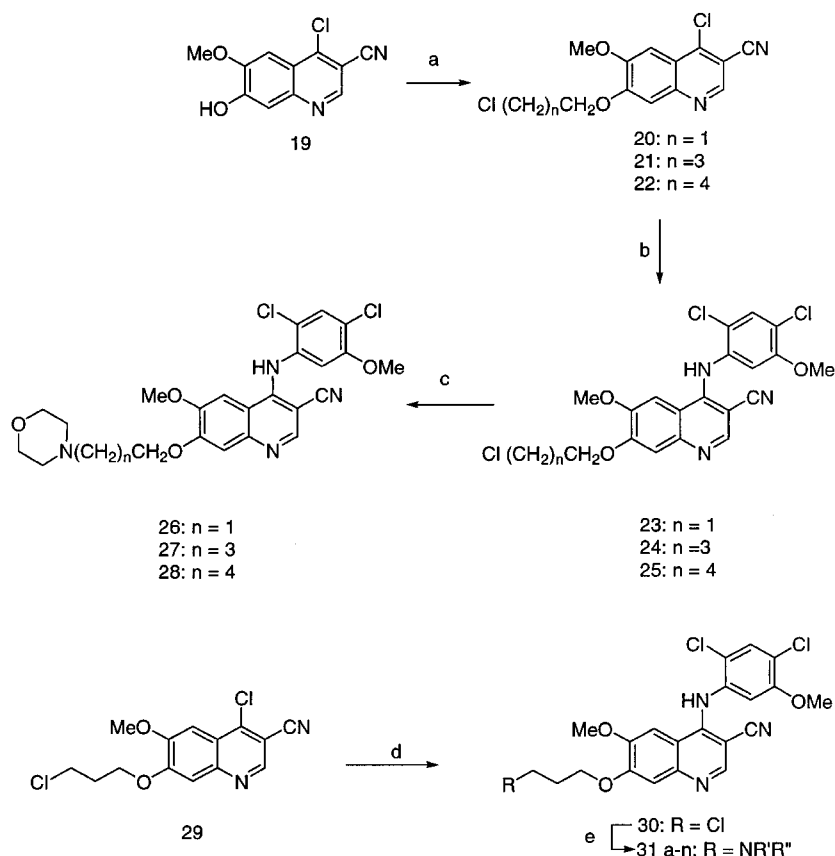
[a] Reagents and conditions: (a) triphenylphosphine, diethyl azodicarboxylate, 0 °C to room temperature, $Cl(CH_2)_nCH_2OH$, tetrahydrofuran; (b) 2,4-dichloro-5-methoxyaniline, pyridine hydrochloride, 2-ethoxyethanol, reflux, or 2,4-dichloro-5-methoxyaniline, sodium hydride, tetrahydrofuran, reflux; (c) morpholine, sodium iodide, either neat at reflux or with ethylene glycol dimethyl ether as cosolvent at 90 °C; (d) 2,4-dichloro-5-methoxyaniline, pyridine hydrochloride, 2-ethoxyethanol, reflux; (e) R″R′NH, sodium iodide, either neat or with ethylene glycol dimethyl ether as cosolvent at elevated temperatures.

with subsequent iron reduction of the nitro group resulting in **14**. Conversion of **14** to the amidine derivative, followed by cyclization with the anion of acetonitrile, provided the 3-quinolinecarbonitrile **15**. Chlorination of **15** with phosphorus oxychloride led to **16**, which was converted to **17** upon treatment with 2,4-dichloro-5-methoxyaniline. Reaction of **17** with morpholine provided the desired **18**.

The preparation of analogues of **2c** wherein the length of the propoxy chain at C-7 was varied is shown in Scheme 5. Treatment of **19**[19] under Mitsunobu conditions using 2-chloroethanol, 4-chlorobutanol, and 5-chloropentanol provided **20**, **21**, and **22**, respectively. Addition of the 2,4-dichloro-5-methoxyaniline headpiece to these intermediates provided **23**, **24**, and **25**, with subsequent reaction with morpholine leading to the desired **26**, **27**, and **28**.

Additional analogues of **2c** where the morpholine group was replaced by other amines were prepared using the route shown in Scheme 5. Reaction of **29**[18,19,28] with 2,4-dichloro-5-methoxyaniline provided **30**. The alkyl chloro group of **30** was then displaced by a variety of amines, under the conditions used to prepare **18**, to yield **31a**−**31n**.

Results and Discussion

SAR for Src Kinase and Src Cellular Assays. As shown in Table 1, 3-quinolinecarbonitrile **1c**, which has a 2,4-dichloro-5-methoxyaniline group at C-4, was al-

most a log order more potent Src kinase inhibitor than the 2,4-dichloro derivative **1a**. Interestingly, replacement of the 2,4-dichloroaniline group of both the quinazoline **3a** and the quinoline **4a** by a 2,4-dichloro-5-methoxyaniline group to provide **3b** and **4b** did not result in increased inhibition of Src kinase activity.

Further investigation of the 2,4-dichloro-5-methoxy headpiece determined that the 2,4-dichloro-5-ethoxy analogue **1d** was much less active, having an IC_{50} of only 1 μM. Decreased activity was also seen with the 5-propoxy derivative **1e**, implying a size constraint at the binding site of the anilino group. The 5-hydroxy derivative **1f** was also less active than **1c**. To determine the requirement for the chloro group at C-2 of the trisubstituted aniline headpiece, analogues varying the group at C-2 and retaining the 4-chloro and 5-methoxy substituents were prepared. Reduced activity in the Src enzymatic assay was seen with the 2-fluoro derivative **1g**, while the larger 2-bromo and 2-methyl derivatives **1h** and **1i** retained the activity of **1c**. A large decrease in activity was also observed with the 4-chloro-3-methoxy derivative **1j**. We earlier reported that the 4-chloro-2-iodo derivative **1k** was a more potent Src inhibitor than **1a**.[16] By analogy, the corresponding 4-chloro-2-iodo-5-methoxy derivative could be expected to be more active than **1c**. Unfortunately, attempts to replace the 4-chloro group of **5** with 4-chloro-2-iodo-5-methoxyaniline resulted in loss of the 2-iodo group, and only **1j** was isolated.

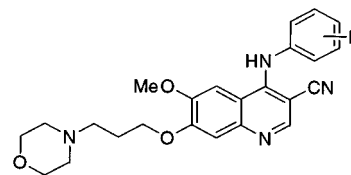**Table 1.** Inhibition of Src Kinase Activity by 6,7-Dimethoxy-4-(phenylamino)-3-quinolinecarbonitriles
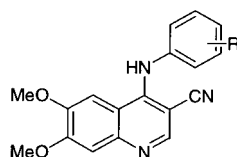
compd	R	Src[a] IC$_{50}$ (nM)
1a[16]	2,4-di-Cl	30
1b	2-Cl, 5-OMe	10
1c	2,4-di-Cl, 5-OMe	4.3
1d	2,4-di-Cl, 5-OEt	1200
1e	2,4-di-Cl, 5-O-n-Pr	>5000
1f	2,4-di-Cl, 5-OH	190
1g	2-F, 4-Cl, 5-OMe	66
1h	2-Br, 4-Cl, 5-OMe	3.0
1i	2-Me, 4-Cl, 5-OMe	4.0
1j	4-Cl, 3-OMe	120
1k[16]	2-I, 4-Cl	6.2
1l	2-Cl, 4-Me, 5-OMe	2.9
1m	2-Cl, 4-Me, 5-OEt	>5000
1n	2,4-di-Me, 5-OMe	4.6
3a[16]	quinazoline analogue of **1a**	250
4a[16]	quinoline analogue of **1a**	84
3b	quinazoline analogue of **1c**	390
4b	quinoline analogue of **1c**	81

[a] IC$_{50}$ values reported for Src inhibition represent the means of at least two separate determinations with typical variations of less than 40% between replicate values.

As was seen with the 2-chloro group, replacement of the 4-chloro group on the 2,4-dichloro-5-methoxyaniline headpiece by a methyl group was not detrimental. While **1l** retained the activity of **1c**, the corresponding 5-ethoxy analogue of **1l**, namely, **1m**, was much less active. This loss of activity parallels that seen with **1d** and **1e**. Replacement of both the 2- and 4-chloro groups of **1c** with methyl groups, as in derivative **1n**, retained the Src inhibitory activity.

Several 3-quinolinecarbonitriles with a methoxy group at C-6, a 3-(morpholin-4-yl)propoxy group at C-7, and trisubstituted aniline groups at C-4, namely, **2c**−**2g**, showed similar activity in the Src enzymatic assay, having IC$_{50}$ values ranging from 0.80 to 1.7 nM (Table 2). These compounds were also tested for their ability to inhibit the proliferation of c-Src-transformed rat fibroblasts growing in suspension.[16] The trisubstituted aniline containing compounds were all more potent than **2a**, with the IC$_{50}$ values for **2c**−**2g** falling within the range of 71−160 nM. As expected from our previous work,[16] **18**, the isomer of **2c** with the 3-(morpholin-4-yl)propoxy group at C-6, was considerably less active in both the enzyme (IC$_{50}$ = 28 nM) and cell (IC$_{50}$ = 1.2 μM) assays.

Attention was next focused on the length of the alkoxy side chain at C-7 of **2c**. As shown in Table 3, the corresponding ethoxy, butoxy, and pentoxy analogues, namely, **26**, **27**, and **28**, were all less potent than **2c** in the enzymatic assay. While **26** was roughly comparable to **2c** in the cell assay, **27** and **28** were about 2-fold less active than **2c**. Since the propoxy group was preferred over other chain lengths, additional analogues of **2c** were prepared wherein the morpholine group was replaced by other amines. The 4-alkylpiperazines **31b**, **31c**, and **31d** had good activity in both the enzymatic and cellular assays. The unsubstituted piperazine derivative **31e** was much less active in the cell assay,

Table 2. Inhibition of Src Kinase Activity and Cell Proliferation by 6-Methoxy-7-(3-morpholinopropoxy)-4-(phenylamino)-3-quinolinecarbonitriles

compd	R	Src[a] IC$_{50}$ (nM)	cells[b] IC$_{50}$ (nM)
2a[16]	2,4-di-Cl	3.8	940
2b	2-Cl, 5-OMe	1.6	150
2c	2,4-di-Cl, 5-OMe	0.80	140
2d	2-Br, 4-Cl, 5-OMe	0.95	71
2e	2-Me, 4-Cl, 5-OMe	1.2	150
2f	2,4-di-Me, 5-OMe	1.7	160
2g	2-Cl, 4-Me, 5-OMe	1.1	100
18	6-O(CH$_2$)$_3$-morpholine, 7-OMe isomer of **2c**	28	1200

[a] IC$_{50}$ values reported for Src inhibition represent the means of at least two separate determinations with typical variations of less than 40% between replicate values. [b] Anchorage-independent cellular assay. IC$_{50}$ values reported represent the means of at least two separate determinations.

Table 3. Inhibition of Src Kinase Activity and Cell Proliferation by Various C-7-Substituted 4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-methoxy-3-quinolinecarbonitriles
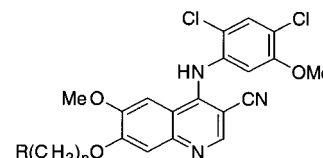
| compd	n	R	Src[a] IC$_{50}$ (nM)	cells[b] IC$_{50}$ (nM)
2c	3	morpholine	0.8	140
26	2	morpholine	2.3	190
27	4	morpholine	1.3	330
28	5	morpholine	2.5	320
31a	3	N-methylpiperazine	1.2	100
31b	3	N-ethylpiperazine	0.77	130
31c	3	cis-3,4,5-tri-Me-piperazine	1.4	220
31d	3	4-n-Pr-piperazine	1.1	160
31e	3	piperazine	1.1	1700
31f	3	4-(CH$_2$)$_2$OH-piperazine	0.76	610
31g	3	4-Me-homopiperazine	1.4	350
31h	3	piperidine	1.2	150
31i	3	4-OH-piperidine	0.64	840
31j	3	2-(1,2,3-triazole)	9.3	1900
31k	3	1-(1,2,3-triazole)	1.9	920
31l	3	1-imidazole	2.1	1400
31m	3	NH(CH$_2$)$_2$-morpholine	3.0	1200
31n	3	NMe(CH$_2$)$_2$NMe$_2$	2.3	650

[a] IC$_{50}$ values reported for Src inhibition represent the means of at least two separate determinations with typical variations of less than 40% between replicate values. [b] Anchorage-independent assay. IC$_{50}$ values reported represent the means of at least two separate determinations.

although it had comparable activity in the enzyme assay. The 4-(2-ethanol)piperazine analogue **31f** had reduced activity in both assays compared to the other 4-alkylpiperazines. The methylene homologue of **31a**, **31g**, had comparable enzyme activity but was about 3-fold less active in cells. While the piperidine analogue **31h** had cell activity comparable to that of **31a**, the 4-hydroxypiperidine analogue **31i** had reduced activity. This parallels the result seen with **31f**, which also

Table 4. Nude Mouse Plasma Levels Following a 50 mg/kg ip Dose
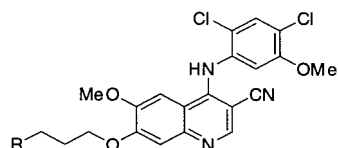
compd	R	plasma concn at 1 h (μg/mL)	plasma concn at 4 h (μg/mL)
2c	morpholine	0.10 ($\pm$0.10)	0.10 ($\pm$0.10)
31a	*N*-methylpiperazine	3.0 ($\pm$1.2)	0.90 ($\pm$0.10)
31k	1-(1,2,3-triazole)	1.3 ($\pm$1.1)	0.30 ($\pm$0.20)

contains a hydroxyl group. Three derivatives with aromatic amines, namely, **31j**, **31k**, and **31l**, all had reduced activity compared to **31a**. Extension of the morpholine group of **2c** by an ethylamine group, as in **31m**, also led to reduced activity. In addition, **31n**, which contains an *N,N,N*-trimethylethylenediamine group, and can be viewed as an "opened piperazine" analogue, also had reduced activity compared to **31a**.

Pharmacokinetic Analysis. As shown in Tables 2 and 3, several compounds had similar activities in the enzymatic assay and also in the cellular assay. To select a compound for further study, including in vivo testing, an evaluation of the plasma levels of three representative compounds was performed. An ip dose of 50 mg/kg **2c**, **31a**, and **31k** was administered to nude mice, and blood samples were drawn after 1 and 4 h. As shown in Table 4, the blood levels at both the 1 and 4 h time points for the *N*-methylpiperazine analogue **31a** were substantially higher than those of **2c**. Although **31k** was not as potent in the enzymatic or cell assay as **2c** or **31a**, this compound was chosen for pharmacokinetic evaluation because it contains a 1-(1,2,3-triazolyl)alkoxy group. In a series of quinazoline VEGFr TK inhibitors reported by Astra-Zeneca,[30] the 1-(1,2,3-triazolyl) group provided superior blood levels compared to a morpholine group. While **31k** had enhanced plasma levels compared to **2c**, these levels were lower than those of **31a**.

Enzyme Selectivity. The lead compound **1a** was previously reported to be selective for Src over several non-Src family kinases, including ErbB-2 and cyclin-dependent kinase 4 (cdk4).[16] When **31a** was tested against ErbB-2, an IC$_{50}$ of 2.6 μM was obtained. In addition, **31a** provided only 20% inhibition of cdk4 activity when tested at a concentration of 10 μg/mL. Further kinase selectivity studies were done in cell-based systems with rat fibroblasts stably transfected with plasmids expressing activated insulin-like growth factor receptor TK (IGFr), platelet-derived growth factor receptor TK (PDGFr), and fibroblast growth factor receptor TK (FGFr). When tested in proliferation assays with these cell lines, **31a** had an IC$_{50}$ of 4.0 μM for the inhibition of PDGFr-mediated cell growth and IC$_{50}$ values greater than 10 μM for the inhibition of IGFr- and FGFr-mediated cell growth. While **31a** was selective for Src over non-SFKs, this compound had an IC$_{50}$ of 410 nM for the inhibition of Fyn-dependent cell proliferation. Therefore, in cell-based assays the selectivity for Src over Fyn, another SFK, is only about 4-fold. This selectivity ratio is similar to that earlier reported for **2a**.[16]
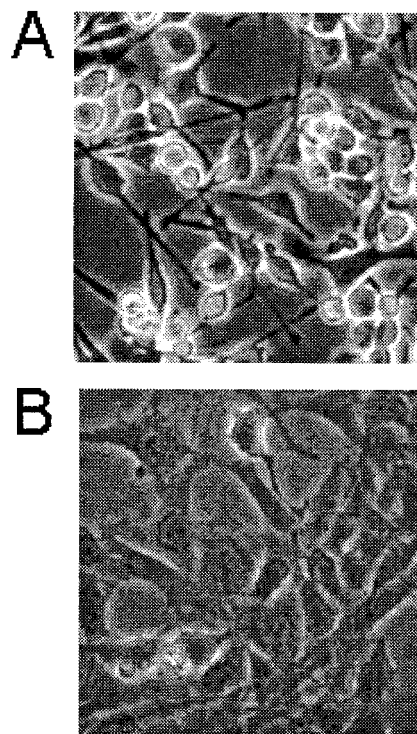

Figure 1. Reversion of Src-transformed fibroblasts to a nontransformed appearance by **31a**. Photomicrographs of rat fibroblasts transformed by a chimeric Prague C v-Src/human c-Src protein. (A) Cells with no compound added. (B) Cells after 1 h of exposure to 1 μM **31a**.
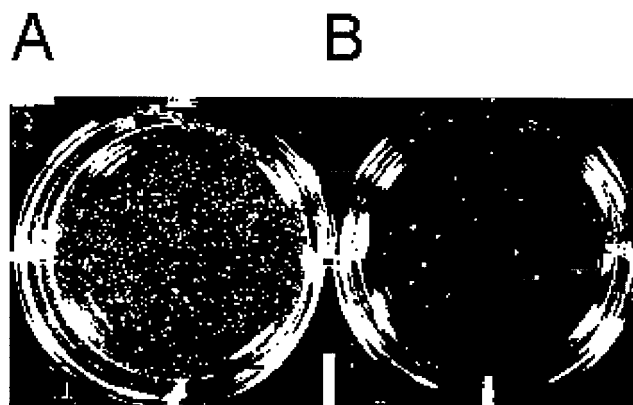

Figure 2. Inhibition of HT29 colony formation in soft agar by **31a**. Cells were plated in soft agar as described in the Experimental Section. (A) Colony formation in agar with no compound added. (B) An equivalent number of cells were plated, and **31a** was added to the medium layered on top of the soft agar layer containing the cells. The concentration of **31a** in the liquid medium was 1 μM, and the liquid medium layer comprised one-third of the total volume of the agar and medium.

Additional Cellular Assays. In an examination of extracts of Src-transformed fibroblasts treated with **31a** by anti-phosphotyrosine immunoblotting, a dose–response relationship for the inhibition of tyrosine phosphorylation was observed that mirrored the IC$_{50}$ for proliferation of these cells in suspension (data not shown). This effect was similar to that previously reported for **2a**.[16] Also, the Src-transformed fibroblasts reverted to a nontransformed morphology after exposure to 1 μM **31a** for 1 h (Figure 1).

The efficacy of anti-sense Src expression in inhibiting the growth of HT29 colon tumors in a nude mouse
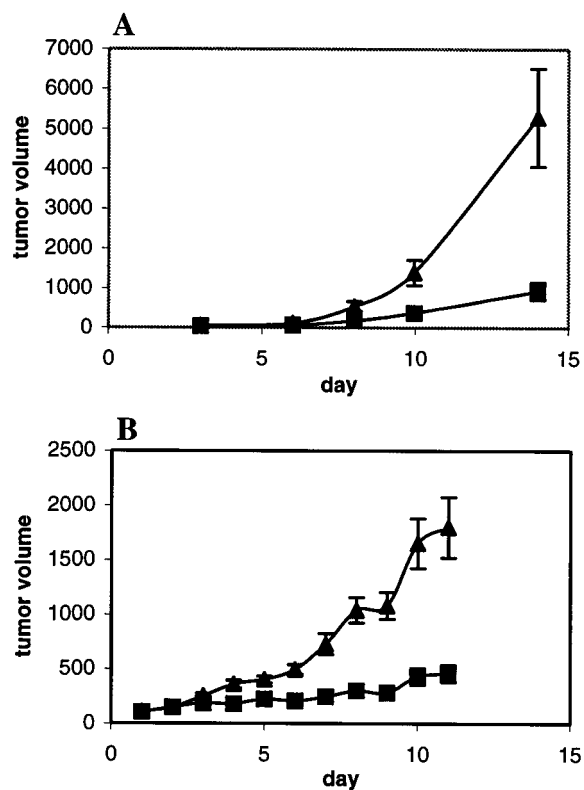
Figure 3. Antitumor activity of **31a** vs a Src-transformed fibroblast xenograft. (A) Unstaged model where mice were treated with **31a** at 30 mg/kg ip bid with dosing beginning just prior to injection of cells (■), vehicle alone (▲). (B) Staged model where mice were treated with **31a** at 25 mg/kg ip bid after the tumors reached 100 mg or more in volume (■), vehicle alone (▲).

xenograft model suggested that the growth of these tumor cells in vivo is dependent upon Src activity.[31,32] When **31a** was tested in an HT29 cell proliferation assay, under adherent conditions, an IC_{50} of only 5 μM was observed, but colony formation in agar was inhibited at submicromolar concentrations (Figure 2). These results imply that **31a** is a potent inhibitor of Src activity in cells and that while HT29 cells do not exhibit a strong dependence for Src activity for their growth in monolayer culture, their growth in agar is Src dependent.

Xenograft Studies. The Src inhibitor **31a** was chosen for evaluation in xenograft models employing the Src-transformed fibroblasts. In an unstaged model, a 30 mg/kg dose of **31a** administered ip twice a day (bid) for 14 days provided a T/C of 18% (Figure 3A). In a staged model, where the tumors were implanted 3 days prior to dosing, a 25 mg/kg dose of **31a** administered ip bid for 10 days provided a T/C of 25% (Figure 3B). These results suggest that **31a** is sufficiently bioavailable to inhibit Src in vivo.

Although **31a** was not a potent inhibitor of the proliferation of HT29 cells during monolayer growth, the ability of **31a** to inhibit HT29 cell growth in agar implied that some aspect of the tumorigenic phenotype of these cells was affected. Therefore, **31a** was tested in a staged xenograft model with HT29 cells. When **31a** was dosed at 30 mg/kg ip bid for 21 days, a statistically significant reduction in tumor growth rate was observed (T/C of 30% (p < 0.01) on day 21). Under these dosing condi-

tions, however, the animals exhibited abdominal bloating. When **31a** was dosed at 25 mg/kg ip bid, a T/C of 30% was observed, with reduced bloating. A decrease in activity was observed with a 10 mg/kg ip bid dose of **31a** (T/C of 44% (p < 0.01) at day 21), and no activity was observed with a 3 mg/kg ip bid dose of **31a**. When dosed orally at 50 mg/kg bid, **31a** inhibited tumor growth (T/C of 35% (p < 0.01) at day 21) with no associated bloating, but increasing the oral dose to 100 mg/kg bid resulted in 40% mortality by day 21. Therefore, while **31a** has significant antitumor activity, this compound does not have a wide therapeutic window in this xenograft model.

Conclusion. Optimization of the C-4 anilino group of a 6,7-dimethoxy-3-quinolinecarbonitrile with a trisubstituted aniline provides enhanced Src inhibitory activity over disubstituted aniline groups. Replacement of the 7-methoxy group of these compounds with an alkoxy group terminating with a basic amine results in increased Src enzymatic and cellular inhibition. Analogue **31a**, which contains a 3-(4-methylpiperazin-1-yl)propoxy group, has an IC_{50} of 1.2 nM in the Src enzymatic assay and an IC_{50} of 100 nM for the inhibition of Src-dependent cell proliferation and is selective for Src over non-Src family kinases. In this series of compounds, the 4-methylpiperazine group provides superior blood levels over a morpholine or 1-(1,2,3-triazole) group. Compound **31a** effectively inhibited tumor growth in xenograft models employing Src-transformed fibroblasts and also showed activity against HT29 xenografts.

Experimental Section

General Methods. Melting points were determined in open capillary tubes on a Meltemp melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded using a NT-300 WB spectrometer. Chemical shifts (δ) are in parts per million referenced to Me$_4$Si. Electrospray (ES) mass spectra were recorded in positive mode on a Micromass Platform spectrometer. Electron impact (EI) and high-resolution mass spectra were obtained on a Finnigan MAT-90 spectrometer. Flash chromatography was performed with Baker 40 μM silica gel. Reactions were carried out under an inert atmosphere, either nitrogen or argon.

4-[(2-Chloro-5-methoxyphenyl)amino]-6,7-dimethoxy-3-quinolinecarbonitrile (1b). A mixture of **5** [17] (120 mg, 0.48 mmol), 6-chloro-*m*-anisidine hydrochloride (103 mg, 0.53 mmol), and pyridine hydrochloride (61 mg, 0.53 mmol) in 6 mL of 2-ethoxyethanol was heated at reflux for 6 h and then concentrated in vacuo. The residue was treated with water and neutralized by addition of sodium carbonate (112 mg). After being stirred for 1.5 h, the aqueous suspension was extracted with dichloromethane. The combined organic phase was washed with brine, dried over sodium sulfate, and then filtered. Removal of the solvent gave a dark residue that was purified by preparative thin-layer chromatography, developing with 5% methanol in dichloromethane, to provide 35 mg (20%) of **1b** as a light brown solid: mp 143–145 °C; ^{1}H NMR (DMSO-d_6) δ 3.77 (s, 3H), 3.86 (s, 3H), 3.93 (s, 3H), 6.98 (dd, J = 9, 3 Hz, 1H), 7.07 (d, J = 3 Hz, 1H), 7.33 (s, 1H), 7.48 (d, J = 9 Hz, 1H), 7.83 (s, 1H), 8.41 (s, 1H), 9.56 (s, 1H); MS (ES) m/z 370.2, 372.2 (M + 1).

Anal. (C$_{19}$H$_{16}$ClN$_3$O$_3$·1.0H$_2$O) C, H, N.

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6,7-dimethoxy-3-quinolinecarbonitrile (1c). A mixture of 2,4-dichloro-5-methoxyaniline (771 mg, 4.0 mmol)[23] and 60% sodium hydride (160 mg, 4.0 mmol) in 25 mL of tetrahydrofuran was heated at reflux for 1 h. After the mixture was cooled to room temperature, **5** (500 mg, 2.0 mmol) was added, and the reaction mixture was heated at reflux overnight. The reaction mixture

was cooled to room temperature, and 40 mg of sodium hydride was added. After being heated at reflux for 5.5 h, the reaction mixture was cooled to room temperature and partitioned between ethyl acetate and water. The organic layer was washed with water, dried over magnesium sulfate, filtered, and concentrated in vacuo. Diethyl ether was added to the residue, and the solid was collected by filtration to provide 556 mg (69%) of **1c** as an off-white solid: mp 258−259 °C; ^{1}H NMR (DMSO-d_6) δ 3.86 (s, 3H), 3.94 (s, 3H), 3.95 (s, 3H) 7.32 (s, 2H), 7.73 (s, 1H), 7.83 (s, 1H), 8.41 (s, 1H), 9.63 (br s, 1H); MS (ES) m/z 404.2, 406.2 (M + 1).

Anal. ($C_{19}H_{15}Cl_2N_3O_3$) C, H, N.

4-[(2,4-Dichloro-5-ethoxyphenyl)amino]-6,7-dimethoxy-3-quinolinecarbonitrile (1d). According to the procedure used to prepare **1c**, reaction of **5** with **8a** provided **1d** in 60% yield as a light yellow solid: mp 254−255 °C; ^{1}H NMR (DMSO-d_6) δ 1.35 (t, J = 7 Hz, 3H), 3.94 (s, 3H), 3.95 (s, 3H), 4.11 (q, J = 7 Hz, 2H), 7.33 (s, 2H), 7.74 (s, 1H), 7.83 (s, 1H), 8.42 (s, 1H), 9.60 (br s, 1H); MS (ES) m/z 417.9, 419.9 (M + 1).

Anal. ($C_{20}H_{17}Cl_2N_3O_3$·0.20H_2O) C, H, N.

4-[(2,4-Dichloro-5-propoxyphenyl)amino]-6,7-dimethoxy-3-quinolinecarbonitrile (1e). According to the procedure used to prepare **1c**, reaction of **5** and **8b** provided **1e** in 57% yield as a white solid: mp 262−265 °C; ^{1}H NMR (DMSO-d_6) δ 0.98 (t, J = 7 Hz, 3H), 1.76 (m, 2H), 3.94 (s, 3H), 3.95 (s, 3H) 4.02 (t, J = 7 Hz, 2H), 7.33 (s, 2H), 7.74 (s, 1H), 7.83 (s, 1H), 8.42 (s, 1H), 9.60 (br s, 1H); MS (ES) m/z 431.9, 433.9 (M + 1).

Anal. ($C_{21}H_{19}Cl_2N_3O_3$) C, H, N.

4-[(2,4-Dichloro-5-hydroxyphenyl)amino]-6,7-dimethoxy-3-quinolinecarbonitrile (1f). A mixture of **5** (250 mg, 1.0 mmol), **8c** (0.2 g, 1.1 mmol), and pyridine hydrochloride (130 mg, 1.1 mmol) in 20 mL of 2-ethoxyethanol was heated at reflux for 6 h. The mixture was diluted with ethyl acetate, washed with saturated aqueous sodium bicarbonate, dried over sodium sulfate, and concentrated in vacuo. The solids were collected by filtration, washing with diethyl ether, to provide 110 mg (28%) of **1f** as a light tan solid: mp 145−150 °C; ^{1}H NMR (DMSO-d_6) δ 3.93 (s, 3H), 3.95 (s, 3H) 6.99 (s, 1H), 7.34 (s, 1H), 7.62 (s, 1H), 7.79 (s, 1H), 8.44 (br s, 1H), 9.50 (br s, 1H); MS (ES) m/z 389.9, 391.9 (M + 1).

Anal. ($C_{18}H_{13}Cl_2N_3O_3$·1.0H_2O) C, H, N.

4-[4-Chloro-2-fluoro-5-methoxyphenyl)amino]-6,7-dimethoxy-3-quinolinecarbonitrile (1g). A mixture of **5** (300 mg, 1.20 mmol), 4-chloro-2-fluoro-5-methoxyaniline (340 mg, 1.94 mmol),[23] and pyridine hydrochloride (135 mg, 1.16 mmol) in 10 mL of 2-ethoxyethanol was heated at reflux for 3 h. After being cooled, the reaction mixture was partitioned between ethyl acetate and saturated sodium bicarbonate. The organic layer was washed with 1.0 N sodium hydroxide, dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was treated with diethyl ether, and the resultant solid was collected by filtration, washing with diethyl ether, to provide 318 mg (68%) of **1g** as a light brown solid: mp 231−233 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.33 (s, 3H), 3.85 (s, 3H), 3.95 (s, 3H), 7.24 (d, J = 7 Hz, 1H), 7.34 (s, 1H), 7.60 (d, J = 10 Hz, 1H), 7.81 (s, 1H), 8.47 (s, 1H), 9.55 (s, 1H); MS (ES) m/z 388.2 (M + 1).

Anal. ($C_{19}H_{15}ClFN_3O_3$) C, H, N.

4-[(2-Bromo-4-chloro-5-methoxyphenyl)amino]-6,7-dimethoxy-3-quinolinecarbonitrile (1h). According to the procedure used to prepare **1c**, reaction of **5** and 2-bromo-4-chloro-5-methoxyaniline,[23] in *N,N*-dimethylformamide, provided, after flash column chromatography, eluting with a gradient of 25−50% ethyl acetate in hexane, **1h** in 22% yield as an off-white solid: mp 254−255 °C; ^{1}H NMR (DMSO-d_6) δ 3.86 (s, 3H), 3.94 (s, 3H), 3.95 (s, 3H), 7.33 (s, 1H), 7.36 (s, 1H), 7.85 (s, 1H), 7.86 (s, 1H), 8.41 (s, 1H), 9.64 (s, 1H); MS (ES) m/z 447.8, 449.7 (M + 1).

Anal. ($C_{19}H_{15}BrClN_3O_3$·0.5H_2O·0.5HC(O)NH_2$) C, H, N.

4-[(4-Chloro-5-methoxy-2-methylphenyl)amino]-6,7-dimethoxy-3-quinolinecarbonitrile (1i). According to the procedure used to prepare **1g**, reaction of **5** with 4-chloro-5-methoxy-2-methylaniline[23] provided, after flash column chro-

matography, eluting with a gradient of 1:1 hexane/ethyl acetate to all ethyl acetate, followed by trituration with diethyl ether and hexane, **1i** in 39% yield as an off-white solid: mp softens at 95 °C; ^{1}H NMR (DMSO-d_6) δ 2.10 (s, 3H), 3.81 (s, 3H), 3.94 (s, 3H) 3.95 (s, 3H), 7.10 (s, 1H), 7.32 (s, 1H), 7.42 (s, 1H), 7.84 (s, 1H), 8.36 (s, 1H), 9.40 (br s, 1H); MS (ES) m/z 384.1, 386.2 (M + 1).

Anal. ($C_{20}H_{18}ClN_3O_3$·0.1(CH_3CH_2)_2O) C, H, N.

6,7-Dimethoxy-4-[(2,4-dimethyl-5-methoxyphenyl)-amino]-3-quinolinecarbonitrile (1j). According to the procedure used to prepare **1g**, reaction of **5** with **10** provided **1j** in 56% yield as an off-white solid: mp 138−140 °C; ^{1}H NMR (DMSO-d_6) δ 2.07 (s, 3H), 2.17 (s, 3H), 3.75 (s, 3H), 3.93 (s, 3H), 3.94 (s, 3H), 6.84 (s, 1H), 7.09 (s, 1H), 7.29 (s, 1H), 7.85 (s, 1H), 8.32 (s, 1H), 9.38 (s, 1H); MS (ES) m/z 364.3 (M + 1).

Anal. ($C_{21}H_{21}N_3O_3$·1.0H_2O) C, H, N.

4-[(2-Chloro-5-methoxy-4-methylphenyl)amino]-6,7-dimethoxy-3-quinolinecarbonitrile (1k). According to the procedure used to prepare **1g**, reaction of **5** with 2-chloro-5-methoxy-4-methylaniline[23] provided **1k** in 34% yield as a white solid: mp 203−204 °C; ^{1}H NMR (DMSO-d_6) δ 2.20 (s, 3H), 3.79 (s, 3H), 3.94 (s, 3H) 3.95 (s, 3H), 7.07 (s, 1H), 7.32 (s, 1H), 7.37 (s, 1H), 7.85 (s, 1H), 8.37 (s, 1H), 9.60 (br s, 1H); MS (ES) m/z 384.2, 386.2 (M + 1).

Anal. ($C_{20}H_{18}ClN_3O_3$·0.5H_2O·0.2CH_3C(O)OCH_2CH_3$) C, H, N.

4-[(2-Chloro-5-ethoxy-4-methylphenyl)amino]-6,7-dimethoxy-3-quinolinecarbonitrile (1l). According to the procedure used to prepare **1g**, reaction of **5** with **9** provided **1l** in 62% yield as a white solid: mp 240−242 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.35 (t, J = 6 Hz, 3H), 2.19 (s, 3H), 3.93 (s, 3H), 3.94 (s, 3H), 4.02 (d, J = 6 Hz, 2H), 7.06 (s, 1H), 7.31 (s, 1H), 7.37 (s, 1H), 7.84 (s, 1H), 8.36 (s, 1H), 9.53 (s, 1H); MS (ES) m/z 398.0 (M + 1).

Anal. ($C_{21}H_{20}ClN_3O_3$) C, H, N.

4-[(4-Chloro-3-methoxyphenyl)amino]-6,7-dimethoxy-3-quinolinecarbonitrile (1m). According to the procedure used to prepare **1g**, reaction of **5** with 4-chloro-*m*-anisidine,[32] using *N,N*-dimethylformamide as the solvent, provided, after flash column chromatography, eluting with a gradient of 25−50% ethyl acetate in hexane, **1m** in 66% yield as a white solid: mp 136−137 °C; ^{1}H NMR (DMSO-d_6) δ 3.85 (s, 3H), 3.93 (s, 3H), 3.96 (s, 3H), 6.82 (dd, J = 8, 2 Hz, 1H), 7.04 (d, J = 2 Hz, 1H), 7.36 (s, 1H), 7.41 (d, J = 8 Hz, 1H), 7.74 (s, 1H), 8.52 (s, 1H), 9.56 (s, 1H); MS (ES) m/z 369.9.

Anal. ($C_{19}H_{16}ClN_3O_3$·1.8 H_2O) C, H, N.

4-[(2-Chloro-5-methoxyphenyl)amino]-6-methoxy-7-[3-(4-morpholinyl)propoxy]-3-quinolinecarbonitrile (2b). According to the procedure used to prepare **1g,** reaction of **11** with 6-chloro-*m*-anisidine hydrochloride provided, after flash column chromatography, eluting with a gradient of 2% methanol in dichloromethane to 5% methanol in dichloromethane, **2b** in 28% yield as a white solid: mp 179−190 °C; ^{1}H NMR (DMSO-d_6) δ 1.91−2.01 (m, 2H), 2.43−2.49 (m, 6H), 3.59 (t, J = 5 Hz, 4H), 3.75 (s, 3H), 3.93 (s, 3H), 4.20 (t, J = 6 Hz, 2H), 6.98 (dd, J = 9, 3 Hz, 1H), 7.07 (d, J = 3 Hz, 1H), 7.32 (s, 1H), 7.46 (d, J = 9 Hz, 1H), 7.82 (s, 1H), 8.40 (s, 1H), 9.55 (s, 1H); MS (ES) m/z 482.9 (M + 1).

Anal. ($C_{25}H_{27}ClN_4O_4$·0.15CH_2Cl_2) C, H, N.

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[3-(4-morpholinyl)propoxy]-3-quinolinecarbonitrile (2c). According to the procedure used to prepare **1c,** reaction of **11** with 2,4-dichloro-5-methoxyaniline[23] provided **2c** in 88% yield as an off-white solid: mp 160−162 °C; ^{1}H NMR (DMSO-d_6) δ 1.92−2.04 (m, 2H), 2.35−2.50 (m, 6H), 3.58 (t, J = 5 Hz, 4H), 3.86 (s, 3H), 3.95 (s, 3H), 4.21 (t, J = 6 Hz, 2H), 7.34 (br s, 2H), 7.75 (s, 1H), 7.83 (s, 1H), 7.82 (s, 1H), 8.42 (s, 1H), 9.62 (s, 1H); MS (ES) m/z 517.0, 518.9 (M + 1).

Anal. ($C_{25}H_{26}Cl_2N_4O_4$) C, H, N.

4-[(2-Bromo-4-chloro-5-methoxyphenyl)amino]-6-methoxy-7-[3-(4-morpholinyl)propoxy]-3-quinolinecarbonitrile (2d). According to the procedure used to prepare **1c,** reaction of **11** with 2-bromo-4-chloro-5-methoxyaniline[23] provided, after flash column chromatography, eluting with a gradient of dichloromethane to 5% methanol in dichloro-

methane, **2d** in 40% yield as an off-white solid: mp 153–155 °C; ^{1}H NMR (DMSO-d_6) δ 1.97 (m, 2H), 2.38 (t, $J = 4$ Hz, 4H), 2.44 (t, $J = 7$ Hz, 2H), 3.59 (t, $J = 4$ Hz, 4H), 3.83 (s, 3H), 3.94 (s, 3H), 4.21 (t, $J = 6$ Hz, 2H), 7.31 (s, 1H), 7.32 (s, 1H), 7.84 (s, 1H), 7.86 (s, 1H), 8.40 (s, 1H), 9.63 (s, 1H); MS (ES) m/z 561.1, 563.1 (M + 1).

Anal. ($C_{25}H_{26}BrClN_4O_4 \cdot 1.0H_2O$) C, H, N.

4-[(4-Chloro-5-methoxy-2-methylphenyl)amino]-6-methoxy-7-[3-(4-morpholinyl)propoxy]-3-quinolinecarbonitrile (2e). According to the procedure used to prepare **1g**, reaction of **11** with 4-chloro-5-methoxy-2-methylaniline[23] provided, after flash column chromatography, eluting with a gradient of 5% methanol in dichloromethane to 10% methanol in dichloromethane, **2e** in 40% yield as an off-white solid: mp 205–206 °C; ^{1}H NMR (DMSO-d_6) δ 1.93–1.99 (m, 2H), 2.10 (s, 3H), 2.33–2.52 (m, 6H), 3.53–3.62 (m, 4H), 3.81 (s, 3H), 3.94 (s, 3H), 4.02 (t, $J = 6$ Hz, 2H), 7.10 (s, 1H), 7.30 (s, 1H), 7.42 (s, 1H), 7.83 (s, 1H), 8.35 (s, 1H), 9.41 (s, 1H); MS (ES) m/z 497.3 (M + 1).

Anal. ($C_{26}H_{29}ClN_4O_4 \cdot 0.20H_2O$) C, H, N.

4-[(2,4-Dimethyl-5-methoxyphenyl)amino]-6-methoxy-7-[3-(4-morpholinyl)propoxy]-3-quinolinecarbonitrile (2f). According to the procedure used to prepare **1g**, reaction of **11** with **10** provided **2f** in 90% yield as an off-white solid: mp softens 83–85 °C; ^{1}H NMR (DMSO-d_6) δ 1.92 (m, 2H), 2.06 (s, 3H), 2.17 (s, 3H), 2.38 (t, $J = 5$ Hz, 4H), 2.43 (t, $J = 7$ Hz, 2H), 3.59 (t, $J = 5$ Hz, 4H), 3.74 (s, 3H), 3.93 (s, 3H), 4.19 (t, $J = 6$ Hz, 2H), 6.84 (s, 1H), 7.09 (s, 1H), 7.29 (s, 1H), 7.84 (s, 1H), 8.31 (s, 1H), 9.37 (s, 1H); MS (ES) m/z 477.3 (M + 1).

Anal. ($C_{27}H_{32}N_4O_4 \cdot 1.2 H_2O$) C, H, N.

4-[(2-Chloro-5-methoxy-4-methylphenyl)amino]-6-methoxy-7-[3-(4-morpholinyl)propoxy]-3-quinolinecarbonitrile (2g). According to the procedure used to prepare **1g**, reaction of **11** with 2-chloro-5-methoxy-4-methylaniline[23] provided **2g** in 43% yield as a white solid: mp 146–148 °C; ^{1}H NMR (DMSO-d_6) δ 1.92–2.04 (m, 2H), 2.20 (s, 3H), 2.33–2.55 (m, 6H), 3.59 (t, $J = 5$ Hz, 4H), 3.79 (s, 3H), 3.94 (s, 3H), 4.20 (t, $J = 6$ Hz, 2H), 7.07 (s, 1H), 7.31 (s, 1H), 7.37 (s, 1H), 7.84 (s, 1H), 8.36 (s, 1H), 9.54 (s, 1H); MS (ES) m/z 497.1 (M + 1).

Anal. ($C_{26}H_{29}ClN_4O_4 \cdot 0.50H_2O$) C, H, N.

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6,7-dimethoxyquinazoline (3b). According to the procedure used to prepare **1c**, reaction of **6**[22] with 2,4-dichloro-5-methoxyaniline[23] provided **3b** in 25% yield as a light yellow solid: mp 223–225 °C; ^{1}H NMR (DMSO-d_6) δ 3.86 (s, 3H), 3.94 (s, 6H), 7.19 (s, 1H), 7.35 (s, 1H), 7.70 (s, 1H), 7.82 (s, 1H), 8.34 (s, 1H), 9.59 (s, 1H); MS (ES) m/z 380.2, 382.2 (M + 1).

Anal. ($C_{17}H_{15}Cl_2N_3O_3 \cdot 0.50 H_2O$) C, H, N.

N-(2,4-Dichloro-5-methoxyphenyl)-6,7-dimethoxy-4-quinolinamine (4b). 2,4-Dichloro-5-methoxyaniline[23] (175 mg, 0.89 mmol) and **7**[24] (100 mg, 0.45 mmol) were added to a mixture of tris(dibenzylidene)acetonedipalladium (41 mg, 0.045 mmol), 2-(dicyclohexylphosphino)-2′-(N,N-dimethylamino)biphenyl (25) (54 mg, 0.14 mmol), and potassium phosphate (142 mg, 0.67 mmol) in 4.5 mL of ethylene glycol dimethyl ether. The mixture was heated at reflux for 2.5 h, cooled to room temperature, and partitioned between saturated aqueous sodium bicarbonate and ethyl acetate. The combined organic extracts were dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography, eluting with a gradient of dichloromethane to 3% methanol in dichloromethane, followed by recrystallization from acetone and hexane, to provide 130 mg (76%) of **4b** as a white solid: mp 165–167 °C; ^{1}H NMR (DMSO-d_6) δ 3.86 (s, 3H), 3.91 (s, 3H), 3.93 (s, 3H), 6.20 (d, $J = 5$ Hz, 1H), 7.22 (s, 1H), 7.26 (s, 1H), 7.70 (s, 1H), 7.74 (s, 1H), 8.25 (d, $J = 5$ Hz, 1H), 8.67 (s, 1H); MS (ES) m/z 379.3.

Anal. ($C_{18}H_{16}Cl_2N_2O_3 \cdot 0.7CH_3COCH_3$) C, H, N.

2,4-Dichloro-5-ethoxyaniline (8a). A mixture of 2,4-dichloro-5-hydroxyacetanilide (1.50 g, 6.8 mmol),[26] ethyl iodide (0.81 mL, 10.2 mmol), and potassium carbonate (1.41 g, 10.2 mmol) in 25 mL of acetone was heated at reflux for 4 h. The reaction mixture was concentrated in vacuo, and the residue was partitioned between ethyl acetate and water. The organic

layer was dried over sodium sulfate and concentrated in vacuo to provide 1.66 g (98%) of 2,4-dichloro-5-ethoxyacetanilide as a white solid.

A mixture of 2,4-dichloro-5-ethoxyacetanilide (1.66 g, 6.6 mmol) and 3 mL of 5 N sodium hydroxide in 5 mL of ethanol and 7 mL of water was heated at reflux for 2 h and then stirred at room temperature for 18 h. After the mixture was heated at reflux for 1 h, the ethanol was removed and the residue was extracted with dichloromethane. The organic layer was dried over sodium sulfate and concentrated to give 1.2 g (88%) of **8a** as a light yellow oil: ^{1}H NMR (DMSO-d_6) δ 1.33 (t, $J = 7$ Hz, 3H), 3.98 (q, $J = 7$ Hz, 2H), 5.47 (br s, 2H), 6.53 (s, 1H), 7.14 (s, 1H); MS (ES) m/z 205.8, 207.7 (M + 1).

2,4-Dichloro-5-propoxyaniline (8b). According to the procedure used to prepare **8a**, reaction of 2,4-dichloro-5-hydroxyacetanilide[26] with n-propyl bromide, followed by basic hydrolysis, provided **8b** in 66% yield as a light yellow oil: ^{1}H NMR (DMSO-d_6) δ 0.98 (t, $J = 7$ Hz, 3H), 1.70 (m, 2H), 3.86 (t, $J = 7$ Hz, 2H), 5.46 (br s, 2H), 6.54 (s, 1H), 7.21 (s, 1H); MS (ES) m/z 219.7, 221.8 (M + 1).

2,4-Dichloro-5-hydroxyaniline (8c). A solution of 2,4-dichloro-5-methoxyaniline (800 mg, 4.0 mmol)[23] in 10 mL of dichloromethane was cooled to −78 °C, and 7.5 mL of 1.0 M boron tribromide in dichloromethane (7.5 mmol) was added. After being stirred at room temperature overnight, the reaction mixture was cooled to −78 °C, and an additional 4.0 mL of 1.0 M boron tribromide in dichloromethane (4.0 mmol) was added. The reaction mixture was stirred at room temperature overnight and then quenched by the addition of 10 mL of water. The mixture was extracted with diethyl ether, and the product was extracted into 2 N sodium hydroxide. The aqueous layer was neutralized (pH 7) with 1 N hydrochloric acid, and the product was extracted into diethyl ether. The organic layer was dried over sodium sulfate, filtered, and concentrated in vacuo to provide 280 mg (39%) of **8c** as an off-white solid: mp 115–116 °C; ^{1}H NMR (DMSO-d_6) δ 5.34 (s, 2H), 6.41 (s, 1H), 7.10 (s, 1H), 9.89 (s, 1H); MS (ES) m/z 176.07, 178.07 (M + 1).

Anal. ($C_6H_5Cl_2NO$) C, H, N.

2-Chloro-5-ethoxy-4-methylaniline (9). 4-Chloro-2-methyl-5-nitrophenol (3.80 g, 20.2 mmol)[26] was dissolved in 60 mL of acetone, and potassium carbonate (3.0 g, 21.7 mmol) and ethyl iodide (2.1 mL, 26.3 mmol) were added. The reaction mixture was heated at reflux for 3.5 h, cooled, and concentrated in vacuo. The residue was partitioned between water and ethyl acetate. The organic layer was washed with water and 2.0 N sodium hydroxide, dried over magnesium sulfate, filtered, and concentrated in vacuo. Diethyl ether and hexanes were added to the residue, and the resultant solid was collected by filtration to give 2.70 g (62%) of 2-chloro-5-ethoxy-4-methylnitrobenzene as an off-white solid.

A mixture of 2-chloro-5-ethoxy-4-methylnitrobenzene (2.96 g, 13.7 mmol) and iron (1.64 g, 29.4 mmol) in 90 mL of 10% aqueous ethanol and 2 mL of concentrated hydrochloric acid was heated at reflux for 5 h. Additional iron (1.00 g, 18.2 mmol) and concentrated hydrochloric acid (1 mL) were added, and the mixture was heated at reflux for an additional 2 h. The mixture was filtered while warm, washing with ethyl acetate. The filtrate was concentrated in vacuo, and the residue was partitioned between ethyl acetate and aqueous saturated sodium bicarbonate. The organic layer was washed with water, dried over magnesium sulfate, filtered, and concentrated in vacuo to provide 2.20 g (86%) of **9** as a waxy yellow-brown solid: ^{1}H NMR (DMSO-d_6) δ 1.31 (t, $J = 7$ Hz, 3H), 1.98 (s, 3H), 3.85 (q, $J = 7$ Hz, 2H), 5.04 (br s, 2H), 6.39 (s, 1H), 6.90 (s, 1H); MS (ES) m/z 186.0, 188.0 (M + 1).

Anal. ($C_9H_{12}ClNO$) C, H, N.

2,4-Dimethyl-5-methoxyaniline (10). 2,4-Dimethyl-5-nitrophenol (4.40 g, 26.3 mmol)[27] was dissolved in acetone (100 mL), and iodomethane (5.51 g, 38.8 mmol) and potassium carbonate (4.14 g, 30.0 mmol) were added. The reaction mixture was heated at reflux for 2 h, cooled to room temperature, and partitioned between water and ethyl acetate. The organic extract was evaporated, and the residue was recrystallized from diethyl ether and hexanes to provide 3.75 g (79%)

of 2,4-dimethyl-5-methoxynitrobenzene as an off-white solid: ^{1}H NMR (DMSO-d_6) δ 2.20 (s, 3H), 2.43 (s, 3H), 3.86 (s, 3H), 7.28 (s, 1H), 7.52 (s, 1H); MS (ES) *m/z* 182.1 (M + 1).

A mixture of 2,4-dimethyl-5-methoxynitrobenzene (1.97 g, 11 mmol) and 10% palladium on carbon (197 mg) in 30 mL of methanol was hydrogenated at 45 psi overnight. The reaction mixture was filtered through a short pad of Celite, and the filtrate was concentrated to provide 1.54 g (93%) of **10** as a light tan solid: mp 80−81°C; ^{1}H NMR (DMSO-d_6) δ 1.94 (s, 3H), 1.95 (s, 3H), 3.65 (s, 3H), 4.58 (br s, 2H), 6.24 (s, 1H), 6.63 (s, 1H); MS (ES) *m/z* 152.1 (M + 1).

Anal. (C$_9$H$_{13}$NO) C, H, N.

Methyl 3-(3-Chloropropoxy)-4-methoxybenzoate (12). A mixture of potassium carbonate (20.1 g, 150 mmol), methyl 3-hydroxy-4-methoxybenzoate (20.0 g, 110 mmol), 3-chloropropyl *p*-toluenesulfonate (27.2 g, 110 mmol),[29] and tricaprylmethylammonium chloride (0.45 g, 1.1 mmol) in 240 mL of acetone was heated at reflux overnight. The solids were removed by filtration and washed with acetone. The filtrate was concentrated, and the residue was dissolved in dichloromethane and washed with 1 N sodium hydroxide, 10% aqueous potassium hydroxide, saturated aqueous sodium bicarbonate, and brine. The organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuo to provide 22.85 g (77%) of **12** as a white solid: mp 46−48 °C; ^{1}H NMR (CDCl$_3$) δ 2.27−2.33 (m, 2H), 3.77 (t, *J* = 5 Hz, 2H), 3.89 (s, 3H), 3.91 (s, 3H), 4.22 (t, *J* = 5 Hz, 2H), 6.89 (d, *J* = 6 Hz, 1H,) 7.58 (d, *J* = 2 Hz, 1H), 7.69 (dd, *J* = 6, 2 Hz, 1H); MS (ES) *m/z* 259.2 (M + 1).

Anal. (C$_{12}$H$_{15}$ClO$_4$) C, H.

Methyl 5-(3-Chloropropoxy)-4-methoxy-2-nitrobenzoate (13). To a solution of **12** (20.63 g, 80 mmol) in 60 mL of acetic acid was added 20 mL of 70% nitric acid dropwise over 20 min. The reaction mixture was heated at reflux for 2 h, poured into ice−water, and extracted with dichloromethane. The organic layer was washed with water, followed by 0.5 N aqueous sodium hydroxide, dried over magnesium sulfate, filtered, and concentrated in vacuo to provide 19.12 g (79%) of **13** as a white solid: mp 63−64 °C; ^{1}H NMR (CDCl$_3$) δ 2.32−2.38 (m, 2H), 3.77 (t, *J* = 5 Hz, 2H), 3.90 (s, 3H), 3.95 (s, 3H), 4.26 (t, *J* = 5 Hz, 2H), 7.11 (s, 1H), 7.45 (s, 1H); MS (APCI) *m/z* 304.1 (M + 1).

Anal. (C$_{12}$H$_{14}$ClNO$_6$) C, H, N.

Methyl 2-Amino-5-(3-chloropropoxy)-4-methoxybenzoate (14). A solution of ammonium chloride (16.83 g, 315 mmol) and iron (11.60 g, 208 mmol) in 55 mL of water and 135 mL of methanol was heated at reflux under mechanical stirring for 10 min. The solution was allowed to cool slightly, and **13** (19.12 g, 63 mmol) dissolved in 140 mL of methanol and 60 mL of water was added in portions. The reaction mixture was heated at reflux for 4 h. After the reaction mixture was cooled to room temperature, dilute aqueous sodium bicarbonate was added, and the insoluble material was removed by filtration, washing with dichloromethane. The aqueous filtrate was extracted with dichloromethane, and the organic extracts were combined and washed with brine. The organic layer was dried over sodium sulfate, filtered, and concentrated in vacuo. Addition of 10:1 hexane/diethyl ether to the residue provided 15.96 g (93%) of **14** as a white solid: 92−93 °C; ^{1}H NMR (DMSO-d_6) δ 2.07−2.13 (m, 2H), 3.74 (s, 3H), 3.75 (s, 3H), 3.77 (t, *J* = 5 Hz, 2H), 3.93 (t, *J* = 5 Hz, 2H), 6.37 (s, 1H), 6.48 (s, 2H), 7.18 (s, 1H); MS (ES) *m/z* 273.8 (M + 1).

Anal. (C$_{12}$H$_{16}$ClNO$_4$) C, H, N.

6-(3-Chloropropoxy)-4-hydroxy-7-methoxy-3-quinolinecarbonitrile (15). A mixture of **14** (8.00 g, 29.0 mmol) and dimethylformamide dimethylacetal (5.18 g, 44.0 mmol) was heated at reflux for 8 h. The reaction was concentrated in vacuo to provide methyl 5-(3-chloropropoxy)-2-{[(dimethylamino)methylene]amino}-4-methoxybenzoate that was used directly without purification.

To an oven-dried flask containing 95 mL of tetrahydrofuran was added 38 mL of 1.6 M *n*-butyllithium (61 mmol) in hexanes. The solution was cooled to −78 °C, and acetonitrile (2.50 g, 61 mmol) in 142 mL of tetrahydrofuran was added dropwise. After the solution was stirred for 15 min, a solution of methyl 5-(3-chloropropoxy)-2-{[(dimethylamino)methylene]amino}-4-methoxybenzoate in 75 mL of tetrahydrofuran was added dropwise over 30 min. The reaction mixture was allowed to stir for 25 min at −78 °C. Acetic acid (5.22 g, 87 mmol) was added, and the reaction was allowed to warm to room temperature. After being stirred overnight, the heterogeneous mixture was concentrated in vacuo and diluted with 250 mL of water. The resulting suspension was stirred at room temperature for 20 min. The solids were collected by filtration and washed with diethyl ether to provide 4.0 g (47%) of **15** as a pale yellow solid: mp > 260 °C; ^{1}H NMR (DMSO-d_6) δ 2.19−2.25 (m, 2H), 3.81 (t, *J* = 5 Hz, 2H), 3.89 (s, 3H), 4.18 (t, *J* = 5 Hz, 2H), 7.05 (s, 1H), 7.47 (s, 1H), 8.57 (s, 1H); MS (ES) *m/z*, 292.8 (M + 1).

Anal. (C$_{14}$H$_{13}$ClN$_2$O$_3$) C, H, N.

4-Chloro-6-(3-chloropropoxy)-7-methoxy-3-quinolinecarbonitrile (16). A mixture of **15** (2.00 g, 6.84 mmol) and phosphorus oxychloride (9.44 g, 61 mmol) was heated at reflux for 1 h. The solution was concentrated, and saturated aqueous sodium bicarbonate was added to the residue. The resultant precipitate was collected by filtration to provide 1.93 g (91%) of **16** as a gray solid: mp 138−140 °C; ^{1}H NMR (DMSO-d_6) δ 2.26−2.33 (m, 2H), 3.85 (t, *J* = 5 Hz, 2H), 4.03 (s, 3H), 4.33 (t, *J* = 5 Hz, 2H), 7.43 (s, 3H), 7.53 (s, 3H), 8.97 (s, 3H); MS (ES) *m/z* 310.8, 312.8 (M + 1).

Anal. (C$_{14}$H$_{12}$Cl$_2$N$_2$O$_2$) C, H, N.

6-(3-Chloropropoxy)-4-[(2,4-dichloro-5-methoxyphenyl)amino]-7-methoxy-3-quinolinecarbonitrile (17). According to the procedure used to prepare **1f**, reaction of **16** with 2,4-dichloro-5-methoxyaniline[23] provided, after flash column chromatography, eluting with 5% methanol in diethyl ether, **17** in 19% yield: mp 100−105 °C; ^{1}H NMR (DMSO-d_6) δ 2.25−2.31 (m, 2H), 3.75−3.86 (m, 5H), 3.96 (s, 3H), 4.26 (t, *J* = 6 Hz, 2H), 7.35 (br s, 2H), 7.75 (s, 1H), 7.87 (s, 1H), 8.42 (s, 1H), 9.62 (s, 1H); MS (ES) *m/z* 466.3, 468.3 (M + 1).

Anal. (C$_{21}$H$_{18}$Cl$_3$N$_3$O$_3$) C, H.

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-7-methoxy-6-[3-(4-morpholinyl)propoxy]-3-quinolinecarbonitrile (18). A mixture of **17** (200 mg, 0.43 mmol) and a catalytic amount of sodium iodide in 2 mL of morpholine was heated at 90 °C overnight. The reaction mixture was partitioned between brine and ethyl acetate. The organic layer was dried over sodium sulfate, filtered, and concentrated in vacuo until a solid began to precipitate out. The solid was collected by filtration to provide 165 mg (74%) of **18** as an off-white solid: mp 181−185 °C; ^{1}H NMR (DMSO-d_6) δ 1.94−2.02 (m, 2H), 2.34−2.43 (m, 4H), 2.46 (t, *J* = 5 Hz, 2H), 3.57 (t, *J* = 3 Hz, 4 H), 3.86 (s, 3H), 3.95 (s, 3H), 4.16 (t, *J* = 6 Hz, 2H), 7.32 (br s, 2H), 7.73 (s, 1H), 7.82 (s, 1H), 7.82 (s, 1H), 8.41 (s, 1H), 9.60 (s, 1H); MS (ES) *m/z* 516.8, 518.8 (M + 1).

Anal. (C$_{25}$H$_{26}$Cl$_2$N$_4$O$_4$) C, H, N.

4-Chloro-7-(2-chloroethoxy)-6-methoxy-3-quinolinecarbonitrile (20). To a mixture of **19** (185 mg, 0.79 mmol),[19] triphenylphosphine (373 mg, 1.42 mmol), and 2-chloroethanol (200 μL, 2.98 mmol) in 20 mL of tetrahydrofuran at 0 °C was added dropwise diethyl azodicarboxylate (230 μL, 1.45 mmol). The reaction mixture was kept at 0 °C for 15 min and then allowed to warm to room temperature. The reaction mixture was concentrated, and the residue was purified by flash column chromatography, eluting with 2:1 hexane/ethyl acetate to provide 166 mg (71%) of **20** as a white solid: 174−176 °C; ^{1}H NMR (DMSO-d_6) δ 4.01−4.09 (m, 2H), 4.05 (s, 3H), 4.54 (t, *J* = 5 Hz, 2H), 7.48 (s, 1H), 7.61 (s, 1H), 9.00 (s, 1H); MS (ES) *m/z* 297.1, 299.1 (M + 1).

Anal. (C$_{13}$H$_{10}$Cl$_2$N$_2$O$_2$) C, H, N.

4-Chloro-7-(4-chlorobutoxy)-6-methoxy-3-quinolinecarbonitrile (21). According to the procedure used to prepare **20**, reaction of **19** with 4-chlorobutanol provided **21**, after flash column chromatography, eluting with 2:1 hexane/ethyl acetate, in 65% yield as a white solid: mp 134−136 °C; ^{1}H NMR (DMSO-d_6) δ 1.88−1.89 (m, 4H), 3.76 (t, *J* = 6 Hz, 2H), 4.02

(s, 3H), 4.28 (t, *J* = 6 Hz, 2H), 7.42 (s, 1H), 7.54 (s, 1H), 8.97 (s, 1H); MS (ES) *m*/*z* 325.1, 327.1 (M + 1).

Anal. (C$_{15}$H$_{14}$Cl$_2$N$_2$O$_2$) C, H, N.

4-Chloro-7-[(5-chloropentyl)oxy]-6-methoxy-3-quino-linecarbonitrile (22). According to the procedure used to prepare **20**, reaction of **19** with 5-chloropentanol provided, after flash column chromatography, eluting with 2:1 hexane/ethyl acetate, **22** in 67% yield as a white solid: mp 139−140 °C; ^{1}H NMR (DMSO-*d*$_6$) δ 1.53−1.63 (m, 2H), 1.77−1.90 (m, 4H), 3.68 (t, *J* = 7 Hz, 2H), 4.01 (s, 3H), 4.23 (t, *J* = 6 Hz, 2H), 7.41 (s, 1H), 7.53 (s, 1H), 8.96 (s, 1H); MS (ES) *m*/*z* 339.1, 341.1 (M + 1).

Anal. (C$_{16}$H$_{16}$Cl$_2$N$_2$O$_2$) C, H, N.

7-(2-Chloroethoxy)-4-[(2,4-dichloro-5-methoxyphenyl)-amino]-6-methoxy-3-quinolinecarbonitrile (23). A mixture of 2,4-dichloro-5-methoxyaniline (153 mg, 0.80 mmol), pyridine hydrochloride (75 mg, 0.65 mmol), and **20** (300 mg, 0.66 mmol) in 30 mL of 2-ethoxyethanol was heated at reflux for 4 h. The reaction mixture was partitioned between ethyl acetate and saturated aqueous sodium bicarbonate. The organic layer was washed with saturated aqueous sodium bicarbonate and brine, then dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography, eluting with 7% methanol in dichloromethane to provide 210 mg (70%) of **23** as a pale yellow solid: mp 218−220 °C; ^{1}H NMR (DMSO-*d*$_6$) δ 3.86 (s, 3H), 3.96 (s, 3H), 4.05 (t, *J* = 5 Hz, 2H), 4.46 (t, *J* = 5 Hz, 2H), 7.33 (s, 1H), 7.37 (s, 1H), 7.74 (s, 1H), 7.86 (s, 1H), 8.42 (s, 1H), 9.65 (br s, 1H); MS (ES) *m*/*z* 452.2, 454.2 (M + 1).

Anal. (C$_{20}$H$_{16}$Cl$_3$N$_3$O$_3$·0.5H$_2$O) C, H.

7-(4-Chlorobutoxy)-4-[(2,4-dichloro-5-methoxyphenyl)-amino]-6-methoxy-3-quinolinecarbonitrile (24). According to the procedure used to prepare **23**, reaction of **21** with 2,4-dichloro-5-methoxyaniline provided **24** in 43% yield as an off-white solid: mp 191−195 °C; ^{1}H NMR (DMSO-*d*$_6$) δ 1.89−1.98 (m, 4H), 3.72−3.79 (m, 2H), 3.86 (s, 3H), 3.95 (s, 3H), 4.17−4.24 (m, 2H), 7.33 (s, 1H), 7.35 (s, 1H), 7.74 (s, 1H), 7.83 (s, 1H), 8.42 (s, 1H), 9.60 (s, 1H); MS (ES) *m*/*z* 480.1, 482.1 (M + 1).

Anal. (C$_{22}$H$_{20}$Cl$_3$N$_3$O$_3$·0.2H$_2$O) C, H, N.

7-[(5-Chloropentyl)oxy]-4-[(2,4-dichloro-5-methoxy-phenyl)amino]-6-methoxy-3-quinolinecarbonitrile (25). A mixture of 2,4-dichloro-5-methoxyaniline (840 mg, 4.37 mmol) and 60% sodium hydride (176 mg, 4.59 mmol) in 30 mL of tetrahydrofuran was heated at reflux for 30 min. The mixture was cooled, and **22** (854 mg, 2.5 mmol) was added. The reaction mixture was heated at reflux overnight and then cooled to room temperature. The residue was partitioned between ethyl acetate and water. The organic layer was washed with water, dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography, eluting with 1:1 hexane/ethyl acetate to provide 317 mg (25%) of **25** as a pale yellow solid: mp 173−175 °C; ^{1}H NMR (DMSO-*d*$_6$) δ 1.49−1.67 (m, 2H), 1.75−1.90 (m, 4H), 3.69 (t, *J* = 7 Hz, 2H), 3.85 (s, 3H), 3.94 (s, 3H), 4.16 (t, *J* = 6 Hz, 2H), 7.32 (br s, 2H), 7.73 (s, 1H), 7.82 (s, 1H), 8.40 (s, 1H), 9.26 (br s, 1H); MS (ES) *m*/*z* 494.1, 496.0 (M + 1).

Anal. (C$_{23}$H$_{22}$Cl$_3$N$_3$O$_3$) C, H, N.

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[2-(4-morpholinyl)ethoxy]-3-quinolinecarbonitrile (26). A mixture of **23** (300 mg, 0.66 mmol), sodium iodide (87 mg, 0.6 mmol), and 1 mL of morpholine in 3 mL of ethylene glycol dimethyl ether was heated at 90 °C overnight. The reaction mixture was partitioned between ethyl acetate and saturated aqueous sodium bicarbonate. The organic layer was washed with saturated aqueous sodium bicarbonate and brine, then dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by preparative thin-layer chromatography, eluting with 15% methanol in dichloromethane to provide **26** as an off-white solid in 45% yield: mp 199−201 °C; ^{1}H NMR (DMSO-*d*$_6$) δ 2.49−2.58 (m, 4H), 2.73−2.84 (m, 2H), 3.59 (t, *J* = 7 Hz, 4H), 3.86 (s, 3H), 3.94 (s, 3H), 4.29 (t,

J = 6 Hz, 2H), 7.33 (s, 1H), 7.38 (s, 1H), 7.75 (s, 1H), 7.83 (s, 1H), 8.42 (s, 1H), 9.61 (s, 1H); MS (ES) *m*/*z* 503.2, 505.2 (M + 1).

Anal. (C$_{24}$H$_{24}$Cl$_2$N$_4$O$_4$·0.60H$_2$O) C, H, N.

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[4-(4-morpholinyl)butoxy]-3-quinolinecarbonitrile (27). According to the procedure used to prepare **26**, reaction of **24** and morpholine provided, after recrystallization from ethyl acetate, **27** as a light yellow solid in 47% yield: mp 158−161 °C; ^{1}H NMR (DMSO-*d*$_6$) δ 1.56−1.69 (m, 2H), 1.75−1.90 (m, 2H), 2.29−2.40 (m, 6H), 3.57 (t, *J* = 5 Hz, 4H), 3.86 (s, 3H), 3.94 (s, 3H), 4.18 (t, *J* = 6 Hz, 2H), 7.33 (s, 2H), 7.74 (s, 1H), 7.83 (s, 1H), 8.42 (s, 1H), 9.60 (s, 1H); MS (ES) *m*/*z* 531.1, 533.2 (M + 1).

Anal. (C$_{26}$H$_{28}$Cl$_2$N$_4$O$_4$ · 0.20 H$_2$O) C, H, N.

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-meth-oxy-7-{[5-(4-morpholinyl)pentyl]oxy}-3-quinolinecarbo-nitrile (28). A mixture of **25** (200 mg, 0.40 mmol) and sodium iodide (70 mg, 0.47 mmol) in 4 mL of morpholine was heated at reflux for 2.5 h and then stirred at room temperature overnight. The reaction mixture was concentrated in vacuo, and the residue was partitioned between ethyl acetate and water. The organic layer was washed with saturated aqueous sodium bicarbonate, dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography, eluting with 3:1 ethyl acetate/ methanol followed by recrystallization from ethyl acetate and diethyl ether to provide 40 mg (18%) of **28** as an off-white solid: mp 144−146 °C; ^{1}H NMR (DMSO-*d*$_6$) δ 1.49−1.60 (m, 4H), 1.75−1.87 (m, 2H), 2.25−2.40 (m, 6H), 3.56 (t, *J* = 4.5 Hz, 4H), 3.85 (s, 3H), 3.93 (s, 3H), 4.15 (t, *J* = 6 Hz, 2H), 7.31 (br s, 2H), 7.73 (s, 1H), 7.82 (s, 1H), 8.40 (s, 1H), 9.65 (br s, 1H); MS (ES) *m*/*z* 545.1, 547.1 (M + 1).

Anal. (C$_{27}$H$_{30}$Cl$_2$N$_4$O$_4$·0.50H$_2$O) C, H, N.

7-(3-Chloropropoxy)-4-[(2,4-dichloro-5-methoxyphenyl)-amino]-6-methoxy-3-quinolinecarbonitrile (30). A mixture of 2,4-dichloro-5-methoxyaniline (1.35 g, 7.0 mmol), pyridine hydrochloride (690 mg, 6.1 mmol), and **29**[28] (2.00 g, 6.4 mmol) in 20 mL of 2-ethoxyethanol was heated at reflux for 2.5 h. The reaction mixture was partitioned between ethyl acetate and saturated aqueous sodium bicarbonate. The organic layer was washed with water, filtered, and concentrated in vacuo until a solid began to appear. This solid was collected by filtration to provide 1.30 g (46%) of **30** as an off-white solid: mp 183−186 °C; ^{1}H NMR (DMSO-*d*$_6$) δ 2.22−2.33 (m, 2H), 3.83 (t, *J* = 6 Hz, 2H), 3.86 (s, 3H), 3.95 (s, 3H), 4.29 (t, *J* = 6 Hz, 2H), 7.34 (s, 1H), 7.37 (s, 1H), 7.75 (s, 1H), 7.85 (s, 1H), 8.42 (s, 1H), 9.64 (s, 1H); MS (ES) *m*/*z* 465.8, 467.8 (M + 1).

Anal. (C$_{21}$H$_{18}$Cl$_3$N$_3$O$_3$) C, H, N.

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[3-(4-methyl-1-piperazinyl)propoxy]-3-quinolinecarbo-nitrile (31a). A mixture of **30** (656 mg, 1.40 mmol) and sodium iodide (210 mg, 1.40 mmol) in 4 mL of *N*-methylpiperazine was heated at 80 °C for 20 h. The reaction mixture was concentrated in vacuo and partitioned between ethyl acetate and saturated aqueous sodium bicarbonate. The organic layer was washed with brine, dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by column chromatography, eluting with 30% methanol in dichloro-methane. The fractions containing product were collected and concentrated in vacuo. Diethyl ether was added to the residue, and the light pink solid was collected by filtration to provide 560 mg (75%) of **31a**: mp 116−120 °C; ^{1}H NMR (DMSO-*d*$_6$) δ 1.89−2.05 (m, 2H), 2.15 (s, 3H), 2.29−2.52 (m, 10H), 3.86 (s, 3H), 3.94 (s, 3H), 4.19 (t, *J* = 6 Hz, 2H), 7.31 (br s, 2H), 7.73 (s, 1H), 7.82 (s, 1H), 8.40 (s, 1H), 9.60 (s, 1H); MS (ES) *m*/*z* 530.2, 532.2 (M + 1).

Anal. (C$_{26}$H$_{29}$Cl$_2$N$_5$O$_3$·2.0H$_2$O) C, H, N.

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-7-[3-(4-ethyl-1-piperazinyl)propoxy]-6-methoxy-3-quinoline-carbonitrile (31b). A mixture of **30** (3.50 g, 7.50 mmol), sodium iodide (1.12 g, 7.50 mmol), and 4.8 mL of *N*-ethyl-piperazine in 5 mL of ethylene glycol dimethyl ether was

heated at 95 °C for 20 h. The reaction mixture was concentrated in vacuo and partitioned between ethyl acetate and saturated aqueous sodium bicarbonate. The organic layer was washed with saturated aqueous sodium bicarbonate, followed by brine, dried over sodium sulfate, filtered, and concentrated in vacuo. Diethyl ether was added to the residue, and the white solid was collected by filtration to provide 1.80 g (44%) of **31b**: mp 102−104 °C; ^{1}H NMR (DMSO-d_6) δ 0.98 (t, $J = 7$ Hz, 3H), 1.88−2.02 (m, 2H), 2.25−2.52 (m, 12 H), 3.86 (s, 3H), 3.94 (s, 3H), 4.19 (t, $J = 6$ Hz, 2H), 7.30 (br s, 2H), 7.72 (s, 1H), 7.82 (s, 1H), 8.39 (s, 1H), 9.60 (s, 1H); MS (ES) *m/z* 544.3, 546.4 (M + 1).

Anal. ($C_{27}H_{31}Cl_2N_5O_3 \cdot 2.0 H_2O$) C, H, N.

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[3-(*cis*-3,4,5-trimethylpiperazinyl)propoxy]-3-quinolinecarbonitrile (31c). According to the procedure used to prepare **31b**, reaction of **30** and *cis*-1,2,6-trimethylpiperazine[33] provided, after flash column chromatography, eluting with 25% methanol in dichloromethane, **31c** in 39% yield as a white solid: mp 98−101 °C; ^{1}H NMR (DMSO-d_6) δ 0.96 (s, 3H), 0.98 (s, 3H), 1.65−1.77 (m, 2H), 1.90−1.98 (m, 2H), 2.07−2.18 (m, 5H), 2.34−2.41 (m, 2H), 2.69−2.78 (m, 2H), 3.86 (s, 3H), 3.94 (s, 3H), 4.19 (t, $J = 6$ Hz, 2H), 7.32 (br s, 2H), 7.74 (s, 1H), 7.83 (s, 1H), 8.41 (s, 1H), 9.60 (s, 1H); MS (ES) *m/z* 558.3, 560.3 (M + 1).

Anal. ($C_{28}H_{33}Cl_2N_5O_3 \cdot 2.0H_2O$) C, H.

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[3-(4-propyl-1-piperazinyl)propoxy]-3-quinolinecarbonitrile (31d). According to the procedure used to prepare **31b**, reaction of **30** and *N*-propylpiperazine provided, after preparative thin-layer chromatography, eluting with 20% methanol in dichloromethane, and subsequent recrystallization from diethyl ether and hexane, **31d** in 15% yield as a white solid: mp 97−101 °C; ^{1}H NMR (DMSO-d_6) δ 0.84 (t, $J = 7$ Hz, 3H), 1.32−1.47 (m, 2H), 1.89−2.03 (m, 2H), 2.15−2.27 (m, 2H), 2.25−2.52 (m, 10 H), 3.86 (s, 3H), 3.94 (s, 3H), 4.19 (t, $J = 6$ Hz, 2H), 7.32 (br s, 2H), 7.74 (s, 1H), 7.83 (s, 1H), 8.41 (s, 1H), 9.61 (s, 1H); MS (ES) *m/z* 558.2, 560.2 (M + 1). Anal. ($C_{28}H_{33}Cl_2N_5O_3 \cdot 2.2H_2O$) C, H.

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-methoxy-7-(3-piperazin-1-ylpropoxy)-3-quinolinecarbonitrile (31e). According to the procedure used to prepare **31b**, reaction of **30** with piperazine provided, after preparative thin-layer chromatography, eluting with 25% methanol in dichloromethane, **31e** in 48% yield as a white solid: mp 123−125 °C; ^{1}H NMR (DMSO-d_6) δ 1.89−2.02 (m, 2H), 2.27−2.37 (m, 4 H), 2.42 (t, $J = 7$ Hz, 2H), 2.72 (t, $J = 5$ Hz, 4H), 3.85 (s, 3H), 3.93 (s, 3H), 4.19 (t, $J = 6$ Hz, 2H), 7.28 (s, 1H), 7.30 (s, 1H), 7.72 (s, 1H), 7.82 (s, 1H), 8.38 (s, 1H), 9.61 (s, 1H); MS (ES) *m/z* 516.2, 518.1 (M + 1).

Anal. ($C_{25}H_{27}Cl_2N_5O_3$) C, H.

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-methoxy-7-{3-[4-(2-hydroxyethyl)piperazin-1-yl]propoxy}-3-quinolinecarbonitrile (31f). According to the procedure used to prepare **31b**, reaction of **30** with 4-(2-hydroxyethyl)piperazine provided **31f** in 75% yield as a white solid: mp 133−137 °C; ^{1}H NMR (DMSO-d_6) δ 1.90−2.01 (m, 2H), 2.31−2.50 (m, 10H), 3.47 (q, $J = 6$ Hz, 2H), 3.86 (s, 3H), 3.94 (s, 3H), 4.18 (t, $J = 6$ Hz, 2H), 4.37 (t, $J = 5$ Hz, 1H), 7.31 (br s, 2H), 7.74 (s, 1H), 7.82 (s, 1H), 8.41 (s, 1H), 9.62 (s, 1H); MS (ES) *m/z* 560.1, 562.1 (M + 1).

Anal. ($C_{27}H_{31}Cl_2N_5O_4 \cdot 1.2H_2O$) C, H, N.

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[3-(4-methyl-[1,4]diazepan-1-yl)propoxy]-3-quinolinecarbonitrile (31g). According to the procedure used to prepare **31b**, reaction of **30** with 1-methylhomopiperazine provided, after preparative thin-layer chromatography, eluting with 20% methanol in dichloromethane, **31g** in 43% yield as a light yellow solid: mp 134−137 °C; ^{1}H NMR (DMSO-d_6) δ 1.65−1.77 (m, 2H), 1.86−1.97 (m, 2H), 2.25 (s, 3H), 2.50−2.69 (m, 10H), 3.86 (s, 3H), 3.94 (s, 3H), 4.19 (t, $J = 6$ Hz, 2H), 7.30 (br s, 2H), 7.73 (s, 1H), 7.82 (s, 1H), 8.40 (s, 1H), 9.61 (s, 1H); MS (ES) *m/z* 544.2, 546.2 (M + 1). Anal. ($C_{27}H_{31}Cl_2N_5O_3 \cdot 1.7 H_2O$) C, H, N.

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[3-(4-piperidin-1-yl)propoxy]-3-quinolinecarbonitrile (31h). According to the procedure used to prepare **31b**, reaction of **30** with piperidine provided, after preparative thin-layer chromatography, eluting with 20% methanol in dichloromethane, **31h** in 61% yield as a white solid: mp 157−161 °C; ^{1}H NMR (DMSO-d_6) δ 1.34−1.45 (m, 2H), 1.45−1.57 (m, 4H), 1.88−2.02 (m, 2H), 2.32−2.49 (m, 6H), 3.86 (s, 3H), 3.94 (s, 3H), 4.19 (t, $J = 6$ Hz, 2H), 7.31 (br s, 2H), 7.73 (s, 1H), 7.83 (s, 1H), 8.40 (s, 1H), 9.62 (s, 1H); MS (ES) *m/z* 515.2, 517.2 (M + 1).

Anal. ($C_{26}H_{28}Cl_2N_4O_3 \cdot 2.7H_2O$) C, H, N.

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-7-[3-(4-hydroxypiperidin-1-yl)propoxy]-6-methoxy-3-quinolinecarbonitrile (31i). According to the procedure used to prepare **31b**, reaction of **30** with 4-hydroxypiperidine provided **31i** as a white solid in 75% yield: mp 122−125 °C; ^{1}H NMR (DMSO-d_6) δ 1.30−1.46 (m, 2H), 1.65−1.78 (m, 2H), 1.88−2.09 (m, 4H), 2.43 (t, $J = 6$ Hz, 2H), 2.67−2.78 (m, 2H), 3.39−3.50 (m, 1H), 3.86 (s, 3H), 3.94 (s, 3H), 4.18 (t, $J = 6$ Hz, 2H), 4.53 (d, $J = 3$ Hz, 1H), 7.31 (br s, 2H), 7.74 (s, 1H), 7.83 (s, 1H), 8.40 (s, 1H), 9.62 (s, 1H); MS (ES) *m/z* 531.0, 533.1 (M + 1).

Anal. ($C_{26}H_{28}Cl_2N_4O_4 \cdot 2.0H_2O$) C, H, N.

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[3-(2*H*-1,2,3-triazol-2-yl)propoxy]-3-quinolinecarbonitrile (31j) and 4-[(2,4-Dichloro-5-methoxyphenyl)-amino]-6-methoxy-7-[3-(1*H*-1,2,3-triazol-1-yl)propoxy]-3-quinolinecarbonitrile (31k). According to the procedure used to prepare **31b**, reaction of **30** with 1,2,3-triazole provided a mixture of **31j** and **31k**. The mixture was separated by preparative thin-layer chromatography, eluting with 5% methanol in dichloromethane. **31j** was obtained as an off-white solid in 30% yield: mp 190−191 °C; ^{1}H NMR (DMSO-d_6) δ 2.35−2.46 (m, 2H), 3.86 (s, 3H), 3.94 (s, 3H), 4.18 (t, $J = 6$ Hz, 2H), 4.64 (d, $J = 7$ Hz, 2H), 7.29 (s, 1H), 7.34 (s, 1H), 7.75 (s, 1H), 7.80 (s, 2H), 7.85 (s, 1H), 8.42 (s, 1H), 9.63 (s, 1H); MS (ES) *m/z* 499.4, 501.3 (M + 1).

Anal. ($C_{23}H_{20}Cl_2N_6O_3$) C, H, N.

31k was obtained as an off-white solid in 39% yield: mp 188−190 °C; ^{1}H NMR (DMSO-d_6) δ 2.33−2.45 (m, 2H), 3.86 (s, 3H), 3.96 (s, 3H), 4.17 (t, $J = 6$ Hz, 2H), 4.60 (d, $J = 7$ Hz, 2H), 7.32 (s, 1H), 7.33 (s, 1H), 7.75 (s, 2H), 7.85 (s, 1H), 8.20 (s, 1H), 8.41 (s, 1H), 9.63 (s, 1H); MS (ES) *m/z* 499.4, 501.3 (M + 1).

Anal. ($C_{23}H_{20}Cl_2N_6O_3 \cdot 0.3H_2O$) C, H, N.

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-7-[3-(1*H*-imidazol-1-yl)propoxy]-6-methoxy-3-quinolinecarbonitrile (31l). A mixture of **30** (200 mg, 0.43 mmol), imidazole (300 mg, 4.3 mmol), sodium hydroxide powder (80 mg, 2.0 mmol), and sodium iodide (30 mg, 0.2 mmol) in 3 mL of *N,N*-dimethylformamide was heated at 80 °C for 2 h. The reaction mixture was cooled, poured into water, and stirred. The resultant solid was collected by filtration, washing with water and diethyl ether. The solid was purified by silica gel chromatography, eluting with a gradient of 3% methanol in dichloromethane to 6% methanol in dichloromethane, to provide 180 mg (84%) of **31l** as a yellow solid: mp 155−170 °C; ^{1}H NMR (DMSO-d_6) δ 2.27−2.44 (m, 2H), 3.87 (s, 3H), 3.95 (s, 3H), 4.26 (t, $J = 6$ Hz, 2H), 4.42 (t, $J = 7$ Hz, 2H), 7.36 (s, 1H), 7.39 (s, 1H), 7.71 (s, 1H), 7.76 (s, 1H), 7.84 (s, 1H), 7.96 (s, 1H), 8.14 (s, 1H), 8.55 (s, 1H), 9.18 (s, 1H); MS (ES) *m/z* 498.0, 500.1 (M + 1).

Anal. ($C_{24}H_{21}Cl_2N_5O_3 \cdot 1.05CH_2Cl_2$) C, H, N.

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-6-methoxy-7-{3-[[2-(4-morpholinyl)ethyl]amino]propoxy}-6-methoxy-3-quinolinecarbonitrile (31m). According to the procedure used to prepare **31b**, reaction of **30** with 4-(2-aminoethyl)-morpholine provided, after preparative thin-layer chromatography, eluting with 20% methanol in dichloromethane, **31m** as an orange solid in 31% yield: mp 75−80 °C; ^{1}H NMR (DMSO-d_6) δ 1.95−2.06 (m, 2H), 2.31−2.44 (m, 6H), 2.72 (t, $J = 6.5$ Hz, 2H), 2.79 (t, $J = 7$ Hz, 2H), 3.86 (s, 3H), 3.94 (s, 3H), 4.23 (t, $J = 6$ Hz, 2H), 4.53 (d, $J = 3$ Hz, 1H), 7.30 (s,

1H), 7.32 (s, 1H), 7.72 (s, 1H), 7.84 (s, 1H), 8.40 (s, 1H), 9.60 (s, 1H); MS (ES) m/z 560.2, 562.1 (M + 1).

Anal. ($C_{27}H_{31}Cl_2N_5O_4\cdot 1.0H_2O$) C, H.

4-[(2,4-Dichloro-5-methoxyphenyl)amino]-7-{3-[((2-(dimethylamino)ethyl)methyl)amino]propoxy}-6-methoxy-3-quinolinecarbonitrile (31n). According to the procedure used to prepare **31a**, reaction of **30** with *N,N,N*-trimethyl-ethylenediamine provided, after preparative thin-layer chromatography, eluting with 20% methanol in dichloromethane, **31n** in 31% yield as a white solid: mp 85−90 °C; ¹H NMR (DMSO-d_6/TFA) δ 2.23−2.38 (m, 2H), 2.89 (s, 6H), 2.93 (s, 3H), 3.30−3.41 (m, 2H), 3.48−3.61 (m, 4H), 3.90 (s, 3H), 4.02 (s, 3H), 4.26−4.35 (m, 2H), 7.53 (s, 1H), 7.57 (s, 1H), 7.89 (s, 1H), 8.14 (s, 1H), 9.12 (s, 1H); MS (ES) m/z 532.1, 534.1 (M + 1).

Anal. ($C_{26}H_{31}Cl_2N_5O_3\cdot 0.6H_2O$) C, H, N.

Biological Evaluation. The protocols for the Src kinase and Src and Fyn cell proliferation assays were previously reported.[16]

Plasma Pharmacokinetic Assay. Compounds were administered to athymic nu/nu female mice (Charles River Laboratories). Each compound was dosed intraperitoneally (ip) into 10 mice with a dose of 50 mg/kg prepared in 2% Tween-80, 5% dextrose/water (D5W). Blood was collected from five animals for each compound at 1 h postdose and from five animals at 4 h postdose. To collect the blood, the mice were anesthetized with Isoflurane gas and bled by cardiac puncture. Blood was collected in heparinized tubes and placed on ice. Blood samples were spun down at 14 000 rpm in a microfuge at 4 °C. The plasma was collected for each sample and stored at −70 °C. The compounds were extracted by combining 100 μL of plasma, 50 μL of an internal standard, and 300 μL of acetonitrile. After centrifugation for 15 min, the supernatant was transferred to a 1.5 mL tube and evaporated at 45 °C. The residue was dissolved in 100 μL of aqueous acetonitrile. The samples were analyzed on an Agilent HP1100 MSD LC/MS instrument using a Prodigy ODS3 column (5 μm, 4.6 × 150 mm) at 1.0 mL/min and 40 °C. The mobile phase consisted of 0.02% trifluoroacetic acid/water (A) and 0.02% trifluoroacetic acid/acetonitrile (B). The samples (25 μL injections) were run using a gradient of 15−70% B in 10 min and were introduced into the electrospray source without splitting to maximize sensitivity. Standards for each compound were prepared in an identical manner, and a calibration curve was generated. The samples were quantitated using both UV (wavelengths ranging from 268 to 349 nm) and single-ion monitoring (ES⁺). The results are reported as the means of five samples.

Colony Formation in Agar. HT29 cells (15 000) were suspended in 1.5 mL of 0.33% agar/DMEM supplemented with 10% fetal bovine serum. This mixture was layered onto 2.5 mL of 0.75% agar/DMEM with 10% fetal bovine serum. After the top agar hardened, 2 mL of medium with **31a** at 1 μM was layered onto the top agar, and the cells were then incubated at 37 °C. The cells were incubated for 4 weeks, with fresh medium without compound added after weeks 2 and 3.

Src-Transformed Fibroblast Xenograft Assay. Src-transformed fibroblasts were grown to confluence and then split 1:2 the day prior to injection. Cells were harvested on ice into growth media and resuspended to a final concentration of 5 million cells/mL. The cells were inoculated into female Balb/c nu/nu (athymic) mice which were 8−10 weeks of age and housed according to the guidelines detailed by the International Animal Care & Use Committee (IACUC). All mouse handling was done in a laminar flow hood, and the mice were housed in microisolator cages according to the procedures defined for handling nude mice. A 0.2 cm³ sample of cell suspension was injected subcutaneously (sc) into the right side of the mouse. For the unstaged model, the animals received compound followed by cells 30 min later, using 15−20 mice per group to compensate for the innate variability in tumor growth rates. In the staged model, the number of mice per group was reduced to 10 mice per group, with initial tumor size ranging from 100 to 150 mg. At the time of staging, typically 3−5 days following inoculation, the mice were dosed with compound or vehicle, 0.5 cm³ ip bid for the noted duration.

The vehicle used was 2% Tween-80 in 5% dextrose/water. All suspensions were sonicated prior to dosing. Tumors were measured on the days indicated using digital-display vernier calipers. The tumor size was calculated using the formula (length × width²)/2 = tumor mass (mg). The conversion from cubic millimeters to milligrams was made assuming unit density. The experiment was ended when the tumors reached 15% of the body weight of the mouse, at which time the animals were sacrificed.

HT29 Xenograft Assay. Human colon carcinoma HT29 cells (American Type Culture Collection, Rockville, MD, no. HTB-38) were grown in vitro. Athymic nu/nu female mice (Charles River, Wilmington, MA), 6−7 weeks of age, were housed in a barrier-type facility under an approved IACUC protocol. The animals were provided ad libitum access to both food and water. A unit of 7×10^6 HT29 cells was injected sc into the flanks of mice. When the tumors attained a mass of between 80 and 120 mg, the mice were randomized into treatment groups (day 0). The mice were treated either ip (5 mice per group) or po (orally) (10 mice per group) twice daily on days 1−20 poststaging with either vehicle control (2% Tween-80 in 5% dextrose/water) or **31a** at the doses indicated for each experiment. The tumor mass was determined every 7 days [(length × width²)/2] for 28 days. The relative tumor growth (mean tumor mass on days 7, 14, 21, and 28 divided by the mean tumor mass on day 0) was determined for each treatment group. Statistical analysis (Student's *t* test) of the log of the relative tumor growth compares the treated group with the control group. A *p* value ($p \leq 0.05$) indicates a statistically significant reduction in the relative tumor growth of the treated group compared to the vehicle control.

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