Synthesis and Src Kinase Inhibitory Activity of a Series of 4-Phenylamino-3-quinolinecarbonitriles

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Screening of a directed compound library in a yeast-based assay identified 4-[(2,4-dichlorophenyl)amino]-6,7-dimethoxy-3-quinolinecarbonitrile (**2a**) as a Src inhibitor. An enzymatic assay established that **2a** was an ATP-competitive inhibitor of the kinase activity of Src. We present here SAR data for **2a** which shows 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 are crucial for optimal activity. Increasing the size of the C-2 substituent of the aniline at C-4 of **2a** from chloro to bromo to iodo resulted in a corresponding increase in Src inhibition. Furthermore, replacement of the 7-methoxy group of **2a** with various 3-heteroalkylaminopropoxy groups provided increased inhibition of both Src enzymatic and cellular activity. Compound **25**, which contains a 3-morpholinopropoxy group, had an IC_{50} of 3.8 nM in the Src enzymatic assay and an IC_{50} of 940 nM for the inhibition of Src-dependent cell proliferation.

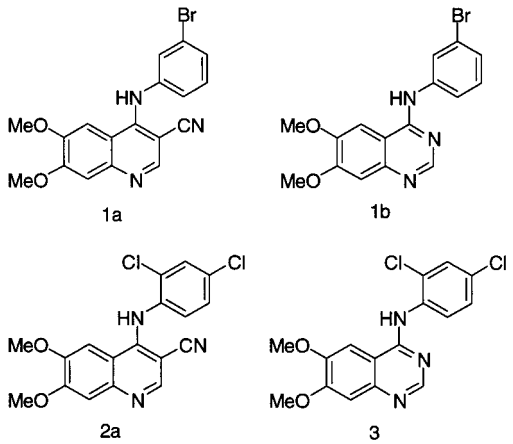
Introduction

Tyrosine kinases (TKs), enzymes that catalyze the specific phosphorylation of tyrosine residues on proteins, can be divided into two classes: the transmembrane growth factor receptor TKs (RTKs) and the cytoplasmic TKs which include Src. Src is a member of the Src family of kinases (SFKs) that share a common structural organization and have a high degree of homology, especially in their ATP-binding regions.[1−4] In addition to Src, the SFKs include Yes, Fyn, Fgr, Lck, Hck, Lyn, Blk, and Yrk. While some SFKs, including Src, Yes, and Fyn, are widely expressed, others such as Lck are limited in their expression.

Src is a potential therapeutic target for the treatment of diverse human disease states. Studies with Src-deficient mice demonstrated that bone resorption by osteoclasts requires Src activity, implying that Src inhibitors might be useful in the treatment of osteoporosis.[5−7] In addition, since Src is overexpressed in colon, breast, hepatic, and pancreatic tumors, as well as in certain B-cell leukemias and lymphomas, Src inhibitors may be efficacious in the treatment of cancer.[3,8−13] Further evidence for an active role for Src in tumor growth comes from experiments where anti-sense *src* expression in ovarian and colon tumor cells inhibited the growth of these cells in mouse xenograft models.[14,15]

Various classes of SFK inhibitors have been reported by several companies including, 5,10-dihydropyrimido-[4,5-*b*]quinolin-4(1*H*)-ones[16] and pyrazolo[3,4-*d*]pyrimidines[17] from Pfizer, pyrrolo[2,3-*d*]pyrimidines from Novartis,[18,19] pyrido[2,3-*d*]pyrimidin-7(8*H*)-ones[20] and 1,6-naphthyridin-2(1*H*)-ones [21] from Parke-Davis, and 4-anilinoquinazolines from Rhone Poulenc Rorer.[22] In some cases, modification of certain substituents on these core structures led to inhibitors of other kinase families. For example, varying the group at C-6 of Parke-Davis's pyrido[2,3-*d*]pyrimidin-7(8*H*)-ones provided inhibitors of either platelet-derived growth factor receptor TK (PDGFr)[23] or fibroblast growth factor receptor TK (FGFr).[24] By varying the aniline substituent at C-4 of their quinazolines, the group at Rhone Poulenc Rorer obtained inhibitors of CSF-1R TK.[25]

We recently reported that 4-[(3-bromophenyl)amino]-6,7-dimethoxy-3-quinolinecarbonitrile (**1a**) was an inhibitor of epidermal growth factor receptor (EGFr) TK,[26,27] showing slightly reduced activity compared to 4-[(3-bromophenyl)amino]-6,7-dimethoxyquinazoline (**1b**), one of the most potent EGFr inhibitors known.[28] Hoping



that the 3-quinolinecarbonitrile core could function as a template for inhibitors of other kinases, we prepared a library of 6,7-dimethoxy-3-quinolinecarbonitriles with various anilino groups at C-4. These compounds were screened in several assays, including a yeast-based Src assay. Yeast strains were prepared that harbor plasmids containing galactose-inducible *src* genes.[29] When grown in medium with galactose, these cells express Src and consequently undergo rapid cell death. These yeast strains were embedded in agar, and when the 3-quino-

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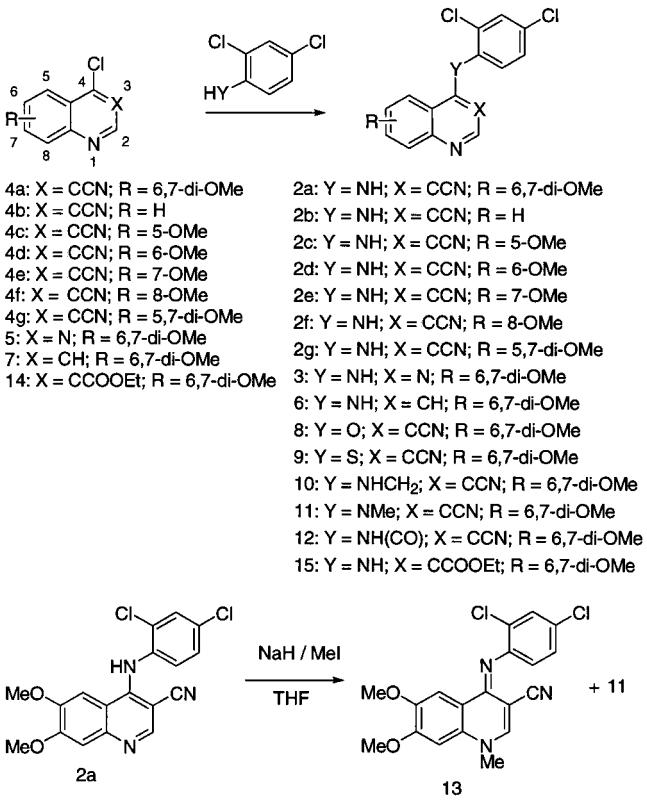
Scheme 1



linecarbonitriles were spotted on the surface, a ring of cell growth implied that the compound was blocking the toxic effect of Src. One of the best compounds in restoring cell proliferation in this assay was **2a**, which contains a 2,4-dichloroanilino group at C-4.[30] The structure of **2a** suggested that this compound was an inhibitor of Src kinase activity, and this was confirmed by an enzymatic assay. The corresponding quinazoline **3** also inhibited Src kinase activity but was 8-fold less active than **2a**. These initial results led us to investigate additional analogues of **2a** as Src kinase inhibitors.

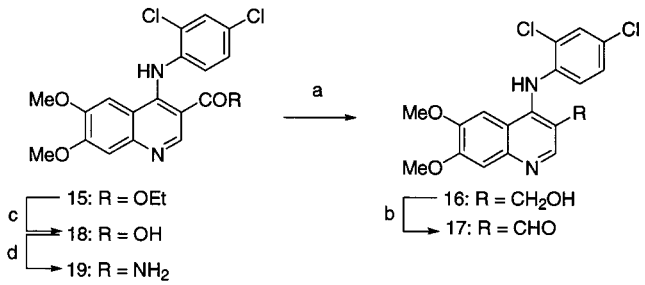
Chemistry

As depicted in Scheme 1, **2a** was prepared by treatment of 4-chloro-6,7-dimethoxy-3-quinolinecarbonitrile (**4a**)[26,27] with 2,4-dichloroaniline. When this reaction was run in ethoxyethanol in the presence of pyridine hydrochloride a 25% yield of **2a** was obtained. This yield was increased to 67% by first generating the anion of 2,4-dichloroaniline with sodium hydride followed by addition of **4a**. The corresponding quinazoline and quinoline analogues, namely **3** and **6**, were obtained from the reaction of 2,4-dichloroaniline with 4-chloro-6,7-dimethoxyquinazoline (**5**)[31] and 4-chloro-6,7-dimethoxyquinoline (**7**),[32] respectively.

The linker group at C-4 of **2a** was varied to include the phenol derivative **8**, the thiophenol derivative **9**, the benzylamine derivative **10**, the methylanilino derviative **11**, and the carboxamide derivative **12**. All of these were prepared from **4a** by standard conditions. Treatment of **2a** with methyl iodide and sodium hydride provided a readily separable mixture of predominately **13**, the 1-methyl analogue, along with the 4-methyl isomer **11**.

To study the effect of the carbonitrile group at C-3, several analogues of **2a** were prepared with alternative

Scheme 2[a]



[a] (a) DIBAL, THF, 0 °C; (b) MnO_2, $CHCl_3$; (c) (1) 0.5 N NaOH, EtOH, reflux, (2) concd HCl; (d) (1) CDI, DMF, THF, 60 °C, (2) aq NH_4OH.

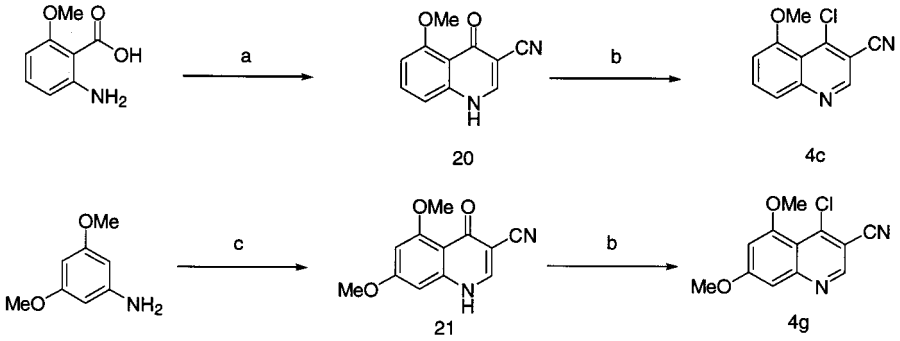
groups at this position. Ethyl 4-chloro-6,7-dimethoxy-3-quinolinecarboxylate (**14**)[33] was treated with 2,4-dichloroaniline in the presence of pyridine hydrochloride to provide **15**. The ester group of **15** was reduced with diisobutylaluminum hydride, as shown in Scheme 2, to provide the 3-hydroxymethyl derivative **16**. Oxidation of **16** with manganese(IV) oxide provided the 3-aldehyde derivative **17**. Additional analogues of **2a** were prepared by hydrolysis of the ester group of **15** to give the 3-carboxylic acid derivative **18**, which was then converted to the primary amide derivative **19** by treatment with 1,1′-carbonyldiimidazole followed by aqueous ammonium hydroxide.

The lead compound **2a** has methoxy substituents at C-6 and C-7 of the quinoline ring. The C-6 and C-7 unsubstituted analogue **2b** was prepared from the known 4-chloro compound **4b**.[34] The mono 5-, 6-, 7-, and 8-methoxy derivatives, **2c**−**2f**, were prepared from the corresponding 4-chloro compounds, **4c**−**4f**. While **4d**−**4f** were previously reported in the literature,[26] **4c** was prepared as shown in Scheme 3. Formation of the amidine derivative of 2-amino-6-methoxybenzoic acid[35] followed by addition of the anion of acetonitrile provided 4-hydroxy-5-methoxy-3-quinolinecarbonitrile (**20**). Reaction of **20** with phosphorus oxychloride in the presence of a catalytic amount of *N*,*N*-dimethylformamide gave **4c**. The 5,7-dimethoxy isomer of **2a**, namely **2g**, was obtained from the corresponding 4-chloro derivative **4g**, which was prepared as shown in Scheme 3. Reaction of 3,5-dimethoxyaniline with ethyl (ethoxymethylene)-cyanoacetate and subsequent thermal cyclization provided **21**. Treatment of **21** with phosphorus oxychloride gave the desired 4-chloro derivative **4g**.

Additional 6,7-dialkoxy analogues of **2a** were prepared as shown in Scheme 4. Demethylation of **2a** with pyridine hydrochloride provided the 6,7-dihydroxy analogue **2h**. To cleanly alkylate the hydroxy groups of **2h**, it was necessary to first completely acylate the molecule, then selectively deacylate the hydroxy groups to give **22**. Alkylation of the hydroxyl groups of **22** with ethyl iodide or *n*-butyl bromide, followed by removal of the acyl group from the 4-nitrogen, provided the 6,7-diethoxy and 6,7-di-*n*-butoxy analogues **2i** and **2j**, respectively.

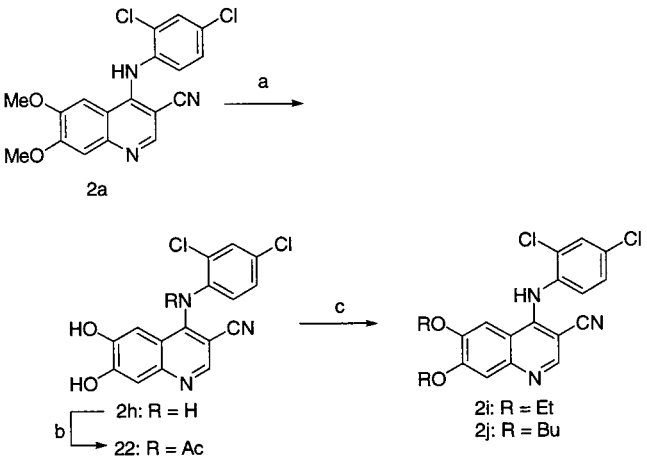
A large number of analogues of **2a** were prepared with various halogen substituents on the 4-anilino group. Compounds **2k**−**2v** were prepared by the reaction of **2a** with the desired aniline in the presence of either pyridine hydrochloride or sodium hydride. The anilines

Scheme 3[a]



[a] (a) (1) DMF−DMA, reflux, (2) *n*-BuLi, MeCN, THF, −78 °C; (b) $POCl_3$, cat. DMF, reflux; (c) (1) EtOCH=C(CO_2Et)CN, (2) Ph-O-Ph, Ph-Ph, reflux.

Scheme 4[a]



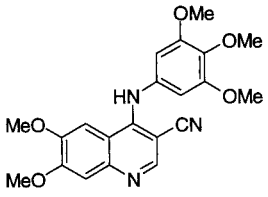
[a] (a) Pyridine hydrochloride, 215−222 °C; (b) (1) Ac_2O, DMAP, pyridine, reflux, (2) $NaHCO_3$, H_2O, MeOH; (c) (1) K_2CO_3, DMF, RX, 60 °C, (2) K_2CO_3, MeOH, reflux.

used included 2-chloro, 4-chloro, all five possible dichloro isomers, and some 2,4-dihaloanilines other than 2,4-dichloro.

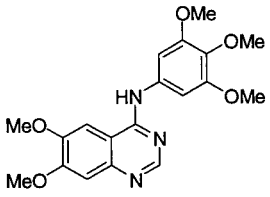
Derivatives of **2a** with water-solublizing groups at C-6 and/or C-7 of the quinoline ring were prepared as shown in Scheme 5. The diol **2h** was converted into the 6,7-di(3-chloropropoxy) derivative which was then treated with morpholine to provide **23**. In this case, unlike the preparations of **2i** and **2j**, the nitrogen atom was not protected in the alkylation reaction, which resulted in the low yield (31%) of **23** from **2h**. The 6-(3-morpholinopropoxy) derivative **24** was obtained by reaction of **4w**[26] with 2,4-dichloroaniline. The isomeric 7-(3-morpholinopropoxy) derivative **25** was obtained in a similar fashion from **4x**.[26] An alternative route to **25** was also used wherein intermediate **4y**[26] was treated with 2,4-dichloroaniline to provide **2y**, which was then reacted with morpholine in the presence of NaI. By varying the heterocyclic amine in the reaction with **2y** from morpholine to *N*-methylpiperazine and 4-hydroxypiperidine, analogues **26** and **27** were obtained.

Results and Discussion

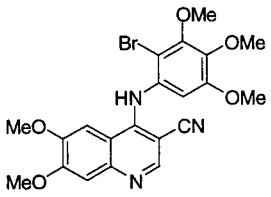
The screening lead **2a** had an average IC_{50} value of 30 nM in the Src enzymatic assay, when run with an ATP concentration of 0.1 mM. In a study where the ATP concentration was increased from 0.1 to 1 to 5 mM, the IC_{50} for **2a** increased from 23 to 130 to 270 nM, suggesting that this compound is an ATP-competitive inhibitor. Compound **2a** was tested against several other kinases, including EGFr-2 (ErbB-2), FGFr, and cdk4 (cyclin-dependent kinase 4). No activity (less than 20% inhibition) was observed against ErbB-2, FGFr, and cdk4 when **2a** was tested at doses of 2, 1, and 10 μg/mL, respectively. When PP1, 4-amino-5-(4-methylphenyl)-7-*tert*-butylpyrazolo[3,4-*d*]pyrimidine, a Src inhibitor reported by Pfizer,[17] was tested under our assay conditions, an IC_{50} value of 35 nM was obtained (Table 1). Therefore the Src inhibitory activity of **2a** is comparable to that of PP1. As mentioned previously, compared to the corresponding quinazoline **3** (IC_{50} value of 250 nM), **2a** exhibited an 8-fold increase in Src inhibitory activity. This result is consistent with our earlier report that 6,7-dimethoxy-4-[(3,4,5-trimethoxyphenyl)amino]-3-quinolinecarbonitrile (**28a**) was a more potent inhibitor of Src activity than the corresponding quinazoline **28b**.[36]



28a



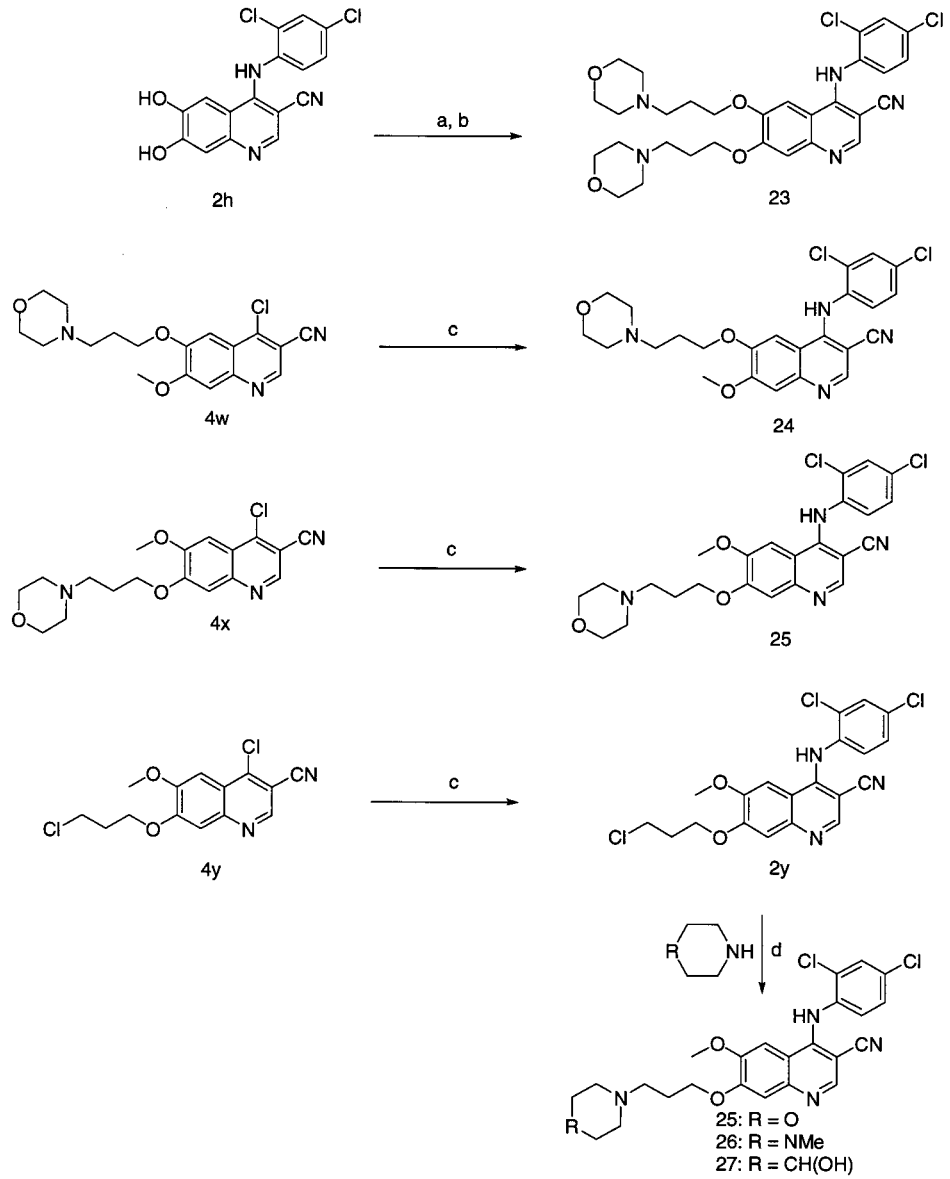
28b



28c

As shown in Table 1, the NH group at C-4 of **2a** is essential for Src inhibitory activity. Substantial decreases in activity were observed with compounds **8**−**12**, the analogues of **2a** with O, S, $NHCH_2$, NMe, and NHC(O) substituents at C-4. The importance of the C-4 NH group is further demonstrated by the lack of activity of the 1-Me isomer **13**. In addition, Table 1 also illustrates that the 3-carbonitrile group of **2a** is required for good activity. The 3-unsubstituted quinoline **6** (IC_{50} value of 84 nM) was less active than **2a**, and while some activity was seen with the 3-aldehyde analogue **17** (IC_{50} value of 250 nM), the 3-ester, alcohol, acid, and amide deriva-

Scheme 5[a]



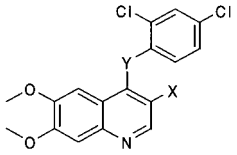
[a] (a) 3-Chloropropyl *p*-toluenesulfonate, tricaprylmethylammonium chloride, K_2CO_3, acetone, reflux; (b) morpholine, 130 °C; (c) 2,4-dichloroaniline, pyridine hydrochloride, 2-ethoxyethanol; (d) NaI, ethylene glycol dimethyl ether, 100 °C.

tives, **15**, **16**, **18**, and **19**, all provided less than 20% inhibition when tested at a concentration of 1 μM. These results correspond with the 4-(3-bromoanilino)quinoline series of EGFr inhibitors, where after the 3-carbonitrile derivative **1a**, the best activity was seen with the 3-aldehyde derivative.[27]

Table 2 shows the effect of varying the 6,7-dimethoxy substituents of **2a**. Removal of both alkoxy groups, as in **2b**, resulted in a dramatic loss of activity. While the 6- and 7-methoxy derivatives **2d** and **2e** were both about 1 log order less active than **2a**, further reductions in activity were observed with the 5-methoxy derivative **2c** and especially the 8-methoxy derivative **2f**. In addition, decreased activity was seen with the 6,7-dihydroxy analogue **2h** and the 6,7-dibutoxy analogue **2j**, but increased activity was seen with the 6,7-diethoxy analogue **2i** (IC_{50} value of 11 nM). This result is in contrast to the 4-[(3-bromophenyl)amino]-3-quinolinecarbonitrile series of EGFr inhibitors wherein reduced activity was seen with the 6,7-diethoxy analogue and the 6,7-dihydroxy analogue had activity comparable to **1a**.[27]

In the study of the 2,4-dichloroanilino group, shown in Table 3, the 2-chloro derivative **2k** was about 3-fold less active than **2a** while the 4-chloro derivative **2l** was about 1.5-fold less active than **2a**. Of the five possible dichloro isomers of **2a**, only the 3,5-dichloro analogue **2p** (IC_{50} value of 1.06 μM) had greatly reduced activity compared to **2a**. Comparing the 4-chloroanilino analogues where the substituent at C-2 was varied from fluoro to bromo to iodo, an increase in Src inhibition was seen with the larger bromo and iodo substituents. In fact, the best activity in this series was seen with **2t** (IC_{50} value of 6.2 nM), which has a 4-chloro-2-iodoanilino group at C-4. However **2u**, the 2-chloro-4-iodo isomer of **2t**, had reduced activity (IC_{50} value of 95 nM) compared to **2a**, while the 2-chloro-4-bromo analogue **2v** had increased activity (IC_{50} value of 15 nM). These results imply that there is a greater steric constraint

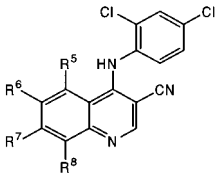
Table 1. Inhibition of Src Kinase Activity by C-3 and C-4 Substituted 6,7-Dimethoxyquinolines[a]



compd	Y	X	Src: IC_{50} (nM) or % inhib at 1 μM
2a	NH	CN	30
3	quinazoline analogue of **2a**		250
6	NH	H	84
8	O	CN	37%
9	S	CN	2200
10	$NHCH_2$	CN	24%
11	NMe	CN	32%
12	NHC(O)	CN	NA[b]
13	1-Me analogue of **2a**		NA[b]
15	NH	COOEt	NA[b]
16	NH	CH_2OH	NA[b]
17	NH	CHO	250
18	NH	COOH	NA[b]
19	NH	$CONH_2$	NA[b]
PP1[c]			35

[a] IC_{50} or % inhibition 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] NA, less than 20% inhibition at 1 μM. [c] Purchased from Calbiochem (San Diego, CA).

Table 2. Inhibition of Src Kinase Activity by Various C-5, C-6, C-7, and C-8 Substituted 4-(2,4-Dichlorophenylamino)-3-quinolinecarbonitriles[a]



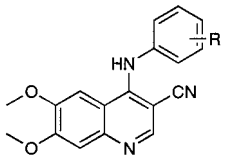
compd	R^5	R^6	R^7	R^8	Src: IC_{50} (nM) or % inhib at 1 μM
2a	H	OMe	OMe	H	30
2b	H	H	H	H	NA[b]
2c	OMe	H	H	H	1200
2d	H	OMe	H	H	320
2e	H	H	OMe	H	200
2f	H	H	H	OMe	NA[b]
2g	OMe	H	OMe	H	52%
2h	H	OH	OH	H	NA[b]
2i	H	OEt	OEt	H	11
2j	H	O-*n*-Bu	O-*n*-Bu	H	160

[a] IC_{50} or % inhibition 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] NA, less than 20% inhibition at 1 μM.

at C-4 of the anilino group than at C-2. Interestingly, we previously reported that addition of a bromo group at C-2 of the anilino group of **28a** caused a decrease in the IC_{50} for Src inhibition from 35 nM for **28a** to 170 nM for **28c**.[36] This discrepancy suggests that the 3,4,5-trimethoxyanilino derivative **28a** has a binding mode that is different from that of **2a**.

Compound **2a** was next evaluated for its ability to block the proliferation of rat fibroblasts stably transfected with a plasmid expressing activated Src. In a proliferation assay measuring the inhibition of the growth of these cells on plastic, an IC_{50} value of 18 μM was obtained for **2a.** Src-transformed fibroblasts exhibit

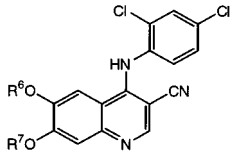
Table 3. Inhibition of Src Kinase Activity By 6,7-Dimethoxy-4-phenylamino-3-quinolinecarbonitriles[a]



compd	R	Src: IC_{50} (nM)	compd	R	Src: IC_{50} (nM)
2a	2,4-diCl	30	**2q**	3,4-diCl	57
2k	2-Cl	95	**2r**	2-F, 4-Cl	53
2l	4-Cl	53	**2s**	2-Br, 4-Cl	10
2m	2,3-diCl	90	**2t**	2-I, 4-Cl	6.2
2n	2,5-diCl	40	**2u**	2-Cl, 4-I	95
2o	2,6-diCl	61	**2v**	2-Cl, 4-Br	15
2p	3,5-diCl	1100			

[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.

Table 4. Inhibition of Src Kinase Activity and Cell Proliferation by 4-(2,4-Dichlorophenylamino)-3-quinolinecarbonitriles



			IC_{50} (μM)		
compd	R^6	R^7	nM Src[a]	Src cells[b]	Fyn cells[b]
2a	Me	Me	30	5.2	>10
23	$(CH_2)_3$-morpholine	$(CH_2)_3$-morpholine	19	3.3	>10
24	$(CH_2)_3$-morpholine	Me	220	>10	>10
25	Me	$(CH_2)_3$-morpholine	3.8	0.94	3.2
26	Me	$(CH_2)_3$-*N*-methyl-piperazine	8.7	1.0	2.9
27	Me	$(CH_2)_3$-4-hydroxy-piperidine	3.5	1.4	4.5

[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 four separate determinations.

anchorage independence that has an absolute requirement for Src, while anchorage-dependent growth, i.e., growth on plastic, does not necessarily rely on Src activity. Therefore, an anchorage-independent growth assay with plates that do not support cell adhesion was used.[37] In this suspension assay, an IC_{50} value of 5.2 μM was obtained for **2a** (see Table 4).

There are several reports in the literature wherein the addition of a 3-morpholinopropoxy group at either C-6 or C-7 of various 4-anilinoquinazoline TK inhibitors resulted in improved water solubility and cellular activity.[38−40] This basic amine tail was added at either the C-6, the C-7, or both the C-6 and C-7 positions of **2a**, to give **23**−**25**, respectively. As shown in Table 4, the most potent of these three compounds was **25**, which had increased activity not only in the suspension assay (IC_{50} of 940 nM) but also in the enzymatic assay (IC_{50} of 3.8 nM). This result suggests that a basic amine tail at C-7 leads to an additional interaction with Src, while this same substitution at C-6 is detrimental. The dose−response curve for **25** in the suspension assay is shown in Figure 1. It should be noted that in the assay measuring the inhibition of cell growth on plastic, an IC_{50} value of 3.2 μM was obtained for **25**. Since addition

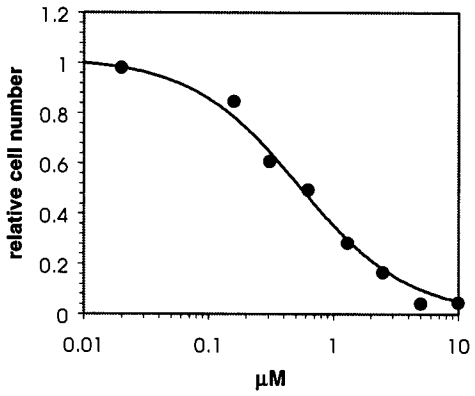


Figure 1. Suspension growth assay with Src-tranformed fibroblasts treated with **25**. Cells were plated into 96-well ultralow binding plates on day 0. On day 1, serial dilutions of compound were added to the indicated concentrations, and proliferation was scored on day 4. Relative cell proliferation was determined using the CellTiter 96 cell proliferation assay kit (Promega). The curve shown is the calculated fit to the data.

of the water solublizing group at C-7 was preferred to addition at C-6, additional derivatives of **25** were prepared, including the *N*-methylpiperazine and 4-hydroxypiperidine analogues **26** and **27**. In the enzymatic assay, **27** had comparable activity to **25**, while **26** exhibited about one-half the activity of **25**. However, in the suspension assay all three compounds had similar activity.

We next studied the effect of **25** on the phosphorylation of cellular proteins in extracts prepared from the Src-transformed fibroblasts. Figure 2A shows a Western blot of these whole cell extracts probed with phosphotyrosine antibody. Compound **25** caused a dramatic reduction of total cell phosphotyrosine at submicromolar concentrations. We also examined the tyrosine phosphorylation of the 80-kDa Src target protein cortactin (Figure 2B).[41] A Western blot of cortactin immunoprecipitates from the Src-transformed fibroblasts extracts treated with **25** indicated that the phosphorylation of cortactin is substantially reduced at 1 μM. Under these conditions of protein loading, none of these phosphotyrosine-containing proteins can be detected on similar blots of extracts from rat fibroblasts transfected with the vector alone (data not shown). Therefore, the tyrosine phosphorylation observed here is Src-dependent, and **25** inhibits Src at sub-micromolar concentrations in cells.

While **2a** is selective for inhibiting Src compared to some RTKs, selectivity for Src over other SFKs would also be desirable. To investigate the activity of these compounds against Fyn, rat fibroblasts transfected with activated Fyn were prepared. As shown in Table 4, compounds **25**−**27** exhibited only about a 3-fold selectivity for inhibition of Src over Fyn. This result is not surprising since the catalytic domain of these two SFKs is highly conserved.

Conclusion

By employing a novel screening strategy, we identified and then optimized potent Src kinase inhibitors. By systematic replacement of the functional groups of the screening lead **2a** we determined that various 4-phenylamino-3-quinolinecarbonitriles are potent inhibitors of the kinase activity of Src. Modification of either the

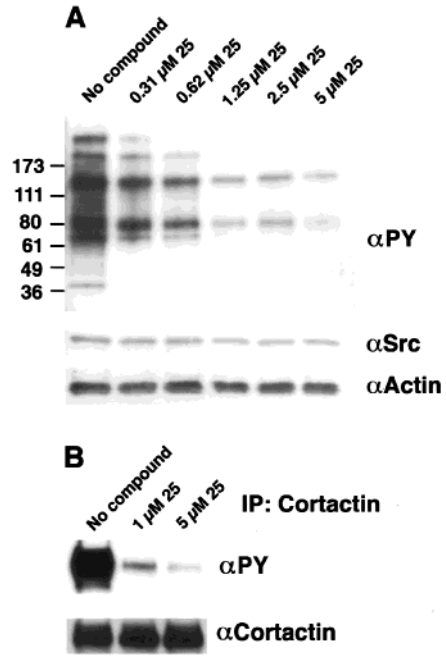


Figure 2. Effect of **25** on Src-dependent tyrosine phosphorylation. Equal numbers of Src-transformed cells were exposed to various concentrations of **25** for 5 h. Extracts were analyzed on a 4−15% SDS−polyacrylamide gradient gel. Blots were probed with antibody to phosphotyrosine (A, top panel). Equal loading was verified by probing equivalent blots with antibodies to Src and actin (A, bottom panel). Lane 1, no compound; lane 2, 0.31 μM **25**; lane 3, 0.62 μM **25**; lane 4, 1.25 μM **25**, lane 5, 2.5 μM **25**; lane 6, 5 μM **25**. (B) Immunoprecipitates of cortactin from whole cell extracts of Src-transformed fibroblasts treated with (lane 1) no compound, (lane 2) 1 μM **25**, (lane 3) 5 μM **25**; probed with antibodies to phosphotyrosine (top panel) and cortactin (bottom panel).

heteroatom at C-4 or the carbonitrile group at C-3 of **2a** results in compounds with greatly diminished activity. We also determined that a 2,4-dihalogen substitution pattern on the 4-phenylamino group is preferred and that larger 2-halo groups are optimal. Addition of a 3-morpholinopropoxy group at C-7 of **2a** provided **25** which has an IC_{50} of 3.8 nM for the inhibition of Src kinase activity and exhibits submicromolar activity in inhibiting both the growth of Src-transformed rat fibroblasts in suspension and the phosphorylation of Src substrate proteins.

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_4Si. 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-[(2,4-Dichlorophenyl)amino]-6,7-dimethoxy-3-quinolinecarbonitrile (2a). A mixture of **4a** (249 mg, 1.0 mmol),[26,27] 2,4-dichloroaniline (194 mg, 1.2 mmol) and pyridine hydrochloride (116 mg, 1.0 mmol) in 12 mL of 2-ethoxyethanol was heated at reflux for 7 h. After cooling, the solvent was removed in vacuo and the residue was treated with 10 mL of a saturated $NaHCO_3$ solution and the suspension was stirred for 15 min. The precipitate was collected by filtration, washing with water

and ether. Drying in vacuo provided 93 mg (25%) of **2a** as a light tan solid: mp 243−244 °C; ^{1}H NMR (DMSO-d_6) δ 3.93 (s, 3H), 3.95 (s, 3H), 7.33 (s, 1H), 7.50 (s, 2H), 7.78 (s, 1H), 7.81 (s, 1H), 8.41 (s, 1H), 9.59 (br s, 1H); MS (ES) *m*/*z* 374.0, 375.9 (M + 1).

Alternative Preparation of 4-[(2,4-Dichlorophenyl)-amino]-6,7-dimethoxy-3-quinolinecarbonitrile (2a). To a suspension of NaH (60% in mineral oil, 800 mg) in 50 mL of DMF was added 2,4-dichloroaniline (3.24 g, 20.0 mmol) and the mixture was stirred at room temperature for 1 h. To the resultant green solution was added **4a** (2.50 g, 10.0 mmol) and the mixture was heated at reflux for 1 h then poured into water and extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. Et_2O was added to the residue and the white solid collected by filtration to provide 2.36 g (67%) of **2a**: ^{1}H NMR (DMSO-d_6) δ 3.93 (s, 3H), 3.95 (s, 3H), 7.33 (s, 1H), 7.50 (br s, 2H), 7.77−7.81 (m, 2H), 8.41 (s, 1H), 9.57 (br s, 1H); MS (ES) *m*/*z* 373.8, 375.8 (M + 1). Anal. ($C_{18}H_{13}Cl_2N_3O_2$·0.5H_2O) C, H, N.

4-[(2,4-Dichlorophenyl)amino]-3-quinolinecarbonitrile (2b). To a suspension of NaH (60% in mineral oil, 130 mg) in 10 mL of THF was added 2,4-dichloroaniline (520 mg, 3.21 mmol) and the mixture was heated to reflux. After cooling, **4b** (300 mg, 1.59 mmol)[34] was added and the mixture was heated at reflux for 30 min then cooled to room temperature and partitioned between water and EtOAc. The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by flash chromatography eluting with 1:1 hexane:EtOAc to provide 263 mg (53%) of **2b** as a tan solid: mp 196−198 °C; ^{1}H NMR ($CDCl_3$) δ 6.96 (s, 1H), 7.03 (d, J = 8 Hz, 1H), 7.23 (dd, J = 9, 2 Hz, 1H), 7.53−7.60 (m, 2H), 7.80−7.87 (m, 2H), 8.12 (d, J = 9 Hz, 1H), 8.80 (s, 1H); MS (ES) *m*/*z* 314.1, 316.0 (M + 1). Anal. ($C_{16}H_9Cl_2N_3$) C, H, N.

4-[(2,4-Dichlorophenyl)amino]-5-methoxy-3-quinoline-carbonitrile (2c). Following the NaH route used to prepare **2a**, **2c** was obtained as a gray solid in 63% yield from **4c**: mp 216−218 °C; ^{1}H NMR (DMSO-d_6) δ 4.06 (s, 3H), 7.20 (d, J = 8 Hz, 1H), 7.48−7.62 (m, 3H), 7.73−7.81 (m, 2H), 8.46 (s, 1H), 10.21 (br s, 1H); MS (ES) *m*/*z* 343.9, 345.8 (M + 1). Anal. ($C_{17}H_{11}Cl_2N_3O$) C, H, N.

4-[(2,4-Dichlorophenyl)amino]-6-methoxy-3-quinoline-carbonitrile (2d). Following the route used to prepare **2b**, **2d** was obtained as an off-white solid in 30% yield from **4d**:[26] mp 160−161 °C; ^{1}H NMR (DMSO-d_6) δ 3.94 (s, 3H), 7.48−7.62 (m, 3H), 7.81−7.91 (m, 3H), 8.45 (s, 1H), 9.73 (s, 1H); MS (ES) *m*/*z* 343.8, 345.8 (M + 1). Anal. ($C_{17}H_{11}Cl_2N_3O$) C, H, N.

4-[(2,4-Dichlorophenyl)amino]-7-methoxy-3-quinoline-carbonitrile (2e). Following the NaH route used to prepare **2a**, **2e** was obtained as an off-white solid in 97% yield from **4e**:[26] mp 192−194 °C; ^{1}H NMR (DMSO-d_6) δ 3.93 (s, 3H), 7.23−7.35 (br s, 2H), 7.45−7.60 (br s, 2H), 7.76 (s, 1H), 8.39 (d, J = 8 Hz, 1H), 8.49 (s, 1H), 9.81 (br s, 1H); MS (ES) *m*/*z* 344.2, 346.2 (M + 1). Anal. ($C_{17}H_{11}Cl_2N_3O$·0.2H_2O) C, H, N.

4-[(2,4-Dichlorophenyl)amino]-8-methoxy-3-quinoline-carbonitrile (2f). Following the NaH route used to prepare **2a**, **2f** was obtained as a light tan solid in 32% yield from **4f**:[26] mp 201−202 °C; ^{1}H NMR (DMSO-d_6) δ 3.90 (s, 3H), 7.34 (d, J = 8 Hz, 1H), 7.41−7.67 (m, 3H), 7.71−8.09 (m, 2H), 8.51 (s, 1H), 9.80 (br s, 1H); MS (ES) *m*/*z* 343.7, 345.5 (M + 1). Anal. ($C_{17}H_{11}Cl_2N_3O$·0.5H_2O) C, H, N.

4-[(2,4-Dichlorophenyl)amino]-5,7-dimethoxy-3-quino-linecarbonitrile (2g). Following the NaH route used to prepare **2a**, **2g** was obtained as an off-white solid in 57% yield from **4g** after flash column chromatography eluting with a gradient of 3:1 to 1:1 hexane:EtOAc: mp 238−239 °C; ^{1}H NMR (DMSO-d_6) δ 3.92 (s, 3H), 4.03 (s, 3H), 6.74 (d, J = 2 Hz, 1H), 6.94 (d, J = 2 Hz, 1H), 7.48 (dd, J = 9, 2 Hz, 1H), 7.54 (d, J = 9 Hz, 1H), 7.77 (d, J = 2 Hz, 1H), 8.39 (s, 1H), 9.97 (s, 1H); MS (ES) *m*/*z* 374.1 (M + 1). Anal. ($C_{18}H_{13}Cl_2N_3O_2$) C, H, N.

4-[(2,4-Dichlorophenyl)amino]-6,7-dihydroxy-3-quino-linecarbonitrile (2h). A mixture of **2a** (600 mg, 1.60 mmol) and pyridine hydrochloride (270 mg, 24.06 mmol) was heated at 215−222 °C for 30 min. After cooling to 100 °C, 3.2 mL of concentrated aqueous NH_4OH was added. The reaction mixture was concentrated in vacuo and 50 mL of water and 1.2 mL of AcOH was added to the residue. The mixture was stirred at room temperature for 30 min. The resultant solids were collected by filtration washing with water and Et_2O. After drying in vacuo, 415 mg (75%) of **2h** was isolated as a white solid: mp 295 °C dec; ^{1}H NMR (DMSO-d_6) δ 6.94 (br s, 1H), 7.39 (br s, 1H), 8.48 (d, J = 8 Hz, 1H), 9.82 (br s, 1H), 10.36 (br s, 1H), 12.40 (br s, 1H); MS (ES) *m*/*z* 345.8, 347.8 (M + 1). Anal. ($C_{16}H_9Cl_2N_3O_2$·0.8H_2O) C, H, N.

4-[(2,4-Dichlorophenyl)amino]-6,7-diethoxyquinoline-3-carbonitrile (2i). To a mixture of **22** (500 mg, 1.29 mmol) and K_2CO_3 (390 mg, 2.82 mmol) in 5 mL of DMF was added ethyl iodide (602 mg, 3.86 mmol). The mixture was heated at 65 °C for 3 h then diluted with water and the pH was adjusted to 5−6 with AcOH. The mixture was extracted into EtOAc and the organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by flash column chromatography eluting with 3:1 hexane:EtOAc to provide 383 mg of a colorless syrup. This material was combined with K_2CO_3 (475 mg, 3.44 mmol) in 10 mL of MeOH and heated at reflux for 90 min then cooled to room temperature and diluted with CH_2Cl_2. The organic layer was concentrated in vacuo in the presence of silica gel. The residue was purified by column chromatography eluting with 2:1 hexane:EtOAc to provide 192 mg (36%) of **2i** as an off-white solid: mp 85−87 °C; ^{1}H NMR (DMSO-d_6) δ 1.42 (t, J = 7 Hz, 6H), 4.22 (m, 4H), 7.32 (s, 1H), 7.51 (br s, 2H), 7.80 (br s, 2H), 8.40 (s, 1H), 9.52 (s, 1H); MS (ES) *m*/*z* 402.1, 404.1 (M + 1). Anal. ($C_{20}H_{17}Cl_2N_3O_3$·0.2H_2O) C, H, N.

4-[(2,4-Dichlorophenyl)amino]-6,7-di-*n*-butoxy-3-quinolinecarbonitrile (2j). To a mixture of **22** (400 mg, 1.03 mmol) and K_2CO_3 (312 mg, 2.26 mmol) in 5 mL of DMF was added *n*-butyl bromide (424 mg, 3.09 mmol). The mixture was heated at 65 °C for 4 h then cooled to room temperature and extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by flash column chromatography eluting with 3:1 hexane:EtOAc to provide 450 mg of product. This material was combined with K_2CO_3 (496 mg, 3.59 mmol) in 10 mL of MeOH and heated at reflux for 90 min then cooled to room temperature and diluted with CH_2Cl_2. The organic layer was concentrated in vacuo in the presence of silica gel. The residue was purified by column chromatography eluting with 2:1 hexane:EtOAc to provide 184 mg (39%) of **2j**: mp 138−140 °C; ^{1}H NMR (DMSO-d_6) δ 0.95 (t, J = 7 Hz, 3H), 1.07 (t, J = 7 Hz, 3H), 1.49−1.62 (m, 4H), 1.73 (m, 2H), 1.90 (m, 2H), 3.84 (t, J = 7 Hz, 2H), 4.18 (t, J = 7 Hz, 2H), 6.69 (s, 1H), 6.79 (d, J = 9 Hz, 1H), 6.82 (s, 1H), 7.14 (dd, J = 9, 2 Hz, 1H), 7.26 (s, 1H), 7.37 (s, 1H), 7.51 (d, J = 2 Hz, 1H), 8.69 (s, 1H); MS (ES) *m*/*z* 458.0, 460.0 (M + 1). Anal. ($C_{24}H_{25}Cl_2N_3O_2$) C, H, N.

4-(2-Chlorophenylamino)-6,7-dimethoxyquinoline-3-carbonitrile (2k). Following the pyridine hydrochloride route used to prepare **2a**, **2k** was obtained as a light tan solid in 48% yield from **4a** after chromatography eluting with 1% MeOH in CH_2Cl_2: mp 227−229 °C; ^{1}H NMR (DMSO-d_6) δ 3.94 (s, 3H), 3.95 (s, 3H), 7.33 (s, 1H), 7.37−7.51 (m, 3H), 7.60 (m, 1H), 7.84 (s, 1H), 8.40 (s, 1H), 9.56 (s, 1H); MS (ES) *m*/*z* 339.9, 341.9 (M + 1). Anal. ($C_{18}H_{14}ClN_3O_2$·0.17CH_2Cl_2) C, H, N.

4-[(4-Chlorophenyl)amino]-6,7-dimethoxy-3-quinolin-ecarbonitrile (2l). Following the pyridine hydrochloride route used to prepare **2a**, **2l** was obtained as a light tan solid in 48% yield from **4a** after chromatography eluting with 1% MeOH in CH_2Cl_2: mp 204−206 °C; ^{1}H NMR (DMSO-d_6) δ 3.92 (s, 3H), 3.96 (s, 3H), 7.26 (d, J = 9 Hz, 2H), 7.36 (s, 1H), 7.45 (d, J = 9 Hz, 2H), 7.72 (s, 1H), 8.51 (s, 1H), 9.51 (s, 1H); MS (ES) *m*/*z* 339.9, 341.9 (M + 1). Anal. ($C_{18}H_{14}ClN_3O_2$) C, H, N.

4-[(2,3-Dichlorophenyl)amino]-6,7-dimethoxy-3-quino-linecarbonitrile (2m). Following the route used to prepare **2b**, **2m** was obtained as a pale yellow solid in 66% yield from **4a**: mp 227−229 °C; ^{1}H NMR (DMSO-d_6) δ 3.93 (s, 3H), 3.95 (s, 3H), 7.34 (s, 1H), 7.39−7.46 (m, 2H), 7.61 (d, J = 6 Hz, 1H), 7.81 (s, 1H), 8.43 (s, 1H), 9.65 (s, 1H); MS (ES) *m*/*z* 374.3, 376.3 (M + 1). Anal. ($C_{18}H_{13}Cl_2N_3O_2$·0.5EtOAc) C, H, N.

4-[(2,5-Dichlorophenyl)amino]-6,7-dimethoxy-3-quinolinecarbonitrile (2n). Following the pyridine hydrochloride route used to prepare **2a**, **2n** was obtained as a light tan solid in 14% yield from **4a** after chromatography eluting with 5% MeOH in CH_2Cl_2: mp 219−220 °C; ^{1}H NMR (DMSO-d_6) δ 3.93 (s, 3H), 3.95 (s, 3H), 7.35 (s, 1H), 7.42−7.52 (m, 1H), 7.59−7.70 (m, 2H), 7.79 (s, 1H), 8.45 (s, 1H), 9.75 (s, 1H); MS (ES) *m*/*z* 373.8, 375.8 (M + 1). Anal. ($C_{18}H_{13}Cl_2N_3O_2 \cdot 0.05Et_2O$) C, H, N.

4-[(2,6-Dichlorophenyl)amino]-6,7-dimethoxy-3-quinolinecarbonitrile (2o). Following the NaH route used to prepare **2a**, **2o** was obtained as a pale yellow solid in 66% yield from **4a**: mp 264−266 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.95 (s, 3H), 3.96 (s, 3H), 7.35 (s, 1H), 7.49 (t, J = 8 Hz, 1H), 7.65 (d, J = 8 Hz, 2H), 7.88 (s, 1H), 8.39 (s, 1H), 9.66 (s, 1H); MS (ES) *m*/*z* 373.9, 375.8 (M + 1). Anal. ($C_{18}H_{13}Cl_2N_3O_2$) C, H, N.

4-[(3,5-Dichlorophenyl)amino]-6,7-dimethoxy-3-quinolinecarbonitrile (2p). Following the pyridine hydrochloride route used to prepare **2a**, **2p** was obtained as a yellow-brown solid in 49% yield from **4a**: mp 228−230 °C; ^{1}H NMR (DMSO-d_6) δ 3.92 (s, 3H), 3.97 (s, 3H), 7.21 (bs, 2H), 7.30 (s, 1H), 7.40 (s, 1H), 7.64 (s, 1H), 8.63 (s, 1H), 9.63 (s, 1H); MS (ES) *m*/*z* 373.8, 375.8 (M + 1). Anal. ($C_{18}H_{13}Cl_2N_3O_2$) C, H.

4-[(3,4-Dichlorophenyl)amino]-6,7-dimethoxy-3-quinolinecarbonitrile (2q). Following the pyridine hydrochloride route used to prepare **2a**, **2q** was obtained as a yellow-brown solid in 42% yield from **4a**: mp 211−215 °C; ^{1}H NMR (DMSO-d_6) δ 3.92 (s, 3H), 3.96 (s, 3H), 7.20 (dd, J = 9, 3 Hz, 1H), 7.39 (s, 1H), 7.47 (d, J = 3 Hz, 1H), 7.61 (d, J = 9 Hz, 1H), 7.68 (s, 1H), 8.58 (s, 1H), 9.60 (s, 1H); MS (ES) *m*/*z* 373.8, 375.8 (M + 1). Anal. ($C_{18}H_{13}Cl_2N_3O_2 \cdot 0.25H_2O$) C, H, N.

4-[(4-Chloro-2-fluorophenyl)amino]-6,7-dimethoxy-3-quinolinecarbonitrile (2r). Following the pyridine hydrochloride route used to prepare **2a**, **2r** was obtained as a tan solid in 52% yield from **4a** after chromatography eluting with EtOAc: mp 184−186 °C; ^{1}H NMR (DMSO-d_6) δ 3.94 (s, 3H), 3.96 (s, 3H), 7.35 (m, 2H), 7.48 (t, J = 9 Hz, 1H), 7.60 (dd, J = 11, 2 Hz, 1H), 7.79 (s, 1H), 8.47 (s, 1H), 9.56 (s, 1H); MS (ES) *m*/*z* 357.9, 359.9 (M + 1). Anal. ($C_{18}H_{13}ClFN_3O_2$) C, H, N.

4-[(2-Bromo-4-chlorophenyl)amino]-6,7-dimethoxy-3-quinolinecarbonitrile (2s). Following the NaH route used to prepare **2a**, **2s** was obtained as an off-white solid in 58% yield from **4a**: mp 256−257 °C; ^{1}H NMR (DMSO-d_6) δ 3.94 (s, 1H), 3.95 (s, 1H), 7.34 (s, 1H), 7.54 (br s, 2H), 7.82 (s, 1H), 7.93 (s, 1H), 8.41 (s, 1H), 9.58 (s, 1H); MS (ES) *m*/*z* 417.8, 419.8 (M + 1). Anal. ($C_{18}H_{13}BrClN_3O_2$) C, H, N.

4-[(4-Chloro-2-iodophenyl)amino]-6,7-dimethoxy-3-quinolinecarbonitrile (2t). Following the NaH route used to prepare **2a**, **2t** was obtained as a white solid in 77% yield from **4a** after chromatography eluting with a gradient of 1:2 to 1:1 EtOAc:hexane: mp 248−249 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.94 (s, 3H), 3.95 (s, 3H), 7.33 (s, 1H), 7.49 (d, J = 8 Hz, 1H), 7.56 (dd, J = 8, 2 Hz, 1H), 7.84 (s, 1H), 8.06 (d, J = 2 Hz, 1H), 8.38 (s, 1H), 9.60 (s, 1H); MS (ES) *m*/*z* 465.9, 467.9 (M + 1). Anal. ($C_{18}H_{13}ClIN_3O_2$) C, H, N.

4-[(2-Chloro-4-iodophenyl)amino]-6,7-dimethoxy-3-quinolinecarbonitrile (2u). Following the NaH route used to prepare **2a**, **2u** was obtained as a pale yellow solid in 32% yield from **4a**: mp 250−253 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.93 (s, 3H), 3.95 (s, 3H), 7.25 (d, J = 8 Hz, 1H), 7.34 (s, 1H), 7.75 (dd, J = 8, 2 Hz, 1H), 7.80 (s, 1H), 7.98 (d, J = 2 Hz, 1H), 8.43 (s, 1H), 9.50 (s, 1H); MS (ES) *m*/*z* 466.0, 468.0 (M + 1). Anal. ($C_{18}H_{13}ClIN_3O_2 \cdot 0.2EtOAc$) C, H, N.

4-[(4-Bromo-2-chlorophenyl)amino]-6,7-dimethoxy-3-quinolinecarbonitrile (2v). Following the route used to prepare **2b**, **2v** was obtained as a tan solid in 63% yield from **4a**: mp 208−210 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.93 (s, 3H), 3.95 (s, 3H), 7.35 (s, 1H), 7.44 (d, J = 8 Hz, 1H), 7.62 (dd, J = 8, 2 Hz, 1H), 7.81 (s, 1H), 7.89 (d, J = 2 Hz, 1H), 8.43 (s, 1H), 9.53 (s, 1H); MS (ES) *m*/*z* 417.7, 419.7 (M + 1). Anal. ($C_{18}H_{13}BrClN_3O_2$) C, H, N.

7-(3-Chloropropoxy)-4-[(2,4-dichlorophenyl)amino]-6-methoxy-3-quinolinecarbonitrile (2y). Following the route used to prepare **2a**, **2y** was obtained as an off-white solid in 49% yield from **4y**:[26,36] mp 183−186 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.70 (m, 2H), 3.83 (t, J = 6 Hz, 2H), 3.95 (s, 3H), 4.29 (t, J = 6 Hz, 2H), 7.37 (s, 1H) , 7.52 (s, 2H), 7.79 (s, 1H), 7.82 (s, 1H), 8.42 (s, 1H), 9.56 (s, 1H); MS (ES) *m*/*z* 438.2 (M + 1). Anal. ($C_{20}H_{16}Cl_3N_3O_2 \cdot 0.25H_2O$) C, H, N.

4-[(2,4-Dichlorophenyl)amino]-6,7-dimethoxyquinazoline (3). To a suspension of **5**[31] (300 mg, 1.34 mmol) in 7 mL of EtOH was added 2,4-dichloroaniline (238 mg, 1.47 mmol) and the mixture was heated at reflux overnight. The reaction mixture was cooled to room temperature and partitioned between 0.5 N NaOH and EtOAc. The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. Et_2O and hexane were added to the residue and the off-white solid was collected by filtration to provide 230 mg (49%) of **3**: mp 220−222 °C; ^{1}H NMR (DMSO-d_6) δ 3.93 (s, 6H), 7.19 (s, 1H), 7.49 (dd, J = 9, 2 Hz, 1H), 7.59 (d, J = 9 Hz, 1H), 7.75 (d, J = 2 Hz, 1H), 7.82 (s, 1H), 8.32 (s, 1H), 9.57 (s, 1H); MS (ES) *m*/*z* 349.8, 351.9 (M + 1). Anal. ($C_{16}H_{13}Cl_2N_3O_2$) C, H, N.

4-Chloro-5-methoxy-3-quinolinecarbonitrile (4c). A mixture of **20** (811 mg, 4.05 mmol) in 10 mL of phosphorus oxychloride and 4 drops of DMF was heated at reflux for 4 h. After cooling, the mixture was concentrated in vacuo and the residue was diluted with ice cold CH_2Cl_2. The organic phase was washed once with ice water, twice with cold saturated sodium carbonate solution and twice with cold brine, then dried over Na_2SO_4. The solution was passed through a pad of silica gel and the filtrate was concentrated in vacuo to give 772 mg of **4c**, as a yellow solid: mp 174−176 °C; ^{1}H NMR (DMSO-d_6) δ 3.97 (s, 3H), 7.03 (d, J = 8 Hz, 1H), 7.28 (d, J = 8 Hz, 1H), 7.77 (t, J = 8 Hz, 1H), 8.70 (s, 1H); MS (ES) *m*/*z* 218.7, 220.8 (M + 1).

4-Chloro-5,7-dimethoxy-3-quinolinecarbonitrile (4g). A mixture of **21** (500 mg, 2.27 mmol) in 2 mL of phosphorus oxychloride and 3 drops of DMF was heated at 110 °C for 2 h. After cooling, the mixture was poured into ice water and neutralized with saturated $NaHCO_3$. The solids were collected by filtration, washing with water. This material was purified by flash column chromatography eluting with a gradient of 3:1 to 1:1 hexane:ethyl acetate to provide 267 mg (92%) of **4g**, as an off-white solid: mp 240−242 °C; ^{1}H NMR (DMSO-d_6) δ 3.31 (s, 3H), 3.97 (s, 3H), 6.90 (d, J = 2 Hz, 1H), 7.15 (d, J = 2 Hz, 1H), 8.99 (s, 1H); MS (ES) *m*/*z* 248.8 (M + 1). Anal. ($C_{12}H_9ClN_2O_2 \cdot 0.1H_2O$) C, H, N.

(2,4-Dichlorophenyl)(6,7-dimethoxyquinolin-4-yl)-amine (6). Following the pyridine hydrochloride route used to prepare **2a**, **6** was obtained as an off-white solid in 21% yield from **7**[32] after chromatography eluting with EtOAc: mp 144−146 °C; ^{1}H NMR (DMSO-d_6) δ 3.91 (s, 3H), 3.92 (s, 3H), 6.16 (d, J = 5 Hz, 1H), 7.26 (s, 1H), 7.46 (d, J = 8 Hz, 1H), 7.52 (dd, J = 8, 2 Hz, 1H), 7.68 (s, 1H), 7.80 (d, J = 2 Hz, 1H), 8.24 (d, J = 5 Hz, 1H), 8.65 (s, 1H); MS (ES) *m*/*z* 349.3, 351.2 (M + 1). Anal. ($C_{17}H_{14}Cl_2N_2O_2$) C, H, N.

4-(2,4-Dichlorophenoxy)-6,7-dimethoxy-3-quinolinecarbonitrile (8). To a melt of 2,4-dichlorophenol (700 mg, 4.29 mmol) and 80 mg of KOH was added **4a** (200 mg, 0.89 mmol). The mixture was heated for 30 min then cooled. Water was added to the reaction mixture followed by EtOAc. The layers were separated and 1 N HCl was added to the aqueous layer. The aqueous layer was extracted with EtOAc and the organic layers were combined, dried over $MgSO_4$, filtered and concentrated in vacuo. Methanol was added to the residue and the light tan solid was collected by filtration to provide 192 mg (58%) of **8**: mp 234−236 °C; ^{1}H NMR (DMSO-d_6) δ 3.90 (s, 3H), 4.01 (s, 3H), 7.24 (d, J = 9 Hz, 1H), 7.38 (s, 1H), 7.44 (dd, J = 9, 2 Hz, 1H), 7.56 (s, 1H), 7.90 (d, J = 2 Hz, 1H), 8.89 (s, 1H); MS (ES) *m*/*z* 374.8, 376.8 (M + 1). Anal. ($C_{18}H_{12}Cl_2N_2O_3 \cdot 0.25H_2O$) C, H, N.

4-(2,4-Dichlorophenylsulfanyl)-6,7-dimethoxy-3-quinolinecarbonitrile (9). To a suspension of **4a** (249 mg, 1.00 mmol) in 10 mL of DMF was added 2,4-dichlorothiophenol (215 mg, 1.20 mmol) resulting in a homogeneous solution. After stirring at room temperature for 30 min, the off-white solid was collected by filtration, washing with hexane, to provide

294 mg (75%) of **9**: mp 201−202 °C; ^{1}H NMR (DMSO-d_6) δ 3.83 (s, 3H), 4.01 (s, 3H), 7.01 (d, J = 9 Hz, 1H), 7.31 (dd, J = 9, 2 Hz, 1H), 7.43 (s, 1H), 7.57 (s, 1H), 7.82 (d, J = 2 Hz, 1H), 9.04 (s, 1H); MS (ES) m/z 390.8, 392.8 (M + 1). Anal. ($C_{18}H_{12}Cl_2N_2O_2S\cdot0.70H_2O$) C, H, N.

4-[(2,4-Dichlorophenylamino)methyl]-6,7-dimethoxy-3-quinolinecarbonitrile (10). A mixture of **4a** (249 mg, 1.00 mmol), 2,4-dichlorobenzylamine (387 mg, 2.20 mmol) and Hunig's base (284 mg, 2.20 mmol) in 7 mL of DMF was heated at reflux for 2.5 h. After cooling to room temperature, the mixture was partitioned between EtOAc and water. The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by flash column chromatography eluting with a gradient of 1:3 to 1:1 EtOAc:hexane to provide 350 mg (90%) of **10** as a light yellow solid: mp 127−130 °C; ^{1}H NMR (DMSO-d_6) δ 3.93 (s, 6H), 5.00 (d, J = 6 Hz, 2H), 7.25 (d, J = 8 Hz, 1H), 7.28 (s, 1H), 7.40 (dd, J = 8, 2 Hz, 1H), 7.70 (d, J = 2 Hz, 1H), 7.74 (s, 1H), 7.95 (s, 1H), 8.31 (s, 1H), 8.36 (t, J = 6 Hz, 1H); MS (ES) m/z 387.9, 389.8 (M + 1). Anal. ($C_{19}H_{15}Cl_2N_3O_2\cdot0.7H_2O\cdot0.6$DMF) C, H, N.

4-(2,4-Dichlorophenylmethylamino)-6,7-dimethoxy-3-quinolinecarbonitrile (11). To a suspension of NaH (60% in mineral oil, 60 mg) in 10 mL of THF was added 2,4-dichloro-*N*-methylaniline[42] (290 mg, 1.64 mmol) and the mixture was heated to reflux. To the resultant yellow suspension was added **4a** (220 mg, 0.88 mmol) and the mixture was heated at reflux overnight. The reaction mixture was cooled to room temperature and partitioned between water and EtOAc. The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. Et_2O was added to the residue and the resultant light tan solid was collected by filtration to provide 182 mg (53%) of **11**: mp 183−186 °C; ^{1}H NMR (DMSO-d_6) δ 3.62 (s, 3H), 3.72 (s, 3H), 3.96 (s, 3H), 7.10 (s, 1H), 7.43 (s, 1H), 7.50−7.58 (m, 2H), 7.65 (d, J = 9 Hz, 1H), 8.78 (s, 1H); MS (ES) m/z 387.9, 389.9 (M + 1). Anal. ($C_{19}H_{15}Cl_2N_3O_2\cdot0.5H_2O$) C, H, N.

2,4-Dichloro-*N*-(3-cyano-6,7-dimethoxyquinolin-4-yl)-benzamide (12). To a suspension of NaH (60% in mineral oil, 80 mg) in 10 mL of DMF was added 2,4-dichlorobenzamide (380 mg, 2.0 mmol) and the mixture was stirred at room temperature for 15 min. To the resultant yellow solution was added **4a** (250 mg, 1.0 mmol) and the mixture was heated at reflux for 2 h. The reaction mixture was cooled and added to a mixture of 1 N HCl and EtOAc. The mixture was filtered and the solid washed with EtOAc to provide 323 mg (80%) of **12**: mp greater than 300 °C; ^{1}H NMR (DMSO-d_6) δ 3.93 (s, 3H), 4.01 (s, 3H), 7.50 (s, 1H), 7.54 (s, 1H), 7.60−7.75 (m, 2H), 7.88 (d, J = 2 Hz, 1H), 9.00 (s, 1H), 11.5 (s, 1H); MS (ES) m/z 401.8, 403.7 (M + 1). Anal. ($C_{19}H_{13}Cl_2N_3O_3\cdot0.25H_2O$) C, H, N.

4-(2,4-Dichlorophenylimino)-6,7-dimethoxy-1-methyl-1,4-dihydro-3-quinolinecarbonitrile (13). To a suspension of NaH (60% in mineral oil, 25 mg) in 6 mL of THF was added **2a** (196 mg, 0.52 mmol) and the mixture was heated to reflux. The bright yellow suspension was cooled slightly and methyl iodide (0.50 mL, 0.80 mmol) was added. Within 2 min a yellow solution formed. The solution was partitioned between EtOAc and water. The organic layer was washed with water, dried over $MgSO_4$, filtered and concentrated in vacuo. Et_2O was added to the residue and the bright yellow solid was collected by filtration to provide 99 mg (49%) of **13**: mp 222−225 °C; ^{1}H NMR (DMSO-d_6) δ 3.80 (s, 3H), 3.83 (s, 3H), 3.96 (s, 3H), 6.91 (d, J = 8 Hz, 1H), 7.03 (s, 1H), 7.23 (dd, J = 8, 2 Hz, 1H), 7.46 (d, J = 2 Hz, 1H), 7.81 (s, 1H), 8.27 (s, 1H); MS (ES) m/z 387.9, 389.9 (M + 1). Anal. ($C_{19}H_{15}Cl_2N_3O_2$) C, H, N.

Concentration of the filtrate gave a residue which by ^{1}H NMR analysis was a 1:1 mixture of **13** and **11**.

Ethyl 4-[(2,4-Dichlorophenyl)amino]-6,7-dimethoxy-3-quinolinecarboxylate (15). Following the pyridine hydrochloride route used to prepare **2a**, **15** was obtained as a white solid in 40% yield from **14**:[33] mp 110−113 °C; ^{1}H NMR (DMSO-d_6) δ 1.30 (t, J = 7 Hz, 3H), 3.54 (s, 3H), 3.95 (s, 3H), 4.26 (q, J = 7 Hz, 2H), 6.83 (d, J = 9 Hz, 1H), 6.88 (s, 1H), 7.27 (dd, J = 9, 2 Hz, 1H), 7.40 (s, 1H), 7.74 (d, J = 2 Hz, 1H), 8.97 (s, 1H), 9.61 (s, 1H); MS (ES) m/z 421.3, 423.3 (M + 1). Anal. ($C_{20}H_{18}Cl_2N_2O_4\cdot0.2H_2O$) C, H, N.

[4-[(2,4-Dichlorophenyl)amino]-6,7-dimethoxy-3-quinolinyl]methanol (16). To a 0 °C mixture of **15** (1.60 g, 3.8 mmol) in 60 mL of THF, 19 mL of 1.0 M diisobutyl aluminum hydride in hexane was added dropwise. After stirring at 0 °C for 30 min, MeOH was added and the reaction mixture was stirred at room temperature for 30 min. The mixture was diluted with 300 mL of THF followed by the addition of 6.12 g of Na_2SO_4 decahydrate. After stirring for an additional 30 min the volatiles were removed in vacuo and the residue was partitioned between EtOAc and water. The aqueous layer was extracted with CH_2Cl_2, and the organic layers were combined, dried over Na_2SO_4, filtered and concentrated in vacuo. Et_2O was added to the residue and the white solid was collected by filtration to provide 600 mg (42%) of **16**: mp 221−222 °C; ^{1}H NMR (DMSO-d_6) δ 3.65 (s, 3H), 3.93 (s, 3H), 4.53 (d, J = 5 Hz, 2H), 5.48 (t, J = 5 Hz, 1H), 6.26 (d, J = 8 Hz, 1H), 6.93 (s, 1H), 7.11 (dd, J = 8, 2 Hz, 1H), 7.39 (s, 1H), 7.60 (d, J = 2 Hz, 1H), 7.98 (s, 1H), 8.71 (s, 1H); MS (ES) m/z 379.2, 381.2 (M + 1). Anal. ($C_{18}H_{16}Cl_2N_2O_3$) C, H, N.

4-[(2,4-Dichlorophenyl)amino]-6,7-dimethoxy-3-quinolinecarbaldehyde (17). A mixture of **16** (560 mg, 1.48 mmol) and manganese dioxide (2.8 g, 33.3 mmol) in 70 mL of $CHCl_3$ was stirred at room temperature for 2 h. The mixture was diluted with 100 mL of $CHCl_3$ and filtered through a pad of Celite, washing with $CHCl_3$. The filtrate was concentrated in vacuo to provide 440 mg (80%) of **17**: mp 168−169 °C; ^{1}H NMR (DMSO-d_6) δ 3.48 (s, 3H), 3.95 (s, 3H), 6.80 (s, 1H), 7.15 (d, J = 9 Hz, 1H), 7.38 (s, 1H), 7.40 (dd, J = 9, 2 Hz, 1H), 7.80 (d, J = 2 Hz, 1H), 8.86 (s, 1H), 10.04 (s, 1H), 10.39 (s, 1H); MS (ES) m/z 377.3, 379.2 (M + 1). Anal. ($C_{18}H_{14}Cl_2N_2O_3$) C, H, N.

4-[(2,4-Dichlorophenyl)amino]-6,7-dimethoxy-3-quinolinecarboxylic Acid Hydrochloride (18). A mixture of **15** (650 mg, 1.55 mmol) and 2.0 mL of 5.0 N NaOH in 30 mL of ethanol was heated at reflux for 1 h. The mixture was cooled and concentrated HCl was added to provide a pH of 2. The EtOH was removed in vacuo and the solids collected by filtration. Washing with water followed by Et_2O provided 600 mg (98%) of **18** as a yellow solid: mp 270−272 °C; ^{1}H NMR (DMSO-d_6) δ 3.53 (s, 3H), 3.98 (s, 3H), 7.06 (s, 1H), 7.46 (d, J = 9 Hz, 1H), 7.52 (s, 1H), 7.54 (dd, J = 9, 2 Hz, 1H), 7.88 (d, J = 2 Hz, 1H), 9.07 (s, 1H), 11.52 (s, 1H); MS (ES) m/z 393.1, 395.1 (M + 1). Anal. ($C_{18}H_{14}Cl_2N_2O_4\cdot$HCl) C, N.

4-[(2,4-Dichlorophenyl)amino]-6,7-dimethoxy-3-quinolinecarboxamide (19). A mixture of **18** (200 mg, 0.51 mmol) and *N*,*N*-carbonyldiimidazole (170 mg, 1.05 mmol) in 5 mL of DMF was heated at 60 °C for 1.5 h. The mixture was cooled and an additional 15 mL of DMF and 10 mL of THF were added. The mixture was heated until a solution was obtained and 30 mL of concentrated aqueous NH_4OH was added. The reaction was stirred overnight at room temperature and the resulting solids were collected by filtration, washing with water, to provide 103 mg (53%) of **19** as a white solid: mp 247−249 °C; ^{1}H NMR (DMSO-d_6) δ 3.49 (s, 3H), 3.94 (s, 3H), 6.62 (d, J = 9 Hz, 1H), 6.69 (s, 1H), 7.22 (dd, J = 9, 2 Hz, 1H), 7.37 (s, 1H), 7.70 (d, J = 2 Hz, 1H), 7.76 (br s, 1H), 8.36 (br s, 1H), 8.94 (s, 1H) 10.70 (s, 1H); MS (ES) m/z 392.2, 394.2 (M + 1). Anal. ($C_{18}H_{15}Cl_2N_3O_3\cdot0.4H_2O$) C, H, N.

4-Hydroxy-5-methoxy-3-quinolinecarbonitrile (20). A mixture of 2-amino-6-methoxybenzoic acid[35] (2.48 g, 14.8 mmol) in 30 mL of dimethylformamide dimethylacetal was heated at reflux for 2 h. After cooling, the solvent was removed in vacuo and the residue was passed through a Magnesol column, eluting with CH_2Cl_2, to afford 2.1 g of the amidine imtermediate that was used the next step without further purification.

A solution of CH_3CN (734 mg, 17.9 mmol) in 10 mL of THF was added dropwise to a solution of *n*-BuLi (2.5 M in hexane, 5.96 mL, 14.9 mmol) in 7 mL of THF at −78 °C. The mixture was stirred at −78 °C for 15 min. A solution of the amidine (1.6 g, 6.8 mmol) in 13 mL of THF was added dropwise and the mixture was stirred at −78 °C for 2 h, and then at room temperature for 2.5 h. The mixture was cooled to −78 °C and AcOH was added dropwise. The mixture was allowed to stir at room temperature overnight. The precipitate was collected

by filtration and washed with water and ether. After drying in vacuo, 477 mg of **20** was obtained as a light tan solid: mp >260 °C; ^{1}H NMR (DMSO-d_6) δ 3.82 (s, 3H), 6.92 (d, J = 8 Hz, 1H), 7.09 (d, J = 8 Hz, 1H), 7.62 (t, J = 8 Hz, 1H), 8.55 (s, 1H); MS (ES) m/z 200.8 (M + 1). Anal. ($C_{11}H_8N_2O_2 \cdot 0.1H_2O$) C, H, N.

5,7-Dimethoxy-4-hydroxy-3-quinolinecarbonitrile (21). A mixture of 3,5-dimethoxyaniline (5.00 g, 32.7 mmol) and ethyl (ethoxymethylene)cyanoacetate (5.52 g, 32.7 mmol) was heated at 120 °C for 45 min. Diethyl ether was added to the cooled reaction mixture and the solids were collected by filtration washing with additional diethyl ether to yield 7.16 g (79%) of ethyl 2-cyano-3-(3,5-dimethoxyphenylamino)acrylate. A mixture of ethyl 2-cyano-3-(3,5-dimethoxyphenylamino)-acrylate (1.00 g, 3.62 mmol) in 50 mL of a 1:1 mixture of biphenyl and diphenyl ether was heated at reflux for 72 h. The reaction mixture was cooled to room temperature and poured into hexane. The solids were collected by filtration washing with hexane to provide 698 mg (88%) of **21** as a tan solid: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 3.82 (s, 3H), 3.90 (s, 3H), 6.49 (d, J = 2 Hz, 1H), 6.58 (d, J = 2 Hz, 1H), 8.83 (s, 1H); MS (ES) m/z 231.2 (M + 1). Anal. ($C_{12}H_{10}N_2O_3 \cdot 0.25H_2O$) C, H, N.

***N*-Acetyl-4-[(2,4-dichlorophenyl)amino]-6,7-dihydroxy-3-quinolinecarbonitrile (22).** A mixture of **2h** (2.38 g, 6.88 mmol), 4-(dimethylamino)pyridine (1.01 g, 8.26 mmol) and acetic anhydride (7.01 g, 68.8 mmol) in 14 mL of pyridine was heated at reflux for 1.5 h. The reaction mixture was concentrated in vacuo and the residue was stirred with 75 mL of MeOH, 7.5 mL of water and 2.89 g of $NaHCO_3$ for 6.5 h. The mixture was concentrated in vacuo, the residue was suspended in water and AcOH was added to provide a pH of 4−5. The resultant solid was collected by filtration and washed with dilute AcOH followed by water to yield 2.68 g of **22** that was not purified.

4-[(2,4-Dichlorophenyl)amino]-6,7-bis(3-morpholin-4-ylpropoxy)-3-quinolinecarbonitrile (23). To a mixture of **2h** (305 mg, 0.88 mmol), 3-chloropropyl *p*-toluenesulfonate[43] (482 mg, 1.94 mmol) and tricaprylmethylammonium chloride (36 mg, 0.09 mmol) in 2 mL of acetone was added potassium carbonate (292 mg, 2.11 mmol) The mixture was heated at reflux for 18 h then concentrated in vacuo. The residue was dissolved in 20% MeOH in $CHCl_3$ and the solution was passed through Magnesol, washing with additional solvent. The filtrate was concentrated in vacuo and acetone was added. A small amount of solid was removed by filtration and the filtrate was concentrated in vacuo. The residue was dissolved in 20 mL of morpholine and heated at 130 °C for 14 h. The morpholine was removed in vacuo and the residue was purified by preparative thin-layer chromatography eluting with a gradient of 2% MeOH in CH_2Cl_2 to 8% MeOH in CH_2Cl_2 to provide 166 mg (31%) of **23** as a colorless syrup: ^{1}H NMR (DMSO-d_6) δ 1.87−2.03 (m, 4H), 2.32−2.43 (m, 8H), 2.46−2.50 (m, 4H), 3.48−3.63 (m, 8H), 4.11−4.27 (m, 4H), 7.33 (s, 1H), 7.51 (br s, 1H), 7.80 (br s, 1H), 8.41 (s, 1H), 9.53 (s, 1H); MS (ES) m/z 600.1, 602.0 (M + 1). Anal. ($C_{30}H_{35}Cl_2N_5O_4 \cdot 1.0H_2O$) C, H, N.

4-[(2,4-Dichlorophenyl)amino]-7-methoxy-6-(3-morpholin-4-ylpropoxy)-3-quinolinecarbonitrile (24). Following the pyridine hydrochloride route used to prepare **2a**, **24** was obtained as an off-white solid in 7% yield from **4w**[26] after chromatography eluting with 5% MeOH in CH_2Cl_2: mp 168−170 °C; ^{1}H NMR (DMSO-d_6) δ 1.91−2.00 (m, 2H), 2.38 (m, 4H), 2.45 (m, 2H), 3.57 (m, 4H), 3.95 (s, 3H), 4.16 (t, J = 6 Hz, 2H), 7.34 (s, 1H), 7.52 (s, 2H), 7.80 (s, 2H), 8.42 (s, 1H), 9.55 (s, 1H); MS (ES) m/z 487.0, 489.1 (M + 1). Anal. ($C_{24}H_{24}Cl_2N_4O_3 \cdot 0.3H_2O$) C, H, N.

4-[(2,4-Dichlorophenyl)amino]-6-methoxy-7-(3-morpholin-4-ylpropoxy)-3-quinolinecarbonitrile (25). Following the pyridine hydrochloride route used to prepare **2a**, **25** was obtained as a brown foam in 23% yield from **4x**[26] after chromatography eluting with a gradient of 2% to 8% MeOH in CH_2Cl_2: ^{1}H NMR (DMSO-d_6) δ 1.91−2.07 (m, 2H), 2.32−2.49 (m, 6H), 3.53−3.65 (m, 4H), 3.94 (s, 3H), 4.21 (t, J = 6 Hz, 2H), 7.34 (s, 1H), 7.52 (br s, 2H), 7.81 (br s, 2H), 8.42 (s, 1H), 9.56 (s, 1H); MS (ES) m/z 486.9, 488.9 (M + 1). Anal. ($C_{24}H_{24}Cl_2N_4O_3 \cdot 0.6H_2O$) C, H, N.

Alternate Preparation of 25 from 2y. A mixture of **2y** (500 mg, 1.15 mmol), morpholine (2.00 g, 23 mmol) and a catalytic amount of sodium iodide in 10 mL of ethylene glycol dimethyl ether was heated at reflux for 6 h. The reaction mixture was then poured into ice water and the pH was adjusted to 8−9 by the addition of saturated $NaHCO_3$. The solids were collected by filtration to provide 547 mg of crude product. A portion (240 mg) of this material was purified by flash column chromatography eluting with 30% MeOH in ethyl acetate to provide 127 mg of **25** as a white solid: mp 180−182 °C.

4-[(2,4-Dichlorophenyl)amino]-6-methoxy-7-[3-(4-methyl-1-piperazinyl)propoxy]-3-quinolinecarbonitrile (26). A mixture of **2y** (250 mg, 0.57 mmol), 1-methylpiperazine (1.5 mL, 13.5 mmol) and a catalytic amount of sodium iodide was heated at 100 °C for 3 days. The reaction mixture was cooled and partitioned between EtOAc and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by preparative thin-layer chromatography, eluting with 30% MeOH in CH_2Cl_2, to provide 95 mg (33%) of **26** as an off-white solid: mp 158−159 °C; ^{1}H NMR (DMSO-d_6) δ 1.92−1.98 (m, 2H), 2,16 (s, 3H), 2.27−2.53 (m, 10H), 3.93 (s, 3H), 4.19 (t, J = 6 Hz, 2H), 7.31 (s, 1H), 7.50 (br s, 2H), 7.80 (m, 2H), 8.41 (s, 1H), 9.56 (br s, 1H); MS (ES) m/z 500.1, 502.1 (M + 1). Anal. ($C_{25}H_{27}Cl_2N_5O_2 \cdot 1.0H_2O$) C, H, N.

4-[(2,4-Dichlorophenyl)amino]-7-[3-(4-hydroxy-1-piperidinyl)propoxy]-6-methoxy-3-quinolinecarbonitrile (27). A mixture of **2y** (300 mg, 0.70 mmol), 4-hydroxy-1-piperidine (93 mg, 0.92 mmol) and a catalytic amount of sodium iodide in 2 mL of ethylene glycol dimethyl ether was heated at 100 °C overnight. The reaction mixture was cooled and partitioned between EtOAc and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by preparative thin-layer chromatography eluting with 30% MeOH in CH_2Cl_2 to provide 105 mg (30%) of **27** as an off-white solid: mp 108−110 °C; ^{1}H NMR (DMSO-d_6) δ 1.32−1.44 (m, 2H), 1.68−1.75 (m, 2H), 1.90−2.09 (m, 4H), 2.43 (t, J = 7 Hz, 2H), 2.68−2.77 (m, 2H), 3.42 (m, 1H), 3.93 (s, 3H), 4.18 (t, J = 6 Hz, 2H), 4.52 (d, J = 4 Hz, 1H), 7.31 (s, 1H), 7.50 (br s, 2H), 7.80 (m, 2H), 8.40 (s, 1H), 9.55 (br s, 1H); MS (ES) m/z 501.1, 503.1 (M + 1). Anal. ($C_{25}H_{26}Cl_2N_4O_3 \cdot 1.0H_2O$) C, H, N.

Construction of Activated Src and Fyn Proteins. A PCR-based scheme was used to construct precise gene fusions to replace the catalytic domain of Prague C v-Src with the catalytic domains of c-Src and FynB. Briefly, the N-terminal 248 amino acids of v-Src was fused to threonine-250 in human c-Src, while a C-terminal fusion was created by joining alanine-517 of human c-Src to glutamine-515 of Prague C v-Src. This fusion thus has the v-Src SH3 and SH2 domains as well as the v-Src carboxyl terminal tail. Similarly, a v-Src/FynB fusion was created by an N-terminal fusion of threonine-250 in v-Src to methionine-251 in human FynB, and at the C-terminus by a junction of serine-518 (FynB) to glutamine-515 (v-Src). These kinases lack all negative regulatory elements in SFKs. (The details of these constructions will be provided on request.) The genes encoding these fusion proteins were cloned downstream of a GAL1/10 promoter in the yeast vector pRS316 and downstream of a tetracycline-repressible promoter in a mammalian expression vector (pTRE; Clontech).

Yeast Screen. The Src gene was cloned into pRS316,[44] modified by addition of an inducible GAL1/10 yeast promoter, and transformed into the yeast strain W303a. Src was toxic when expressed, i.e., when cells were grown in medium containing galactose. For the compound screen, cells were grown overnight in minimal medium (0.67% yeast nitrogen base, with amino acid and adenine supplements) containing the repressing carbon source glucose. 100 μL of this saturated culture was added to 1.2 L of liquefied 2% agar minus uracil minimal medium (50 °C) containing 0.5% galactose. The mixture was plated in Nunc Bioassay dishes (#240835). When the agar solidified, compounds were spotted onto the plates

(compounds from six 96-well plates onto one assay plate). Plates were then incubated at 30 °C for 3−5 days. Compounds that promoted growth were scored as hits.

Src Kinase Assay. Src kinase activity was measured in an ELISA format (Roche Diagnostics tyrosine kinase assay kit). Src (3 units/reaction; Upstate Biotechnologies), reaction buffer (50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 0.1 mM EGTA, 0.5 mM Na_3VO_4) and cdc2 substrate peptide were added to compound and incubated at 30 °C for 10 min. The reaction was started by the addition of ATP to a final concentration of 100 μM, incubated at 30 °C for 1 h and stopped by addition of EDTA. Instructions from the manufacturer were followed for subsequent steps. Compounds were tested in duplicate and the value given is an average of at least two determinations.

Cell Culture. Rat2 fibroblasts (ATCC CRL-1764) were cotransfected with pTet-Off (Clontech) and pTRE, pTRE Src, or pTRE Fyn. Stable cell lines were obtained by selection in G418. Highly transformed cells were selected by two sequential cloning steps in soft agar. The Src- and Fyn-transformed cells were morphologically distinct, exhibited anchorage independence and exhibited high levels of cellular protein phosphorylation on tyrosine.

Proliferation Assays. Cells grown on plastic (anchorage-dependent assays) were plated at a density of 1000 cells/well in 96-well dishes (Costar 3599) on day 0. Compound was added on day 1 and cells were incubated until day 4 when MTS reagent (Promega CellTiter 96) was added and optical density at 490 nm determined. Anchorage-independent (suspension) growth was measured in ultralow binding plates (Costar 3474) in a similar manner except that 5000 cells/well in a 96-well dish were seeded. We observed no cell attachment under these conditions. The parent Rat2 line grew poorly in the low-binding plates.

Protein Preparations. Equal numbers of cells were exposed to compound for 5 h to overnight. (At higher concentrations of compound (5 μM), the transformed morphology was completely reverted in about 1 h.) Cells were harvested after three washes with ice-cold phosphated-buffered saline pH 7.4 (PBS; Gibco) to which sodium orthovanadate (Fisher Scientific) was added to 0.5 mM. Lysates were prepared in ice-cold RIPA buffer (PBS with 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS)), supplemented with aprotinin and protease inhibitor cocktail (Calbiochem). Lysates were clarified by centrifugation (20 min at 14 000 rpm in an Eppendorf 5417R microcentrifuge), and then frozen in dry ice/ethanol. Lysates from $\sim 4 \times 10^6$ cells were diluted 1:10 in Laemmli buffer (BioRad) and analyzed by SDS−PAGE. Antibodies to phosphotyrosine (4G10) and Src (GD11) were obtained from Upstate Biotechnologies, and antibody to actin was obtained from Boehringer Mannheim (Roche Diagnostic).

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Supporting Information Available: Elemental analysis data for compounds **2a**−**y**, **3**, **4g**, **6**, **8**−**13**, **15**−**21**, **23**−**27**. This material is available free of charge via the Internet at http://pubs.acs.org.

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