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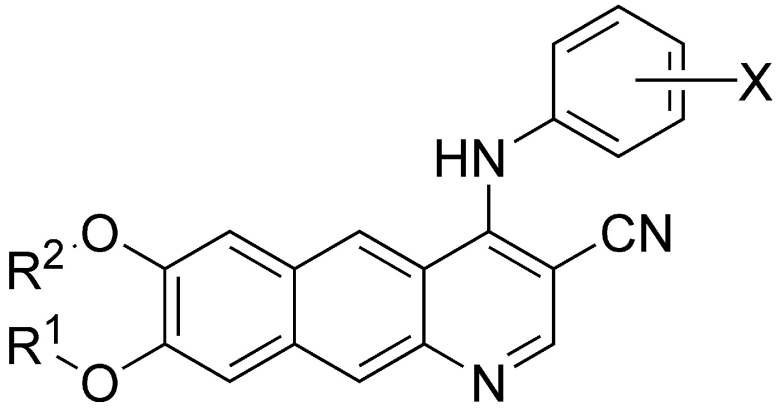

$R^1 = CH_3$, $R^2 = -(CH_2)_2$-NRR

$R^1 = -(CH_2)_2$-NRR, $R^2 = CH_3$

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4-Anilino-7,8-dialkoxybenzo[*g*]quinoline-3-carbonitriles as Potent Src Kinase Inhibitors

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It has been previously reported that appropriately substituted 4-anilinoquinoline-3-carbonitriles are potent inhibitors of Src kinase, with biological activity in vitro and in vivo. Structural modifications to these compounds have been explored, providing the 4-anilinobenzo[*g*]quinoline-3-carbonitriles as a series with enhanced Src inhibitory properties. The synthesis and structure−activity relationships of these 4-anilino-7,8-dialkoxybenzo[*g*]quinoline-3-carbonitriles are presented here. Analogues with cyclic basic amine groups attached via ethoxy linkages at the C-8 position were the most active in vitro, with subnanomolar IC_{50} values against Src kinase observed for a majority of the compounds synthesized. Compound **17d** was more potent in vitro than the analogously substituted 4-anilinoquinoline-3-carbonitrile SKI-606, which is undergoing evaluation in clinical trials. The most potent analogue synthesized was **17a**, with an IC_{50} of 0.15 nM against Src kinase and with an IC_{50} of 10 nM against Src-transformed fibroblasts. Molecular modeling studies provided a rationale for the exceptional activity observed for these compounds, with favorable van der Waals interactions playing the major role. Compound **17c** was found to be highly selective for Src kinase when tested against a panel of other kinases, with modest selectivity versus the Src family kinases Lyn and Fyn. Following ip dosing at 50 mg/kg, analogues **17c** and **17d** were shown to have plasma levels that significantly exceeded the cellular IC_{50} values against Src-transformed fibroblasts. In an Src-transformed fibroblast xenograft model, both compounds exhibited a significant inhibition of tumor growth.

Introduction

Protein tyrosine kinases (TKs) catalyze the phosphorylation of a tyrosine residue on a substrate protein, a process important for cell growth and differentiation. Src kinase is a member of a structurally homologous group of nonreceptor TKs present in the cytoplasm known as the Src family of kinases.[1] Src itself participates in signaling pathways controlling proliferation, differentiation, and migration.[2] It has been demonstrated that Src is overexpressed or constitutively active in a variety of human tumors, including those derived from colon, breast, pancreas, liver, brain, and bladder.[3] Inhibition of Src kinase has been shown to decrease the hypoxic induction of VEGF, a protein that is upregulated in angiogenesis.[4a,b] Thus, the evidence suggests that the inhibition of Src activity may prove to be useful for therapeutic intervention in cancer. Other studies have provided evidence that Src inhibition might be effective in the treatment of other diseases, such as osteoporosis[5a,b] and stroke.[6]

Efforts by researchers to discover small-molecule inhibitors of Src kinase have generated several classes of structures as potent leads, including purines,[7] pyrido[2,3-*d*] pyrimidines,[8] pyrrolo[2,3-*d*]pyrimidines,[9a−e] pyrazolo[2,3-*d*]pyrimidines,[9e,10] indolin-2-ones,[11] 2-methylpyrimidin-4-ylamino)thiazole-5-carboxamides,[12a,b] 4-anilino-5,10-dihydropyrimido[4,5-*b*]quinolines,[13] 4-anilinoquinazolines,[14] and 4-anilino-3-quinolinecarbonitriles.[15a−j]

Optimization of a series of 6,7-dialkoxy substituted 4-anilino-3-quinolinecarbonitriles provided SKI-606 as a potent inhibitor of Src kinase, with oral activity in xenograft models.[15c,g] Modifications of the 3-quinolinecarbonitrile core were explored to provide 8-anilinoimidazo[4,5-*g*]quinoline-7-carbonitriles,[16] 4-anilinobenzothieno[3,2-*b*]pyridine-3-carbonitriles and 4-anilinobenzofuro[3,2-*b*]pyridine-3-carbonitriles,[17] 4-anilinobenzo[*b*]-naphthyridine-3-carbonitriles,[18] 4-anilinobenzo[*g*]quinoline-3-carbonitriles,[19] and 7-anilinothieno[3,2-*b*]pyridine-6-carbonitriles[20a,b] as Src kinase inhibitors. Of these, the most potent were the 4-anilinobenzo[*g*]quinoline-3-carbonitriles with 7,8-dimethoxy substituents. The 7,8-dimethoxy substituted compound **1** (Chart 1) was more active in Src enzyme and cellular assays than the correspondingly substituted quinoline-3-carbonitrile.[19] We set out to further improve the solubility and activity of these 4-anilinobenzo[*g*]quinoline-3-carbonitriles by adding cyclic basic amines via the C-7 or C-8 positions. Preliminary presentations have highlighted the exceptional activity of several of these analogues against Src kinase.[21a−c]

The present work describes the synthesis and detailed SARs of these 4-anilino-7,8-dialkoxybenzo[*g*]quinoline-3-carbonitriles. Structurally, they are described by the general formula **2** shown in Chart 1.

Chemistry

In our initial synthesis of the target compounds, we chose a pathway that would provide the compounds as

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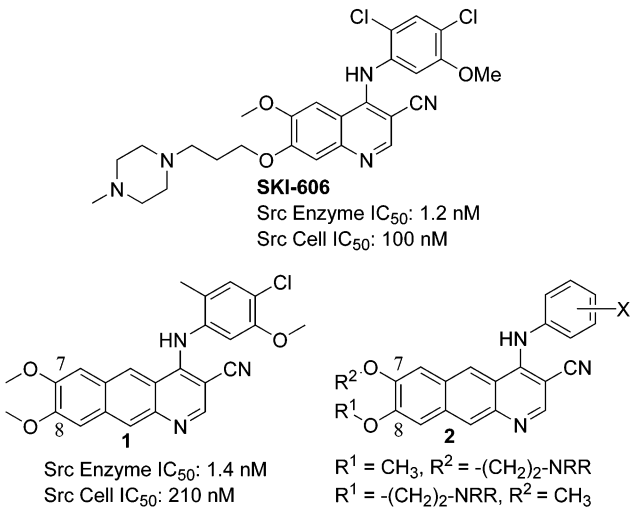
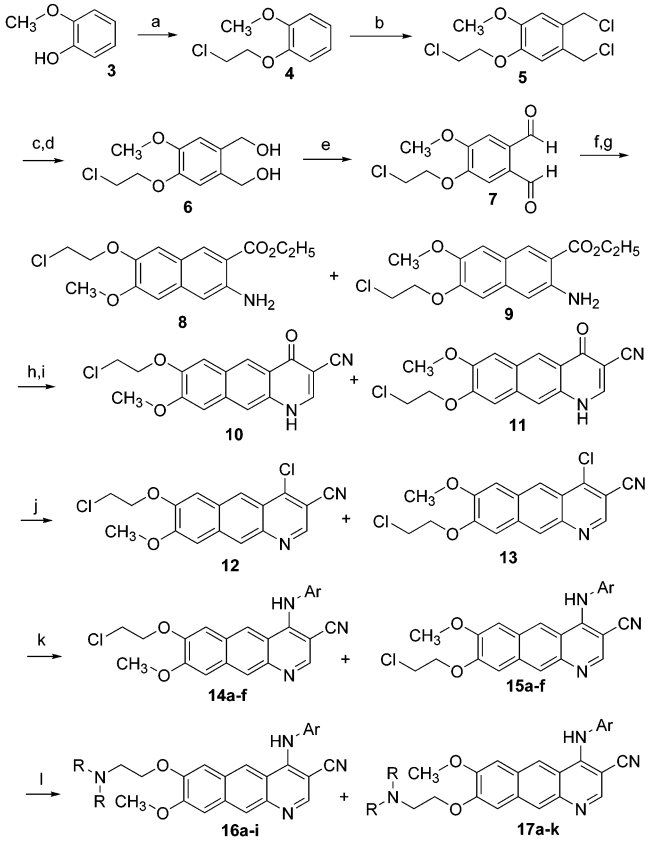
Chart 1



a regioisomeric mixture at the C-7 and C-8 positions. It was anticipated that each product mixture would be separable by chromatographic methods to provide two analogues for biological evaluation. On the basis of the fact that water-solubilizing substituents at the C-7 position of the 4-anilinoquinoline-3-carbonitriles provide optimal in vitro activity,[15b] it seemed likely that the C-8 position would similarly be optimal for the 4-anilinobenzo[*g*]quinoline-3-carbonitriles. However, we decided to confirm this before embarking on the challenge of carrying out a regioselective synthesis of the target compounds. To compensate for the larger benzo[*g*]-quinoline-3-carbonitrile core, we chose to attach the cyclic amine substituents via ethoxy linkers rather than the propoxy linkers generally used for the quinoline-3-carbonitrile scaffold.

The general method by which most of the 7,8-substituted 4-anilinobenzo[*g*]quinoline-3-carbonitriles were synthesized is outlined in Scheme 1. The reaction of 2-methoxyphenol **3** with 2-chloroethyl *p*-toluenesulfonate provided 1-(2-chloroethoxy)-2-methoxybenzene **4**. On heating with formaldehyde and hydrochloric acid (gas), **4** was converted to the 4,5-dichloromethyl substituted intermediate **5**, which on treatment with sodium acetate in acetic acid and subsequent hydrolysis with ammonia-saturated methanol gave **6**. Swern oxidation of the diol **6** yielded phthalaldehyde **7**. Reaction of **7** with an excess of ethyl 3-nitropropanoate and sodium ethoxide in ethanol provided an inseparable mixture of 3-nitro-2-naphthoate[22] intermediates, which were reduced by catalytic hydrogenation to provide 3-amino-2-naphthoates **8** and **9** (approximately 1:1 mixture). Synthesis of the 3-nitro-2-naphthoate intermediates was generally carried out on a small scale (<3 g) because larger scale reactions occasionally failed to provide the desired products, instead resulting in the complete decomposition of starting materials. Compounds **8** and **9** were heated in dimethylformamide dimethylacetal to provide the corresponding dimethylaminomethyleneamine intermediates, which were immediately reacted with the anion of acetonitrile to provide benzoquinoline-3-carbonitriles **10** and **11**.[15b] Chlorination of **10** and **11** was carried out with phosphorus oxychloride to yield the 4-chloro substituted intermediates **12** and **13**. Compounds **14** and **15** were

Scheme 1. Preparation of **16a**−**i** (Table 2) and **17a**−**k** (Table 3)[a]



[a] Reagents and reaction conditions: (a) chloroethyl tosylate, K_2CO_3, 2-butanone, reflux, 2 days; (b) formaldehyde, ether, HCl gas addition, 0 °C, 9 h, room temperature, 16 h; (c) NaOAc, acetic acid, reflux, 2 h; (d) NH_3, MeOH, 0−5 °C, 15 h; (e) oxalyl chloride, DMSO, CH_2Cl_2, −78 °C, 30 min, then NEt_3, 10 min; (f) NaOEt, $O_2NCH_2CH_2CO_2Et$, EtOH, 0−5 °C, 10 min, then room temperature, 16 h; (g) H_2 (Parr, 50 lb/in^2), DMF, 2 h; (h) DMF-dimethyl acetal, reflux, 16 h; (i) CH_3CN, *n*-BuLi, THF, −78 °C, 30 min; (j) $POCl_3$, reflux, 20 min; (k) $ArNH_2$, pyridine−HCl, 2-ethoxyethanol, 135 °C, 30 min to 1 h; (l) RRNH, NaI, neat, reflux 30 min, or in $CH_3OCH_2CH_2OCH_3$, reflux, 3.5 h to 4 days.

obtained by heating **12** and **13** with the appropriate aniline[15c] and pyridine hydrochloride in 2-ethoxyethanol. The final products **16** and **17** were obtained following addition of the cyclic basic amines at the C-7 and C-8 positions. These compounds all proved to be readily separable by silica gel chromatography (CH_2Cl_2/MeOH or EtOAc/MeOH). In every case, analogues **17** were the more polar of the two isomers. For selected analogues, two-dimensional rotating-frame Overhauser enhancement spectroscopy (ROESY) NMR data were obtained on the isolated compounds to confirm the structural assignments. While this work was in progress, a 12-step reaction sequence was elucidated to regioselectively provide compound **17c**.[23] This chemistry was utilized for the synthesis of **17c** and **17j** as further confirmation that the structures of the separated isomers had been correctly assigned, as well as providing sufficient quantities of **17c** for in vivo studies.

Molecular Modeling

To gain insight on how the binding of the 4-anilinobenzo[*g*]quinoline-3-carbonitriles to Src kinase would compare to the 4-anilinoquinoline-3-carbonitriles, mo-

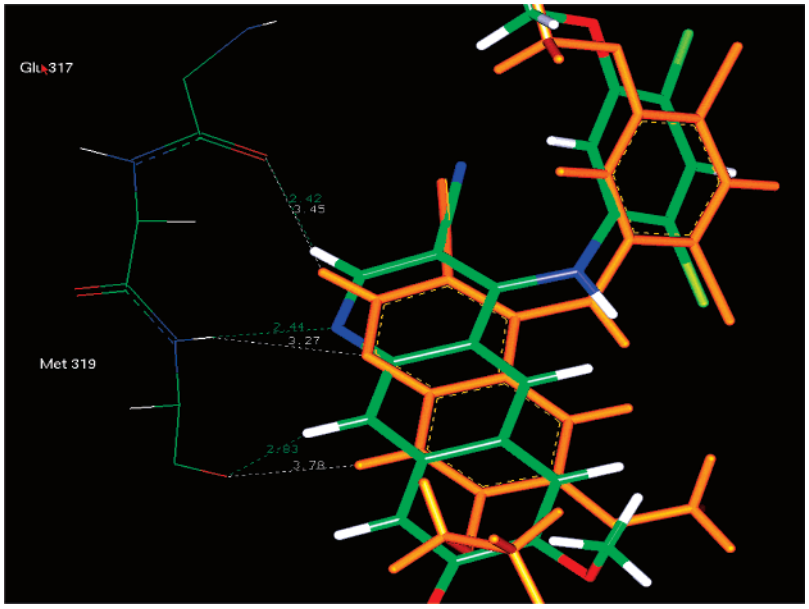


Figure 1. Binding orientations of **17d** (atoms are colored by atom type with the carbon atoms colored green) and SKI-606 (atoms are colored orange) to the hinge region within the ATP-binding site of Src kinase.

Table 1. Calculated Binding Energies of **17d** and SKI-606

structure	total energy (kcal)	screened electrostatic energy (kcal) (Coulomb + solvation)	hydrophobic energy (kcal)	van der Waals energy (kcal)	rotational penalty (kcal)
17d	−28.0	3.6	−8.2	−29.0	5.6
SKI-606	−24.6	4.5	−7.8	−27.6	6.3

lecular modeling studies were carried out using a homology model (a crystal structure of the active form of c-Src was not available) based on the active form of the Lck kinase domain (PDB ID: 1QPE). Compounds **17d** and SKI-606 were chosen as representative examples possessing the same aniline and cyclic amine substituents. The scoring function utilized (described in the Experimental Section) consists of a Poisson−Boltzmann based treatment of the electrostatic interactions in the protein−ligand complex. The binding mode presented here is selected on the basis of binding interactions that result in optimal energetics as given in Table 1. The screened electrostatics term in this model measures the gain in favorable electrostatic interactions between protein and ligand and includes a penalty for buried polar groups due to the cost of removing the ligand from solvent. The hydrophobic term is the energetic reward for the buried hydrophobic interactions. With tighter protein−ligand binding, a greater amount of surface area is expected to be buried away from solvent, making the interaction more energetically favorable. A rotational penalty term is computed as follows: (0.7)(number of rotatable bonds). This is an approximate measure of the internal coordinate entropy of the ligand.

The largest differences in energy metrics for **17d** and SKI-606 were apparent in the screened electrostatics and the van der Waals terms. Notably, the gain in van der Waals contributions was more significant than the corresponding gain in the screened electrostatics. That is, a greater overall contribution from favorable nonbonded interactions between protein and ligand was predicted for **17d** than for SKI-606.

The corresponding binding orientations for the two ligands are shown in Figure 1. This showed that the more favorable electrostatic energy could be associated with the closer approach of the **17d** core (versus the SKI-606 core) with the protein's hinge region residues. Favorable interactions between the core ring nitrogen and the backbone NH of Met 319 were significant in our model. Additionally, the more optimal interaction of the Tyr 318 ring with the core of **17d** enhanced the van der Waals energy term for the interaction of the protein−ligand complex. In the low-energy pose shown (Figure 2), the center aromatic ring of the tricyclic core approached the Tyr 318 ring to within 3.5 Å. In contrast, the closest approach of the bicyclic core of SKI-606 to the Tyr 318 ring was 4.0 Å. Allowing for the movement of both protein and ligand, a greater area of aromatic surface on the tricyclic core was available for interaction with the Tyr 318 aromatic ring in comparison with the bicyclic core of SKI-606. Last, a slight enhancement of binding energy was afforded by the smaller rotational penalty assigned to the less flexible C-8 substituent of ligand **17d**.

Biological Results

SAR for the Src Kinase and Src Cellular Assays. The inhibitory activity of compounds **16a**−**i** (Table 2) and **17a**−**m** (Table 3) against Src kinase was measured using an enzyme linked immunosorbent assay (ELISA).[15b] A Src-transformed fibroblast line was used to measure antiproliferative cellular activity.[15b] A clear trend immediately observed from the data was that the analogues bearing cyclic basic amine groups attached via ethoxy linkers at C-8 (compounds **17**) were significantly more potent Src kinase inhibitors than analogous compounds with cyclic amines attached at the C-7 position (compounds **16**). Furthermore, compounds **17** were all exceptionally active, with the majority of the

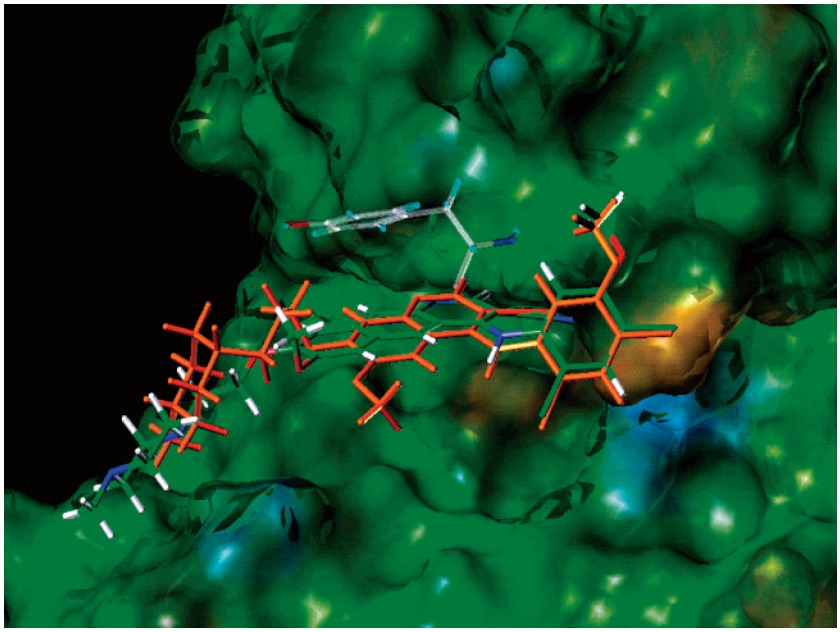
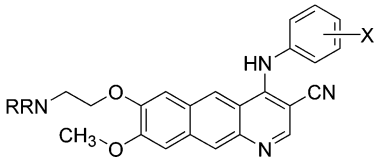


Figure 2. van der Waals surface view of Src kinase domain with SKI-606 (all atoms are colored orange) and **17d** (atoms are colored by atom type with the carbon atoms colored green) docked. The surface is shown in translucent mode such that the location of the Tyr 318 side chain is apparent. The atoms of the Tyr residue are colored by atom type with the carbon atoms colored white.

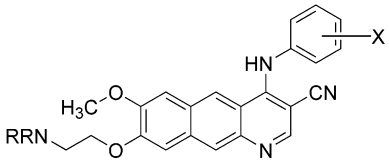
Table 2. Inhibition of Src Kinase Activity and Cell Proliferation by SKI-606, **1**, and 4-Anilinobenzo[*g*]quinoline-3-carbonitriles Substituted with Cyclic Basic Amine Groups Attached at C-7 (**16a**−**i**)[a]



compd	RRN	X	IC_{50} (nM) Src ELISA	IC_{50} (nM) Src cell
SKI-606			1.2	100
1			1.4	210
16a	morpholine	2-CH_3, 4-Cl, 5-OCH_3	2.1	71
16b	4-methylpiperazine	2-CH_3, 4-Cl, 5-OCH_3	1.5	220
16c	morpholine	2,4-diCl, 5-OCH_3	1.8	230
16d	4-methylpiperazine	2,4-diCl, 5-OCH_3	1.1	260[b]
16e	4-hydroxypiperidine	2,4-diCl, 5-OCH_3	2.1	1600
16f	morpholine	2-Cl, 4-CH_3, 5-OCH_3	2.5	110
16g	4-methylpiperazine	3,4,5-triOCH_3	2.6	2000
16h	morpholine	3,4,5-triOCH_3	1.2	180
16i	morpholine	2,4-diCl	13	970

[a] IC_{50} values reported represent the mean of at least two separate determinations. [b] IC_{50} values are from a single determination.

Table 3. Inhibition of Src Kinase Activity and Cell Proliferation by 4-Anilinobenzo[*g*]quinoline-3-carbonitriles Substituted with Cyclic Basic Amine Groups Attached at C-8 (**17a**−**k**)[a]



compd	NRR	X	IC_{50} (nM) Src ELISA	IC_{50} (nM) Src cell
17a	morpholine	2-CH_3, 4-Cl, 5-OCH_3	0.15	10
17b	4-methylpiperazine	2-CH_3, 4-Cl, 5-OCH_3	0.31	42
17c	morpholine	2,4-diCl, 5-OCH_3	0.46	37
17d	4-methylpiperazine	2,4-diCl, 5-OCH_3	0.29	61
17e	4-hydroxypiperidine	2,4-diCl, 5-OCH_3	0.72	350
17f	morpholine	2-Cl, 4-CH_3, 5-OCH_3	0.33	19
17g	4-hydroxypiperidine	2-Cl, 4-CH_3, 5-OCH_3	0.55	28
17h	morpholine	2-Cl, 4-F, 5-OCH_3	0.48	22
17i	4-methylpiperazine	3,4,5-triOCH_3	0.69	270
17j	morpholine	3,4,5-triOCH_3	0.22[b]	50
17k	morpholine	2,4-diCl	2.0	48

[a] IC_{50} values reported represent the mean of at least two separate determinations. [b] IC_{50} values are from a single determination.

synthesized analogues having subnanomolar activity against Src kinase and having exceptional cellular activity as well (IC_{50} < 100 nM). Overall, these compounds were more potent than the correspondingly substituted 4-anilinoquinoline-3-carbonitriles.[15a−c] For example, analogue **17d** was more potent in vitro than SKI-606, the analogously substituted 3-quinolinecarbonitrile. As analogously observed with the 4-anilinoquinoline-3-carbonitriles, basic amine groups attached to C-8 of the benzoquinoline template enhanced both enzyme and cellular activity. Compounds **17a** and **17b** were more potent against Src kinase and Src-transformed fibroblasts than the 7,8-dimethoxy substituted **1**, while **16a** and **16b** had overall activity comparable to that of **1**.

The synthesized analogues utilized substituted anilines that were previously explored in SAR studies of the 4-anilinoquinoline-3-carbonitriles.[15a,c,f] The differences between the activities of these analogues within a series were relatively small, although the 2-methyl-4-chloro-5-methoxyaniline group of **17a** appeared to provide the best activity (Src enzyme IC_{50} = 0.15 nM; Src cell IC_{50} = 10 nM). The 2,4,5-trisubstituted anilines and the 3,4,5-trimethoxyaniline groups appeared to provide comparable activity, with significantly better activity against Src kinase than the corresponding 2,4-dichloro substituted analogues **16i** and **17k**. Of the cyclic basic

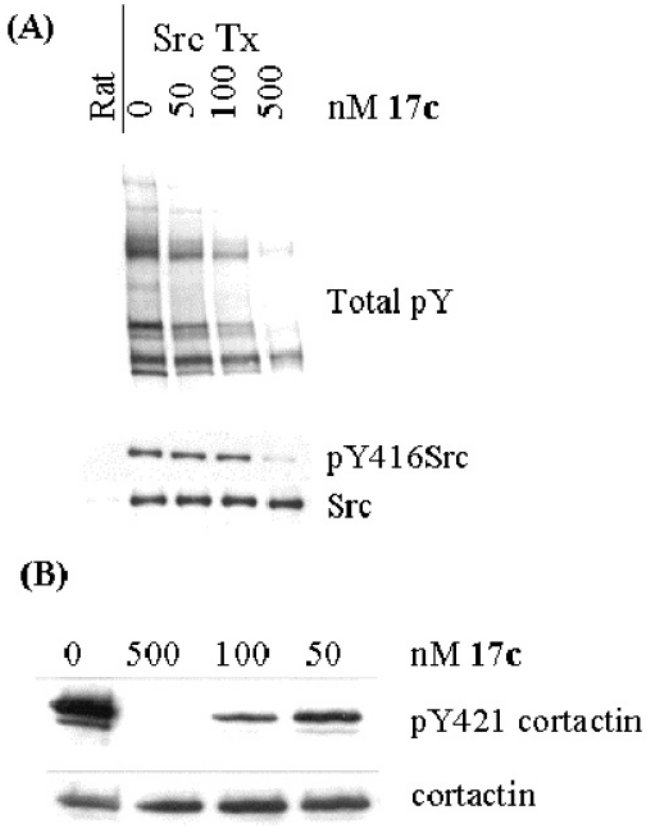


Figure 3. Src-transformed rat fibroblasts were treated with **17c** for 4 h at the indicated concentrations. (A) Blots of whole cell lysates were probed with antibodies to phosphotyrosine (top), pY416 Src (middle), and Src (bottom). The lane labeled "Rat" has cell lysate from untransformed rat fibroblasts. (B) Blots of whole cell lysates probed with antibody to pY421 cortactin (top) or cortactin (bottom).

amine groups that were explored, morpholine provided better cellular potency (**16a**, **16c**, **16h**, **17a**, **17c**, **17f**, **17j**) than the 4-methylpiperazine or the 4-hydroxypiperidine substituted analogues.

Inhibition of Src-Dependent Tyrosine Protein Phosphorylation. Compound **17c**, which possesses the 2,4-dichloro-5-methoxyanilino C-4 substituent of SKI-606, was selected as a representative analogue for further evaluation on cellular phosphorylation of Src-dependent proteins in Src-transformed fibroblasts. Immunoblots of whole cell extracts from cells treated with **17c** were probed with phosphotyrosine antibodies. As shown in Figure 3A, **17c** reduced tyrosine phosphorylation of many cellular proteins with a clear dose response over a concentration range of 50−500 nM. To examine the effects of **17c** treatment on a specific Src substrate protein, we examined tyrosine phosphorylation of cortactin (Figure 3B), whose phosphorylation was also reduced by treatment with 50−500 nM **17c**. In contrast, the autophosphorylation of Src was diminished at relatively high concentrations of this compound. Similar observations were obtained with SKI-606.[15c,24] These studies demonstrated that **17c** inhibited Src-dependent tyrosine phosphorylation of cellular proteins at concentrations that correlated with inhibition of anchorage-independent growth.

Kinase Selectivity. Analogue **17c** was found to be selective for Src versus several other kinases, including ErbB-2 (IC_{50} = 2.5 μM), EGFr (IC_{50} = 2.7 μM), cdk4 (IC_{50} > 10 μM), MK2 (IC_{50} > 20 μM), AKT (IC_{50} > 10 μM), and IKK (no inhibition at 3.6 μM). Other kinase studies were carried out in cell-based systems with rat fibroblasts.[15b,g] In Abl transformed rat fibroblasts, **17c** was found to have modest activity, with an IC_{50} of 0.49 μM.[21b] In contrast, SKI-606 was a more potent inhibitor, with an IC_{50} of 0.09 μM. As was observed for SKI-606, **17c** demonstrated good selectivity versus non-Src family kinases but modest selectivity versus selected Src family kinases. Thus, for example, **17c** had IC_{50} values of 0.14 μM versus Lyn-transformed fibroblasts and 0.42 μM versus Fyn-transformed fibroblasts, respectively. This represents 3.8-fold and 11.4-fold selectivity of **17c** for Src over Lyn and Fyn in the cellular proliferation assays.

Additional Cellular Assays. The antiproliferative activities of compounds **17c** and **17d** were determined using HT29 cells, whose growth in vivo is reduced by administration of SKI-606[15c] and antisense Src.[25] When the HT29 cells were grown on plastic, little inhibition was observed at concentrations lower than 5 μM for both compounds. A similar result was observed when **17c** and **17d** were tested against Colo205 cells.[24] However, submicromolar concentrations of **17c** and **17d** were sufficient to significantly decrease HT29 cellular proliferation on soft agar (Figure 4). This activity mirrors that observed with SKI-606.[15c]

To determine the effect of **17c** on Src activity in human colon tumor cells, we examined the effect of **17c** on tyrosine phosphorylation of focal adhesion kinase (FAK) in Colo205 cells. FAK is phosphorylated by Src on a number of tyrosines, including Y925. As shown in Figure 5, phosphorylation of FAK on Y925 in Colo205 colon tumor cells was reduced in a dose-dependent manner by **17c** at 100, 500, and 1000 nM, consistent with the inhibition of Src activity observed in fibroblasts.

Plasma Level Determination. To determine whether **17c** and **17d** would have sufficient exposure levels to warrant investigation of their antitumor properties in vivo, the plasma levels of both compounds were determined. The mice were dosed ip at 50 mg/kg with each compound, and plasma levels were measured at four time points (Table 4). The results show that both compounds had substantial blood levels up to 24 h after dosing, with **17c** initially showing higher exposure levels than **17d** but a more rapid drop in plasma concentration over time. Both compounds had sustained plasma levels that significantly exceeded the IC_{50} for Src-transformed cellular proliferation for at least 8 h and were therefore evaluated in vivo.

Xenograft Studies. Src inhibitors **17c** and **17d** were evaluated in xenograft models employing Src-transformed fibroblasts.[15c] When these compounds were administered daily at 50 mg/kg ip from day 0 to day 7, significant tumor growth inhibition was observed (Figure 6). The two compounds showed similar efficacy, with a *T*/*C* of 25% for **17c** and a *T*/*C* of 20% for **17d** at day 7. While significantly reducing tumor growth, some abdominal bloating and moderate weight loss (∼15%) was observed with this dosing regimen.

Conclusion

On the basis of the activities observed with modified 4-anilinoquinoline-3-carbonitrile cores, the 4-anilinoben-

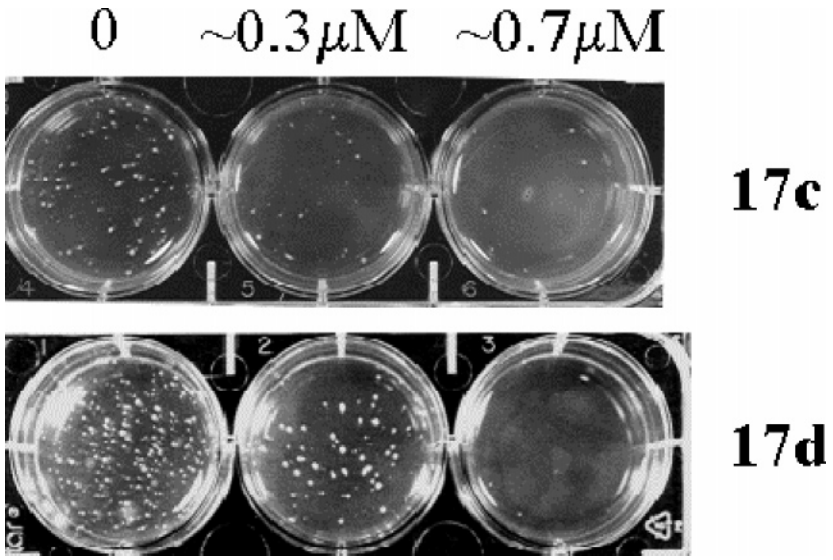


Figure 4. Inhibition of HT29 colony formation in soft agar by **17c** and **17d**. HT29 tumor cells were suspended in a 0.4% agar-medium mix and plated onto a 0.7% hard agar-medium underlay. After the top layer had solidified, medium containing compound was added such that the final equilibrium concentration of compound was the indicated value. Fresh medium was added every week. This photograph was taken 4 weeks after plating.

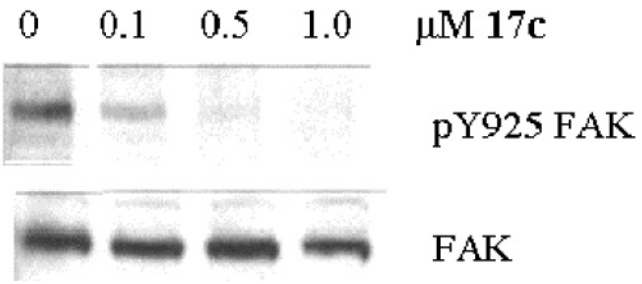


Figure 5. Phosphorylation of FAK on Y925 in Colo205 cells is diminished by **17c**. Colo205 cells were treated with **17c** for 4 h at the indicated concentration. Blots of whole cell lysates were probed with antibody to pY925 FAK (top) or FAK (bottom).

Table 4. Nude Mouse Plasma Levels Following a Single Dose of 50 mg/kg ip

compd	0.5 h (μg/mL)	4 h (μg/mL)	8 h (μg/mL)	24 h (μg/mL)
17c	2.1 ± 0.7	1.9 ± 1.3	0.3 ± 0.2	0.02 ± 0.02
17d	1.3 ± 0.5	0.63 ± 0.06	0.70 ± 0.08	0.24 ± 0.09

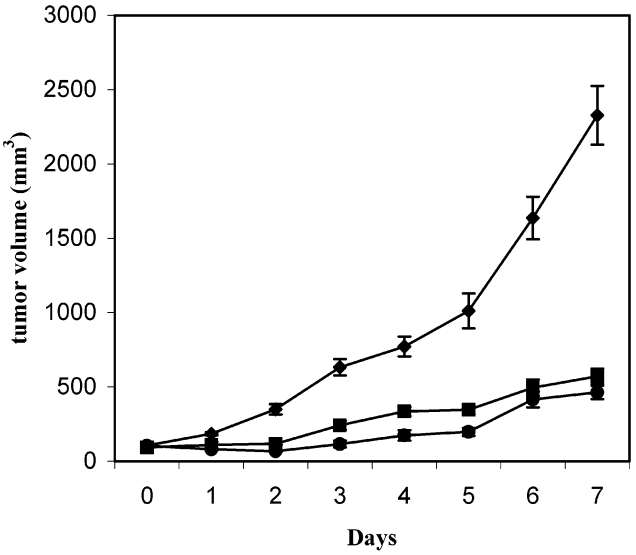


Figure 6. Inhibition of Src-transformed fibroblast tumor growth in nude mice by **17c** (■) and **17d** (●) administered at 50 mg/kg ip in 2% Tween-80 in 5% dextrose/water once a day versus vehicle (◆) alone, at days 0−7.

zo[*g*]quinoline-3-carbonitrileswere chosen as the most promising series to optimize further. We have demonstrated here that adding the appropriate water-solubilizing substituents to these tricyclic benzo[*g*]quinolinecarbonitriles provided a series of analogues with exceptional potency as Src kinase inhibitors. This represents the only core modification we have explored that resulted in a series with greater activity than the similarly substituted 6,7-dialkoxy-4-anilinoquinoline-3-carbonitriles. A 12-step reaction sequence provided target compounds **16** and **17** as a separable mixture of products. Variation at the C-4 and C-7/C-8 positions was introduced in the final two steps from advanced intermediates **12** and **13**.

As anticipated, the SAR of the tricyclic series largely mirrored that of the 4-anilinoquinoline-3-carbonitrile compounds. Thus, the C-8 position proved to be optimal for the attachment of a cyclic basic amine group. As was observed for the 4-anilinoquinoline-3-carbonitriles, cyclic amines (**17a** and **17b**) enhanced both enzyme and cellular activity versus an analogue possessing a methoxy group at C-8 (**1**). While compounds with several 2,4,5-substituted anilines at the C-4 position provided similar activity to those possessing the 3,4,5-trimethoxyaniline moieties, these were all more active than the 2,4-dichloroaniline substituted **16i** and **17k**.

Molecular modeling studies indicated that two modes of protein−ligand interactions, namely, nonbonded and electrostatic interactions, appear to be the major contributors to differences in the binding of **17d** and SKI-606 with the Src protein pocket. Since our computational studies indicated that the van der Waals energy metrics were more discriminating, we attributed the enhanced potency of **17d** primarily to the more optimal van der Waals protein−ligand interactions. Additionally, in structure-based studies, it appeared that the more flexible tailpiece at the 7-position of SKI-606 was often engaged in favorable intramolecular nonbonded

interactions, while the corresponding tailpiece of **17d** was more significantly involved in favorable nonbonded interactions with the nearby protein residues. Taking these factors into account, these studies provide a clear rationale for the strong binding of analogues such as **17d** to Src kinase.

Because **17c** and **17d** demonstrated potent cell activity and good blood exposures on ip dosing at 50 mg/kg, we carried out in vivo studies against Src-transformed fibroblasts in nude mice. Both analogues showed good activity in this model when dosed daily at 50 mg/kg (*T*/*C* of 25% and 20% at day 7, respectively). We have therefore shown that these compounds have sufficient bioavailability when dosed ip to provide significant in vivo activity at 50 mg/kg. In addition to having potent biological activity, these 4-anilinobenzo[*g*]quinolinecarbonitriles have provided important structural information with regard to discovering compounds with enhanced binding to Src kinase.

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 determined at 400 MHz on a DRX-400 spectrometer or at 300 MHz on an NT-300 WB spectrometer, with chemical shifts (δ) reported in parts per million referenced to Me_4Si. Electrospray (ES) mass spectra were recorded in positive mode on a Micromass Platform or an LCT spectrometer. Electron impact (EI) mass spectra were obtained on a Finnigan MAT-90 spectrometer. All reagents and solvents obtained from commercial suppliers were used without further purification. Reactions were carried out under nitrogen as an inert atmosphere. Flash chromatography was carried out with Baker 40 μM silica gel.

1-(2-Chloroethoxy)-2-methoxybenzene (4). A mixture of 52.88 g (0.426 mol) of guaiacol, 100 g (0.426 mol) of chloroethyl tosylate, 88.3 g (0.639 mol) of powdered potassium carbonate, and 600 mL of 2-butanone was stirred mechanically and heated at reflux for 2 days. The mixture was filtered, and the solid was rinsed with 2-butanone. After evaporation of the filtrate, the residue was taken up in ether and washed with 1 N NaOH to remove unreacted guaiacol. The ether layer was dried over sodium sulfate, filtered, and evaporated to give an oil that slowly crystallized. Isolation from cold cyclohexane gave 41.47 g (52%) of **4** as a white solid, mp 42−43 °C. ^{1}H NMR ($CDCl_3$): δ 6.85−7.02 (m, 4H), 4.28 (t, J = 6.3 Hz, 2H), 3.87 (s, 3H), 3.84 (t, J = 6.3 Hz, 2H). MS, *m*/*z*: 187.4 $(M + H)^+$. Anal. ($C_9H_{11}ClO_2$) C, H.

1-(2-Chloroethoxy)-4,5-bis(chloromethyl)-2-methoxybenzene (5). To a solution of 55.99 g (300 mmol) of **4** in 250 mL of 1,4-dioxane was added 40 mL of concentrated hydrochloric acid while stirring at 0 °C. While HCl gas was bubbled in, 30 mL of 35% formalin was added. After 45 min, another equal volume of formalin was added. The addition of HCl gas was continued for 6 h, and the ice bath was removed after 2 h and allowed to warm to ambient temperature. The reaction mixture was stirred overnight at ambient temperature. The green reaction mixture was then cooled in an ice bath, and the resulting solid was filtered and washed with cold dioxane/water (2.5:1). Silica gel chromatography of the crude solid, eluting with 2:1 hexanes/dichloromethane, provided 36.35 g (42%) of **5** as a white solid, mp 117−118 °C. ^{1}H NMR ($CDCl_3$): δ 6.92 (s, 1H), 6.91 (s, 1H), 4.70 (s, 2H), 4.69 (s, 2H), 4.29 (t, J = 6.2 Hz, 2H), 3.90 (s, 3H), 3.84 (t, J = 6.2 Hz, 2H). MS, *m*/*z*: 282.0 $(M + H)^+$. Anal. ($C_{11}H_{13}Cl_3O_2$) C, H.

[4-(2-Chloroethoxy)-2-(hydroxymethyl)-5-methoxyphenyl]methanol (6). To a solution of 5.67 g (20 mmol) of **5** in 75 mL of acetic acid was added a solution of 3.5 g of anhydrous sodium acetate (42.7 mmol) in 100 mL of acetic acid. This mixture was refluxed with stirring for 2 h. Solids were removed by filtration and washed with acetic acid. The filtrate was evaporated to approximately 30 mL, then poured into water and extracted with ether. The organic phase was washed with aqueous sodium carbonate, water, and brine. After drying over sodium sulfate, the solution was filtered and evaporated to give 5.69 g (86%) of 2-[(acetyloxy)methyl]-4-(2-chloroethoxy)-5-methoxybenzyl acetate as a white solid, mp 79−80 °C. ^{1}H NMR ($CDCl_3$): δ 6.96 (s, 1H), 6.94 (s, 1H), 5.14 (s, 2H), 5.12 (s, 2H), 4.29 (t, J = 6.2 Hz, 2H), 3.89 (s, 3H), 3.84 (t, J = 6.2 Hz, 2H), 2.09 (s, 3H), 2.08 (s, 3H). MS (EI), *m*/*z*: 329.72 (M^+). Anal. ($C_{15}H_{19}ClO_6$) C, H.

A solution of 14.0 g of the 2-[(acetyloxy)methyl]-4-(2-chloroethoxy)-5-methoxybenzyl acetate (42.3 mmol) in 600 mL of methanol was stirred and cooled in an ice bath while ammonia gas was bubbled in, until the solution was saturated. The flask was stoppered and stored in the refrigerator for 15 h. The reaction mixture was evaporated to give a white solid that was dried and chromatographed on a silica gel column, eluting with 2:1 hexanes/ethyl acetate, to give 9.87 g (95%) of **6** as a white solid, mp 93−94 °C. ^{1}H NMR ($CDCl_3$): δ 6.94 (s, 1H), 6.93 (s, 1H), 4.68 (br s, 4H), 4.29 (t, J = 6.2 Hz, 2H), 3.88 (s, 3H), 3.83 (t, J = 6.2 Hz, 2H), 2.77 (br s, 1H), 2.71 (br s,1H). MS, *m*/*z*: 264.10 $(M + NH_4)^+$. Anal. ($C_{11}H_{15}ClO_4$) C, H.

4-(2-Chloroethoxy)-5-methoxyphthalaldehyde (7). To a 500 mL three-neck round-bottom flask fitted with mechanical stirrer, thermometer, and addition funnel was added 100 mL of dry methylene chloride and 8 mL (91.7 mmol) of oxalyl chloride under nitrogen. This was cooled to −78 °C in a dry ice/acetone bath. Then 13.6 mL (191.6 mmol) of DMSO in 25 mL of dry methylene chloride was added dropwise. After complete addition it was further stirred for 5 min. Then 9.87 g (40.0 mmol) of **6** in 10 mL of dry methylene chloride (with enough DMSO added to dissolve the solid) was added dropwise. The reaction mixture was stirred for an additional 30 min, and then 100 mL of triethylamine was added slowly at −78 °C. After being stirred for 10 min, the solution was allowed to warm to room temperature, and then 200 mL of ice/water was added. Following separation of the layers, the aqueous layer was extracted with methylene chloride (2 × 100 mL). The organic layers were combined, dried over $MgSO_4$, filtered, and evaporated to give the crude product as a solid. This solid was slurried with cold methanol and filtered, washed with cold methanol, then dried in vacuo to give 6.37 g (66%) of **7** as a yellow solid, mp 113−114 °C. ^{1}H NMR ($CDCl_3$): δ 7.49 (s, 1H), 7.47 (s, 1H), 4.43 (t, J = 5.9 Hz, 2H), 4.03 (s, 3H), 3.91 (t, J = 5.9 Hz, 2H). MS, *m*/*z*: 242.0 $(M + H)^+$. Anal. ($C_{11}H_{11}ClO_4$) C, H.

Ethyl 3-Amino-7-(2-chloroethoxy)-6-methoxy-2-naphthoate (8) and Ethyl 3-Amino-6-(2-chloroethoxy)-7-methoxy-2-naphthoate (9). A mixture of 25 g (0.21 mol) of 3-nitropropionic acid, 300 mL of absolute ethanol, and 10 drops of concentrated sulfuric acid was refluxed overnight. The reaction mixture was evaporated, and the residue was partitioned between water and diethyl ether. The ether layer was washed with water, aqueous sodium bicarbonate solution, and brine and then dried over sodium sulfate. Following the removal of ether in vacuo, the product was distilled as a clear liquid to provide 21.54 g (69%) of ethyl 3-nitropropionate as a clear oil, bp 160−165 °C at 120 mmHg. ^{1}H NMR ($CDCl_3$): δ 4.66 (t, J = 6.1 Hz, 2H), 4.18 (q, J = 7.1 Hz, 2H), 2.98 (t, J = 6.1 Hz, 2H), 1.28 (t, J = 7.1 Hz, 3H).

To a solution of 2.43 g (16.5 mmol) of ethyl 3-nitropropionate in 15 mL of absolute ethanol cooled in an ice bath was added 20 mL of 1 N sodium ethoxide in ethanol dropwise over 10 min, keeping the temperature at 0−5 °C. A slurry of 2.43 g (10.0 mmol) of **7** in 5 mL of ethanol was added. The ice bath was removed, and the reaction mixture was stirred for 16 h. The mixture was transferred to a beaker with 300 mL of water and neutralized with acetic acid to pH 4. A solid was collected and washed first with water, then with 40 mL of cold ethanol. The solid was dried in vacuo to provide 2.48 g (70%) of ethyl 7-(2-chloroethoxy)-6-methoxy-3-nitro-2-naphthoate and ethyl 6-(2-chloroethoxy)-7-methoxy-3-nitro-2-naphthoate (~1:1 mixture) as a yellow solid, mp 119−129 °C dec. ^{1}H NMR ($CDCl_3$):

δ 8.27 (s, 1H), 8.05 (s, 1H), 7.24, 7.23 and 7.22 (3s, 2H), 4.37−4.45 (m, 4H), 4.03 (s, 3H), 3.95 (t, J = 6.0 Hz, 2H), 1.38 (t, J = 7.1 Hz, 3H). MS, *m/z*: 354.2 $(M + H)^+$. Anal. ($C_{16}H_{16}ClNO_6$) C, H, N.

A 1.60 g (4.52 mmol) portion of ethyl 7-(2-chloroethoxy)-6-methoxy-3-nitro-2-naphthoate and ethyl 6-(2-chloroethoxy)-7-methoxy-3-nitro-2-naphthoate (~1:1 mixture) was heated in 100 mL of absolute ethanol until it dissolved. The solution was allowed to cool to room temperature, and 0.2 g of 10% palladium on carbon was added. Hydrogenation was carried out in a Parr apparatus at 50 psi for 2 h. The reaction mixture was filtered through Celite, and the filter cake was rinsed with ethanol. The filtrate and washes were combined and evaporated to give **8** and **9** (~1:1 mixture) as a greenish yellow solid, 1.28 g (87%), mp 104−108 °C. ^{1}H NMR ($CDCl_3$): δ 8.34 and 8.32 (2s, 1H), 7.06 and 7.03 (2s, 1H), 6.85 and 6.84 (2s, 1H), 6.82 (s, 1H), 5.52 (br s, 2H), 4.30−4.43 (m, 4H), 3.97 and 3.93 (2s, 3H), 3.89 (t, J = 6.6 Hz, 2H), 1.44 (t, J = 7.1 Hz, 3H). MS, *m/z*: 324.3 $(M + H)^+$. Anal. ($C_{16}H_{18}ClNO_4$) C, H, N.

7-(2-Chloroethoxy)-8-methoxy-4-oxo-1,4-dihydrobenzo[*g*]quinoline-3-carbonitrile (10) and 8-(2-Chloroethoxy)-7-methoxy-4-oxo-1,4-dihydrobenzo[*g*]quinoline-3-carbonitrile (11). A 648 mg portion (2.0 mmol) of ethyl 3-amino-7-(2-chloroethoxy)-6-methoxy-2-naphthoate and ethyl 3-amino-6-(2-chloroethoxy)-7-methoxy-2-naphthoate (~1:1 mixture) and 5 mL of dimethylformamide dimethylacetal were heated to reflux using an oil bath. The mixture was kept at reflux overnight. Solvent was removed in vacuo to provide crude ethyl 6-(2-chloroethoxy)-3-{[(*E*)-(dimethylamino)methylidene]amino}-7-methoxy-2-naphthoate and ethyl 7-(2-chloroethoxy)-3-{[(*E*)-(dimethylamino)methylidene]amino}-6-methoxy-2-naphthoate (~1:1 mixture) as a dark-red mixture that was used in the next step without further purification.

To 2.5 mL of dry tetrahydrofuran at −78 °C was added 1.8 mL of 2.5 M *n*-butyllithium (4.4 mmol). Then 0.24 mL (4.6 mmol) of dry acetonitrile in 4.5 mL of dry tetrahydrofuran was added dropwise over 10 min. This was stirred an additional 15 min at −78 °C. Then the ethyl 6-(2-chloroethoxy)-3-{[(*E*)-(dimethylamino)methylidene]amino}-7-methoxy-2-naphthoate and ethyl 7-(2-chloroethoxy)-3-{[(*E*)-(dimethylamino)-methylidene]amino}-6-methoxy-2-naphthoate (~1:1 mixture) was dissolved in 3 mL of tetrahydrofuran and added dropwise over 15 min. The reaction mixture was stirred for 30 min at −78 °C. Then the reaction was quenched with 0.57 mL (10 mmol) of glacial acetic acid, and the mixture was warmed to room temperature. A 10 mL portion of water was added to the yellow mixture. The solids were filtered, washed with water, and dried to give 0.502 g of **10** and **11** (~1:1 mixture) as a yellow-green solid (76%) that was used in the next step without further purification, mp 260−73 °C dec. ^{1}H NMR (DMSO-d_6): δ 8.68 (s, 1H), 8.62 and 8.61 (2s, 1H), 7.95 and 7.94 (2s, 1H), 7.62 and 7.61 (2s, 1H), 7.49 and 7.47 (2s, 1H), 4.37−4.47 (m, 2H), 4.00−4.11 (m, 2H), 3.96 and 3.93 (2s, 3H). MS, *m/z*: 329.5 $(M + H)^+$.

4-Chloro-8-methoxy-7-(2-chloroethoxy)benzo[*g*]quinoline-3-carbonitrile (12) and 4-Chloro-7-methoxy-8-(2-chloroethoxy)benzo[*g*]quinoline-3-carbonitrile (13). To a slurry of 1.11 g (3.38 mmol) of **10** and **11** (~1:1 mixture) and 5 mL of phosphorus oxychloride was added 0.15 mL of anhydrous dimethylformamide. This was stirred and heated to reflux for 20 min using an oil bath, followed by concentration in vacuo. The dark residue was quenched with 30 mL of cold water. The solid formed was collected, washed with water, and dried to give 1.02 g (87%) of **12** and **13** (~1:1 mixture) as a greenish yellow solid, mp 195−209 °C dec. ^{1}H NMR ($CDCl_3$): δ 8.88 (s, 1H), 8.66 and 8.65 (2s, 1H), 8.52 and 8.51 (2s, 1H), 7.33 (s, 1H), 7.30 and 7.29 (2s, 1H), 4.46−4.52 (m, 2H), 4.09 and 4.08 (2s, 3H), 3.96−4.00 (m, 2H). MS, *m/z*: 347.3 $(M + H)^+$. Anal. ($C_{17}H_{12}Cl_2N_2O_2$) C, H, N.

4-(4-Chloro-5-methoxy-2-methylanilino)-8-methoxy-7-(chloroethoxy)benzo[*g*]quinoline-3-carbonitrile (14a) and 4-(4-Chloro-5-methoxy-2-methylanilino)-7-methoxy-8-(chloroethoxy)benzo[*g*]quinoline-3-carbonitrile (15a). A mixture of 248 mg (0.714 mmol) of **12** and **13** (~1:1 mixture), 10 mg of pyridine hydrochloride, 150 mg (0.874 mmol) of 4-chloro-5-methoxy-2-methylaniline,[15c] and 5 mL of 2-ethoxyethanol was stirred and heated to 135 °C. After 1 h, the mixture was cooled to room temperature, the reaction was quenched with 0.2 mL of triethylamine, and the mixture was concentrated in vacuo. The residue was dissolved in 1:1 hexane/ethyl acetate with added dichloromethane and chromatographed on silica gel, eluting with 1:1 hexane/ethyl acetate and then ethyl acetate to provide 0.282 g of **14a** and **15a** (~1:1 mixture) as a dull-yellow solid (81%), mp 132−168 °C dec. ^{1}H NMR ($CDCl_3$): δ 8.66 and 8.65 (2s, 1H), 8.41 and 8.40 (2s, 1H), 8.19 and 8.17 (2s, 1H), 7.36 (s, 1H), 7.25 and 7.24 (2s, 1H), 7.12 (s, 1H), 7.02 (br s, 1H), 6.76 (s, 1H), 4.48 and 4.41 (2t, J = 6.1 Hz, 2H), 4.07 and 4.01 (2s, 3H), 3.95 and 3.98 (2t, J = 6 0.1 Hz, 2H), 3.77 (s, 3H), 2.28 (s, 3H). MS, *m/z*: 482.0 $(M + H)^+$. Anal. ($C_{25}H_{21}Cl_2N_3O_3 \cdot H_2O$) C, H, N.

4-(2,4-Dichloro-5-methoxyanilino)-8-methoxy-7-(chloroethoxy)benzo[*g*]quinoline-3-carbonitrile (14b) and 4-(2,4-Dichloro-5-methoxyanilino)-7-methoxy-8-(chloroethoxy)benzo[*g*]quinoline-3-carbonitrile (15b). A mixture of 1.10 g (3.17 mmol) of **12** and **13** (~1:1 mixture), 50 mg of pyridine hydrochloride, 742 mg (3.86 mmol) of 2,4-dichloro-5-methoxyaniline,[15c] and 25 mL of 2-ethoxyethanol was stirred and heated to 135 °C. After 1 h, the mixture was cooled to room temperature, the reaction was quenched with 1.0 mL of triethylamine, and the mixture was concentrated in vacuo. The residue was dissolved in 95:5 methylene chloride/methanol and chromatographed on silica gel, eluting with 1:1 hexane/ethyl acetate. The product was precipitated from ethyl acetate to provide 0.760 g (48%) of **14b** and **15b** (~1:1 mixture) as a dull-yellow solid, mp 239−255 °C dec. ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 8.94 (br s, 1H), 8.77 (br s, 1H), 8.59 (br s, 1H), 8.36 (br s, 1H), 7.54 (br s, 1H), 7.28 (br s, 1H), 7.26 (br s, 1H), 6.96 (br s, 1H), 4.40−4.49 (m, 2H), 3.94−4.07 (m, 5H), 3.86 (s, 3H). MS, *m/z*: 502.2 $(M + H)^+$. Anal. ($C_{24}H_{18}Cl_3N_3O_3 \cdot 0.3H_2O$) C, H, N.

8-(2-Chloroethoxy)-4-(2-chloro-5-methoxy-4-methylanilino)-7-methoxybenzo[*g*]quinoline-3-carbonitrile (14c) and 7-(2-Chloroethoxy)-4-(2-chloro-5-methoxy-4-methylanilino)-8-methoxybenzo[*g*]quinoline-3-carbonitrile (15c). Following the route used to prepare **14a** and **15a**, **14c** and **15c** (~1:1 mixture) were obtained as a yellow solid in 64% yield from the reaction of **12** and **13** with 2-chloro-5-methoxy-4-methylaniline,[15c] mp 204−212 °C. ^{1}H NMR (DMSO-d_6): δ 10.05 (s, 2H), 9.04 (s, 2H), 8.44 (s, 2H), 6.35 (s, 2H), 7.55 (s, 2H), 7.36 (s, 4H), 7.14 (s, 2H), 4.44 (s, 4H), 4.07 (s, 4H), 3.98 (s, 6H), 3.78 (s, 6H), 2.19 (s, 6H). MS, *m/z*: 482.0 $(M + H)^+$. Anal. ($C_{25}H_{21}Cl_2lN_3O_3$) C, H, N.

8-(2-Chloroethoxy)-4-(2-chloro-4-fluoro-5-methoxyanilino)-7-methoxybenzo[*g*]quinoline-3-carbonitrile (14d) and 7-(2-Chloroethoxy)-4-(2-chloro-4-fluoro-5-methoxyanilino)-8-methoxybenzo[*g*]quinoline-3-carbonitrile (15d). Following the route used to prepare **14a** and **15a**, **14d** and **15d** (~1:1 mixture) were obtained as a yellow solid in 59% yield from the reaction of **12** and **13** with 2-chloro-4-fluoro-5-methoxyaniline,[15c] mp 193−204 °C. ^{1}H NMR ($CDCl_3$): δ 8.70 (s, 1H), 8.44 and 8.43 (2s, 1H), 8.28 and 8.27 (2s, 1H), 7.31 and 7.28 (2s, 1H), 7.20 (s, 1H), 7.11 (s, 1H), 6.89 and 6.87 (2s, 1H), 4.49 and 4.42 (2t, J = 6.0 Hz, 2H), 4.08 and 4.03 (2s, 3H), 3.98 and 3.96 (2t, J = 6.0 Hz, 2H), 3.79 (s, 3H). MS, *m/z*: 486.0, 488.1 $(M + H)^+$. Anal. ($C_{24}H_{18}Cl_2FN_3O_3 \cdot 0.3H_2O$) C, H, N.

7-(2-Chloroethoxy)-8-methoxy-4-(3,4,5-trimethoxyanilino)benzo[*g*]quinoline-3-carbonitrile (14e) and 8-(2-Chloroethoxy)-7-methoxy-4-(3,4,5-trimethoxyanilino)-benzo[*g*]quinoline-3-carbonitrile (15e). A mixture of 500 mg of **12** and **13** (1.4 mmol), 277 mg of 3,4,5-trimethoxyaniline[15a] (1.5 mmol), and 200 mg of pyridine hydrochloride (1.7 mmol) in 14 mL of 2-ethoxyethanol was heated at reflux for 0.5 h. When the mixture cooled, a solid precipitated from solution, which was collected by filtration. Subsequent washing with water and hexanes provided 700 mg (99% yield) of a yellow

solid mixture of **14e** and **15e** (~1:1 mixture), which was used in the next step without further purification.

7-(2-Chloroethoxy)-4-(2,4-dichloroanilino)-8-methoxybenzo[*g*]quinoline-3-carbonitrile (14f) and 8-(2-Chloroethoxy)-4-(2,4-dichloroanilino)-7-methoxybenzo[*g*]quinoline-3-carbonitrile (15f). A mixture of 300 mg of **12** and **13** (0.87 mmol), 153 mg of 2,4-dichloroaniline (0.94 mmol), and 100 mg of pyridine hydrochloride (0.87 mmol) in 10 mL of 2-ethoxyethanol was heated at reflux for 1 h. The reaction mixture was cooled to room temperature and added to saturated aqueous sodium bicarbonate. The solid was collected by filtration and partitioned between ethyl acetate and 1 N aqueous sodium hydroxide. The organic layer was washed with 1 N aqueous sodium hydroxide, dried over magnesium sulfate, filtered, and concentrated in vacuo. The crude solid was passed through a silica gel column, eluting with 99:1 dichloromethane/methanol to provide 194 mg of **14f** and **15f** (~1:1 mixture), which was directly used in the next step.

4-(4-Chloro-5-methoxy-2-methylanilino)-8-methoxy-7-[2-(4-morpholinyl)ethoxy]benzo[*g*]quinoline-3-carbonitrile (16a) and 4-(4-Chloro-5-methoxy-2-methylanilino)-7-methoxy-8-[2-(4-morpholinyl)ethoxy]benzo[*g*]quinoline-3-carbonitrile (17a). A mixture of 318 mg (0.66 mmol) of **14a** and **15a** (~1:1 mixture), 100 mg of sodium acetate and 5 mL of morpholine was stirred and heated to 130 °C using an oil bath. After 30 min the mixture was allowed to cool to room temperature. The reaction mixture was concentrated in vacuo and the residue was purified by silica gel chromatography, eluting with 95:5 methylene chloride/methanol to yield 153 mg (43%) of **16a** as a yellow solid, mp 191−194 °C dec, and 82 mg (23%) of **17a** as a yellow wax.

16a. ^{1}H NMR ($CDCl_3$): δ 8.65 (s, 1H), 8.39 (s, 1H), 8.17 (s, 1H), 7.35 (br s, 1H), 7.27 (s, 1H), 7.22 (s, 1H), 7.10 (s, 1H), 6.75 (s, 1H), 4.28 (t, J = 5.7 Hz, 2H), 4.05 (s, 3H), 3.75 (s, 3H), 3.78−3.73 (m, 4H), 2.94 (t, J = 5.7 Hz, 2H), 2.67−2.62 (br s, 4H), 2.27 (s, 3H). MS, *m/z*: 533.1 $(M + H)^+$. Anal. ($C_{29}H_{29}ClN_4O_4 \cdot 0.5H_2O$) C, H, N.

17a. ^{1}HNMR($CDCl_3$): δ 8.57 (s, 1H), 8.40 (s, 1H), 8.30 (s, 1H), 7.76 (br s, 1H), 7.25 (s, 1H), 7.18 (s, 1H), 7.08 (s, 1H), 6.80 (s, 1H), 4.32 (t, J = 5.7 Hz, 2H), 3.92 (s, 3H), 3.75 (s, 3H), 3.77−3.74 (m, 4H), 2.95 (t, J = 5.7 Hz, 2H), 2.67−2.60 (br s, 4H), 2.19 (s, 3H). MS 533.1 $(M + H)^+$. Anal. ($C_{29}H_{29}ClN_4O_4 \cdot H_2O$) C, H, N.

4-(4-Chloro-5-methoxy-2-methylanilino)-7-methoxy-8-[2-(4-methyl-1-piperazinyl)ethoxy]benzo[*g*]quinoline-3-carbonitrile (16b) and 4-(4-Chloro-5-methoxy-2-methylanilino)-8-methoxy-7-[2-(4-methyl-1-piperazinyl)ethoxy]-benzo[*g*]quinoline-3-carbonitrile (17b). A mixture of 205 mg (0.425 mmol) of **14a** and **14b** (~1:1 mixture), 0.15 mL (1.35 mmol) of 1-methylpiperazine, and 50 mg (0.34 mmol) of sodium iodide in 5 mL of ethylene glycol dimethyl ether was heated at 90 °C for 4 days under nitrogen. The mixture was cooled, the solvent was removed in vacuo, and the resulting residue was stirred with saturated aqueous sodium bicarbonate. The crude solid was collected by filtration, washed with water, and dried in vacuo. Purification was carried out by silica gel chromatography, eluting with a gradient of 92:8 to 85:15 methylene chloride/methanol to yield 100 mg (43%) of **16b** as a yellow solid, mp 121−135 °C, and 68 mg (29%) of **17b** as a yellow solid, mp 122−137 °C.

16b. ^{1}H NMR (DMSO-d_6): δ 9.86 (s, 1H), 9.01 (s 1H), 8.42 (s, 1H), 8.32 (s, 1H), 7.57 (s, 1H), 7.43 (s 1H), 7.31 (s, 1H), 7.16 (s, 1H), 4.27 (t, J = 5.6 Hz, 2H), 3.96 (s, 3H), 3.81 (s, 3H), 2.81 (t, J = 5.8 Hz, 2H), 2.54 (m, 4H), 2.37 (br s, 4H), 2.18 (s, 3H), 2.14 (s, 3H). MS, *m/z*: 546.4 $(M + H)^+$. Anal. ($C_{30}H_{32}ClN_5O_3 \cdot 1.6CH_2Cl_2$) C, H, N.

17b. ^{1}HNMR (DMSO-d_6): δ 9.86 (s, 1H), 9.0 (s 1H), 8.43 (s, 1H), 8.34 (s, 1H), 7.53 (s, 1H), 7.43 (s 1H), 7.33 (s, 1H), 7.16 (s, 1H), 4.27 (t, J = 5.6 Hz, 2H), 3.97 (s, 3H), 3.81 (s, 3H), 2.81 (t, J = 5.8 Hz, 2H), 2.55 (m, 4H), 2.36 (br s, 4H), 2.17 (s, 3H), 2.14 (s, 3H). MS, *m/z*: 546.4 $(M + H)^+$. Anal. ($C_{30}H_{32}ClN_5O_3 \cdot 1.0CH_2Cl_2$) C, H, N.

4-(2,4-Dichloro-5-methoxyanilino)-8-methoxy-7-[2-(4-morpholinyl)ethoxy]benzo[*g*]quinoline-3-carbonitrile (16c) and 4-(2,4-Dichloro-5-methoxyanilino)-7-methoxy-8-[2-(4-morpholinyl)ethoxy]benzo[*g*]quinoline-3-carbonitrile (17c). A mixture of 0.436 g (0.87 mmol) of **14b** and **15b** (~1:1 mixture), 2.0 mL (23.0 mmol) of morpholine, and 0.05 g of sodium iodide (0.34 mmol) in 2.0 mL of ethylene glycol dimethyl ether was heated at 90 °C for 3.5 h under nitrogen. The mixture was cooled, solvent was removed in vacuo, and the resulting residue was stirred with saturated aqueous sodium bicarbonate. The crude solid was collected by filtration, washed with water, and dried in vacuo. Purification was carried out by silica gel chromatography, eluting with a gradient of 97:3 to 90:10 ethyl acetate/methanol to yield 0.241 g (50%) of **16c** as a yellow solid, mp 210−212 °C, and 0.203 g (42%) of **17c** as a yellow solid, mp 207−214 °C.

16c. ^{1}H NMR (DMSO-d_6 + TFA): δ 9.35 (s, 1H), 9.25 (s 1H), 8.43 (s, 1H), 7.91 (s, 1H), 7.78 (s 1H), 7.63 (s, 1H), 7.53 (s, 1H), 4.65 (m, 2H), 4.06 (s, 3H), 4.04−3.97 (m, 2H), 3.91 (s, 3H), 3.84−3.63 (m, 6H), 3.34 (t, J = 10.6 Hz, 2H). MS, *m/z*: 553.3 $(M + H)^+$. Anal. ($C_{28}H_{26}Cl_2N_4O_4 \cdot 0.15CH_3CO_2C_2H_5$) C, H, N.

17c. ^{1}H NMR (DMSO-d_6 + TFA): δ 9.35 (s, 1H), 9.24 (s 1H), 8.42 (s, 1H), 7.91 (s, 1H), 7.82 (s 1H), 7.61 (s, 1H), 7.48 (s, 1H), 4.66 (m, 2H), 4.07 (s, 3H), 4.04−3.97 (m, 2H), 3.90 (s, 3H), 3.83−3.63 (m, 6H), 3.34 (m, 2H). MS, *m/z*: 553.3 $(M + H)^+$. Anal. ($C_{28}H_{26}Cl_2N_4O_4 \cdot 2.0H_2O$) C, H, N.

4-(2,4-Dichloro-5-methoxyanilino)-8-methoxy-7-[2-(4-methyl-1-piperazinyl)ethoxy]benzo[*g*]quinoline-3-carbonitrile (16d) and 4-(2,4-Dichloro-5-methoxyanilino)-7-methoxy-8-[2-(4-methyl-1-piperazinyl)ethoxy]benzo-[*g*]quinoline-3-carbonitrile (17d). Following the route used to prepare **16c** and **17c**, the reaction of **14b** and **15b** with 1-methylpiperazine provided **16d** as a yellow solid in 33% yield (after purification by silica gel chromatography, eluting with dichloromethane/methanol), mp 141−150 °C, and **17d** as a yellow solid in 45% yield, mp 132−135 °C.

16d. ^{1}H NMR (DMSO-d_6 + TFA): δ 9.34 (s, 1H), 9.23 (s 1H), 8.42 (s, 1H), 7.91 (s, 1H), 7.77 (s 1H), 7.61 (s, 1H), 7.51 (s, 1H), 4.63 (m, 2H), 4.03 (s, 3H), 3.90 (s, 3H), 3.81−3.31 (m, 10H), 2.94 (s, 3H). MS, *m/z*: 566.3 $(M + H)^+$. Anal. ($C_{29}H_{29}Cl_2N_5O_3 \cdot 0.9CH_2Cl_2$) C, H, N.

17d. ^{1}H NMR (DMSO-d_6 + TFA): δ 9.35 (s, 1H), 9.23 (s 1H), 8.42 (s, 1H), 7.91 (s, 1H), 7.80 (s 1H), 7.60 (s, 1H), 7.48 (s, 1H), 4.64 (m, 2H), 4.03 (s, 3H), 3.90 (s, 3H), 3.81−3.4 (m, 10H), 2.94 (s, 3H). MS, *m/z*: 566.3 $(M + H)^+$ Anal. ($C_{29}H_{29}Cl_2N_5O_3 \cdot 0.5CH_2Cl_2$) C, H, N.

4-(2,4-Dichloro-5-methoxyphenylamino)-7-[2-(4-hydroxypiperidin-1-yl)ethoxy]-8-methoxybenzo[*g*]quinoline-3-carbonitrile (16e) and 4-(2,4-Dichloro-5-methoxyphenylamino)-8-[2-(4-hydroxypiperidin-1-yl)ethoxy]-7-methoxybenzo[*g*]quinoline-3-carbonitrile (17e). Following the route used to prepare **16b** and **17b**, the reaction of **14b** and **15b** with 4-hydroxypiperidine provided **16e** as a yellow solid in 7% yield, mp 150−160 °C, and **17e** as a yellow solid in 25% yield, mp 193−198 °C dec.

16e. ^{1}H NMR(DMSO-d_6): δ 9.37 (s, 1 H), 9.28 (s, 1H), 8.46 (s, 1H), 7.89 (s, 1H), 7.78 (s, 1H), 7.64 (s, 1H), 7.53 (d, J = 3.90 Hz, 1H), 4.68−4.60 (m, 2H), 4.08 (s, 3H), 4.02−3.97 (m, 1H), 3.92 (s, 3H), 3.77−3.67 (m, 3H), 3.57−3.38 (m, 2H), 3.27−3.17 (m, 1H), 2.10−1.79 (m, 3H), 1.74−1.63 (m, 1H). MS, *m/z*: 566.7 $(M + H)^+$. Anal. ($C_{29}H_{28}Cl_2N_4O_4 \cdot 2.5H_2O$) C, H, N.

17e. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.39 (s, 1H), 9.29 (s, 1H), 8.46 (s, 1H), 7.89 (s, 1H), 7.83 (d, J = 3.8 Hz, 1H), 7.64 (s, 1H), 7.49 (s, 1H), 4.70−4.62 (m, 2H), 4.06 (s, 3H), 4.03−3.96 (m, 1H), 3.93 (s, 3H), 3.78−3.67 (m, 3H), 3.57−3.49 (m, 1H), 3.49−3.39 (m, 1H), 3.29−3.19 (m, 1H), 2.11−2.03 (m, 1H), 2.03−1.92 (m, 1H), 1.88−1.82 (m, 1H), 1.75−1.64 (m, 1H). MS, *m/z*: 566.8 $(M + H)^+$. Anal. ($C_{29}H_{28}Cl_2N_4O_4 \cdot 1.1CH_3OH$) C, H, N.

4-(2-Chloro-5-methoxy-4-methylanilino)-8-methoxy-7-[2-(4-morpholinyl)ethoxy]benzo[*g*]quinoline-3-carbonitrile (16f) and 4-(4-Chloro-5-methoxy-2-methylanilino)-7-methoxy-8-[2-(4-morpholinyl)ethoxy]benzo[*g*]quinoline-3-carbonitrile (17f). Following the route used to prepare **16c**

and **17c**, the reaction of **14c** and **15c** with morpholine provided **16d** as a yellow solid in 48% yield, mp 240−241 °C, and **17d** as a yellow solid in 47% yield, mp 220−222 °C.

16f. ^{1}H NMR (DMSO-d_6 + TFA-d): δ 9.36 (s, 1H), 9.24 (s 1H), 8.43 (s, 1H), 7.81 (s, 1H), 7.51 (s, 2H), 7.35 (s 1H), 4.65 (m, 2H), 4.07 (s, 3H), 4.03 (s, 1H), 3.83 (s, 3H), 3.76−3.64 (m, 7H), 3.35 (m, 2H), 2.27 (s, 3H). MS, *m/z*: 533.0 $(M + H)^+$. Anal. ($C_{29}H_{29}ClN_4O_4$) C, H, N.

17f. ^{1}H NMR (DMSO-d_6 + TFA-d): δ 9.38 (s, 1H), 9.25 (s 1H), 8.42 (s, 1H), 7.83 (s, 1H), 7.51 (s, 1H), 7.46 (s, 1H), 7.35 (s 1H), 4.67 (m, 2H), 4.07 (s, 1H), 4.05 (s, 3H), 3.84 (s, 3H), 3.79−3.65 (m, 7H), 3.35 (m, 2H), 2.27 (s, 3H). MS, *m/z*: 533.0 $(M + H)^+$. Anal. ($C_{29}H_{29}ClN_4O_4 \cdot 2.2H_2O$) C, H, N.

4-(2-Chloro-5-methoxy-4-methylanilino)-8-[2-(4-hydroxypiperidin-1-yl)ethoxy]-7-methoxybenzo[*g*]quinoline-3-carbonitrile (17g). Following the route used to prepare **16b** and **17b**, the reaction of **14c** and **15c** with 4-hydroxypiperidine provided **17g** as a yellow solid in 51% yield, mp 210−215 °C. ^{1}H NMR (DMSO-d_6): δ 9.38 (s, 1H), 9.24 (s, 1H), 8.42 (s, 1H), 7.82 (d, J = 3.5 Hz, 1H), 7.51 (s, 1H), 7.47 (s, 1H), 7.34 (s, 1H), 4.64 (q, J = 5.5 Hz, 2H), 4.05 (s, 3H), 3.99−3.95 (m, 1H), 3.84 (s, 3H), 3.76−3.65 (m, 3H), 3.55−3.48 (m, 1H), 3.46−3.36 (m, 1H), 3.27−3.16 (m, 1H), 2.27 (s, 3H), 2.10−1.79 (m, 3H), 1.72−1.61 (m, 1H). MS, *m/z*: 546.8 $(M + H)^+$. Anal. ($C_{30}H_{31}ClN_4O_4 \cdot 1.25H_2O \cdot 0.25HCl$) C, H, N.

4-(2-Chloro-4-fluoro-5-methoxyanilino)-7-methoxy-8-[2-(4-morpholinyl)ethoxy]benzo[*g*]quinoline-3-carbonitrile Hydrochloride (17h). A mixture of 243 mg (0.50 mmol) of **14d** and **15d** (~1:1 mixture) and 100 mg (0.67 mmol) of sodium iodide in 5 mL of morpholine was heated to reflux for 30 min. After cooling to room temperature, the product mixture was evaporated, then chromatographed on silica gel, eluting with 9:1 ethyl acetate/methanol. This gave 76 mg (26%) of **17h** as a yellow solid, mp 206−222 °C dec. ^{1}H NMR (DMSO-d_6): δ 9.36 (s, 1H), 9.26 (s, 1H), 8.43 (s, 1H), 7.84 (s, 1H), 7.78 (d, J = 10.9 Hz, 1H), 7.68 (d, J = 8.3 Hz, 1H), 7.47 (s, 1H), 4.66 (m, 2H), 4.10−3.98 (m, 6H), 3.89 (s, 3H), 3.81−3.73 (m, 4H), 3.73−3.61 (m, 3H), 3.24−3.44 (s, 2H). MS, *m/z*: 537.1 $(M + H)^+$. Anal. ($C_{28}H_{26}ClFN_4O_4 \cdot 1.5H_2O \cdot 1.2HCl$) C, H, N.

8-Methoxy-7-[2-(4-methyl-1-piperazinyl)ethoxy]-4-(3,4,5-trimethoxyanilino)benzo[*g*]quinoline-3-carbonitrile (16g) and 7-Methoxy-8-[2-(4-methyl-1-piperazinyl)ethoxy]-4-(3,4,5-trimethoxyanilino)benzo[*g*]quinoline-3-carbonitrile (17i). A mixture of 350 mg (0.70 mmol) of **14e** and **15e** (~1:1 mixture) was combined with 1.4 g (14 mmol) of *N*-methylpiperazine and 10 mg (0.07 mmol) of sodium iodide in 5 mL of ethylene glycol dimethyl ether. The suspension was heated at reflux for 26 h, then cooled to room temperature and poured into saturated aqueous sodium bicarbonate. The solid was collected by filtration, washing with water. Flash column chromatography, eluting with a gradient of 95:5 to 4:1 methylene chloride/methanol, followed by washing with ethyl acetate and hexane, gave 142 mg (36%) of **16g** as a light-yellow solid, mp 115−120 °C, and 127 mg (32% yield) of **17i** as a light-yellow solid, mp 95−97 °C.

16g. ^{1}H NMR (DMSO-d_6): δ 9.91 (s, 1H), 8.93 (s, 1H), 8.49 (s, 1H), 8.34 (s, 1H), 7.53 (s, 1H), 7.34 (s, 1H), 6.70 (s, 2H), 4.25 (t, J = 5.5 Hz, 2H), 3.97 (s, 3H), 3.78 (s, 6H), 3.69 (s, 3H), 2.81 (t, J = 5.5 Hz, 2H), 2.55−2.38 (m, 8H), 2.18 (s, 3H). MS, *m/z*: 558.3 $(M + H)^+$. Anal. ($C_{31}H_{35}N_5O_5 \cdot 1.05EtOAc$) C, H, N.

17i. ^{1}H NMR (DMSO-d_6): δ 9.92 (s, 1H), 8.95 (s, 1H), 8.49 (s, 1H), 8.32 (s, 1H), 7.57 (s, 1H), 7.31 (s, 1H), 6.70 (s, 2H), 4.28 (t, J = 5.9 Hz, 2H), 3.96 (s, 3H), 3.79 (s, 6H), 3.69 (s, 3H), 2.81 (t, J = 5.5 Hz, 2H), 2.55−2.38 (m, 8H), 2.17 (s, 3H). MS, *m/z*: 558.2 $(M + H)^+$. Anal. ($C_{31}H_{35}N_5O_5 \cdot 5.0H_2O$) C, H, N.

8-Methoxy-7-[2-(4-morpholinyl)ethoxy]-4-[(3,4,5-trimethoxyphenyl)amino]benzo[*g*]quinoline-3-carbonitrile (16h) and 7-Methoxy-8-[2-(4-morpholinyl)ethoxy]-4-(3,4,5-trimethoxyanilino)benzo[*g*]quinoline-3-carbonitrile (17j). Following the route used to prepare **16g** and **17i**, the reaction of **14e** and **15e** with morpholine provided **16h** as a yellow solid in 37% yield, mp 135−138 °C, and **17j** as a yellow solid in 37% yield, mp 127−130 °C.

16h. ^{1}H NMR (DMSO-d_6): δ 9.92 (s, 1H), 8.91 (s, 1H), 8.47 (s, 1H), 8.32 (s, 1H), 7.52 (s, 1H), 7.35 (s, 1H), 6.67 (s, 2H), 4.28 (t, J = 4.3 Hz, 2H), 3.97 (s, 3H), 3.78 (s, 6H), 3.69 (s, 3H), 3.61 (t, J = 3.5 Hz, 4H), 2.81 (t, J = 4.5 Hz, 2H), 2.54 (t, J = 3.5 Hz, 4H). MS, *m/z*: 544.9 $(M + H)^+$. Anal. ($C_{31}H_{35}N_5O_5 \cdot 1.0CH_2Cl_2$) C, H, N.

17j. ^{1}H NMR (DMSO-d_6): δ 9.92 (s, 1H), 8.94 (s, 1H), 8.47 (s, 1H), 8.30 (s, 1H), 7.56 (s, 1H), 7.31 (s, 1H), 6.67 (s, 2H), 4.29 (t, J = 4.4 Hz, 2H), 3.95 (s, 3H), 3.78 (s, 6H), 3.69 (s, 3H), 3.61 (t, J = 3.4 Hz, 4H), 2.82 (t, J = 4.4 Hz, 2H), 2.53 (t, J = 3.5 Hz, 4H). MS, *m/z*: 544.9 $(M + H)^+$. Anal. ($C_{31}H_{35}N_5O_5 \cdot 1.5H_2O$) C, H, N.

4-(2,4-Dichloroanilino)-8-methoxy-7-[2-(4-morpholinyl)-ethoxy]benzo[*g*]quinoline-3-carbonitrile (16i) and 4-(2,4-Dichloroanilino)-7-methoxy-8-[2-(4-morpholinyl)ethoxy]-benzo[*g*]quinoline-3-carbonitrile (17k). A 194 mg (0.41 mmol) portion of **14f** and **15f** was combined with 1.0 mL of morpholine (11.5 mmol) and 10 mg of sodium iodide (0.07 mmol) in 3 mL of ethylene glycol dimethyl ether. The suspension was heated at reflux for 8 h, then cooled to room temperature and poured into saturated aqueous sodium bicarbonate. The solid was collected by filtration, washing with water. Flash column chromatography, eluting with a gradient of 95:5 to 4:1 ethyl acetate/methanol, gave 62 mg (29%) of **16i** as a yellow solid, mp 115−119 °C dec, and 52 mg (24%) of **17k** as a yellow solid, mp 250−252 °C dec.

16i. ^{1}H NMR (DMSO-d_6, TFA): δ 9.29 (s, 1H), 9.20 (s, 1H), 8.40 (s, 1H), 7.94 (d, J = 2.8 Hz, 1H), 7.76 (s, 1H), 7.74 (d, J = 10.4 Hz, 1H), 7.65 (dd, J = 8.4, 2.0 Hz, 1H), 4.62 (m, 2H), 7.52 (s, 1H), 4.09−3.98 (m, 5H), 3.79−3.57 (m, 6H), 3.38−3.23 (m, 2H). MS, *m/z*: 522.7 $(M + H)^+$. Anal. ($C_{27}H_{24}Cl_2N_4O_3 \cdot 1.0H_2O$) C, H, N.

17k. ^{1}H NMR (DMSO-d_6, TFA): δ 9.30 (s, 1H), 9.22 (s, 1H), 8.40 (s, 1H), 7.94 (d, J = 2.4 Hz, 1H), 7.81 (s, 1H), 7.75 (d, J = 8.4 Hz, 1H), 7.65 (dd, J = 8.4, 2.4 Hz, 1H), 7.47 (s, 1H), 4.64 (m, 2H), 4.08−3.97 (m, 5H), 3.79−3.58 (m, 6H), 3.37−3.24 (m, 2H). MS, *m/z*: 522.7 $(M + H)^+$. Anal. ($C_{27}H_{24}Cl_2N_4O_3 \cdot 1.0H_2O$) C, H, N.

Molecular Modeling. The structures of **17d** and SKI-606 were minimized using the MMFF force field in Sybyl, a software package of Tripos (Sybyl 6.9; Tripos, Inc., St. Louis, MO), and conformationally expanded using the Openeye Scientific Software package, Omega (Open Eye Scientific Software, Santa Fe, NM) with a 1.0 Å rmsd cutoff to filter out degenerate conformers. A homology model of the active form of the Src kinase domain was constructed using the active form of the Lck kinase domain as a template. The software package of Andrej Sali, MODELLER (Accelrys, Inc., San Diego, CA), was used to build the homology model. The Openeye Scientific Software package FRED (Open Eye Scientific Software, Santa Fe, NM) was used for the docking studies. In summary, FRED does not use stochastic sampling to dock the ligand conformers; rather, a shape-based matching of all possible orientations of each conformer is optimized in accordance with the selected scoring function. The PLP or piecewise linear potential function was used to score the poses docked using FRED, consisting of a steric matching function with a less stringent charge matching function of protein−ligand interactions. The top 10 poses obtained from the FRED docking procedure are rescored with a Poisson−Boltzmann molecular mechanics scoring function (PBMM-SA).[26] The free energy of protein−ligand binding is desribed as

$$\Delta G_{\text{bind}}^{\text{solv}} = \Delta G_{\text{bind}}^{\text{gas}} - \Delta G_{\text{solv}}^{\text{P}} - \Delta G_{\text{solv}}^{\text{L}} + \Delta G_{\text{solv}}^{\text{PL}}$$

where the first term at the right of the equal sign is the gas-phase protein−ligand binding energy and is obtained via molecular mechanics calculations. The remainder of the terms represent the solvated free energy terms for the protein and ligand individually as well as the protein−ligand complex. The solvated free energy terms are evaluated explicitly via Poisson−Boltzmann/surface area calculations. Solvation and hydrophobic effects are handled by this scoring function, which is used to rescore or fine-tune the docked views.

Biological Materials and Methods. Lysate preparation was carried out as previously described.[15b]

Antibodies. The 4G10 antibody to phosphotyrosine, monoclonal antibody to Src (GD11), and cortactin were obtained from Upstate Biotechnologies. Antibodies to focal adhesion kinase (FAK), PY925 FAK, PY421 cortactin, and PY418 Src were obtained from BioSource.

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Supporting Information Available: Elemental analysis data for compounds **4**−**15**, **16a**−**i**, and **17a**−**k**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Schwartzberg, P. L. The many faces of Src: multiple functions of a prototypical tyrosine kinase. *Oncogene* **1998**, *17*, 1463−1468.

(2) Thomas, S. M.; Brugge, J. S. Cellular functions regulated by Src family kinases. *Annu. Rev. Cell Dev. Biol.* **1997**, *13*, 513−609.

(3) Tsygankov, A. Y.; Shore, S. K. SRC: Regulation, role in human carcinogenesis and pharmacological inhibitors. *Curr. Pharm. Des.* **2004**, *10*, 1745−1756.

(4) (a) Mukhopadhyay, D.; Tsiokas, L.; Zhou, X. M.; Foster, D.; Brugge, J. S.; Sukhatme, V. P. Hypoxic induction of human vascular endothelial growth factor expression through c-Src activation. *Nature* **1995**, *375*, 577−581. (b) Schlessinger, J. New roles for Src kinases in control of cell survival and angiogenesis. *Cell* **2000**, *100*, 293−296.

(5) (a) Missbach, M.; Altmann, E.; Susa, M. Tyrosine kinase inhibition in bone metabolism. *Curr. Opin. Drug Discovery Dev.* **2000**, *3*, 541−548. (b) Susa, M.; Teti, A. Tyrosine kinase Src inhibitors: potential therapeutic applications. *Drug News Perspect.* **2000**, *13*, 169−175.

(6) Paul, R.; Zhang, Z. G.; Eliceiri, B. P.; Jiang, Q.; Boccia, A. D.; Zhang, R. L.; Chopp, M.; Cheresh, D. A. Adhesion events in angiogenesis. *Nat. Med.* **2001**, *7*, 222−227.

(7) (a) Wang, Y.; Metcalf, C. A., III; Shakespeare, W. C.; Sundaramoorthi, R.; Keenan, T. P.; Bohacek, R. S.; van Schravandijk, M. R.; Violette, S. M.; Narula, S. S.; Dalgarno, D. C.; Haraldson, C.; Keats, J.; Liou, S.; Mani, U.; Pradeepan, S.; Ram, M.; Adams, S.; Weigele, M.; Sawyer, T. K. Bone-targeted 2,6,9-trisubstituted purines: novel inhibitors of Src tyrosine kinase for the treatment of bone diseases. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3067−3070. (b) Wang, Y.; Lianping, X.; Metcalf, C. A.; Shakespeare, W.; Sundaramoorthi, R.; Bohacek, R.; Narula, S.; Wardwell, S.; Iuliucci, J.; Weigele, M.; Dalgarno, D.; Boyce, B.; Sawyer, T. Signal transduction pathways and bone diseases: novel bone-targeted Src kinase inhibitors with dual antiresorptive and bone anabolic properties. *Abstracts of Papers*, 227th National Meeting of the American Chemical Society, Anaheim, CA, March 28 through April 1, 2004; American Chemical Society: Washington, DC, 2004; MEDI-346. (c) Sundaramoorthi, R.; Shakespeare, W. C.; Metcalf, C. A.; Wang, Y.; Liu, S.; Bohacek, R. S.; Narula, S. S.; Keats, J. A.; Wardwell, S.; Iuliucci, J.; Weigele, M.; Dalgarno, Sawyer, T. K. Design and synthesis of novel bone-targeted Src tyrosine kinase inhibitor drug discovery. *Abstracts of Papers,* 227th National Meeting of the American Chemical Society, Anaheim, CA, March 28 through April 1, 2004; American Chemical Society: Washington, DC, 2004; MEDI-038.

(8) (a) Klutchko, S. R.; Hamby, J. M.; Boschelli, D. H.; Wu, Z.; Kraker, A. J.; Amar, A. M.; Hartl, B. G.; Shen, C.; Klohs, W. D.; Steinkampf, R. W.; Driscoll, D. L.; Nelson, J. M.; Elliott, W. L.; Roberts, B. J.; Stoner, C. L.; Vincent, P. W.; Dykes, D. J.; Panek, R. L.; Lu, G. H.; Major, T. C.; Dahring, T. K.; Hallak, H.; Bradford, L. A.; Showalter, H. D. H.; Doherty, A. M. 2-Substituted aminopyrido[2,3-*d*]pyrimidin-7(8*H*)-ones. Structure−activity relationships against selected tyrosine kinases and in vitro and in vivo anticancer activity. *J. Med. Chem.* **1998**, *41*, 3276−3292. (b) Kraker, A. J.; Hartl, B. G.; Amar, A. M.; Barvian, M. R.; Showalter, H. D. H.; Moore, C. W. Biochemical and cellular effects of c-Src kinase-selective pyrido[2,3-*d*]pyrimidine tyrosine kinase inhibitors. *Biochem. Pharmacol.* **2000**, *60*, 885−898.

(9) (a) Missbach, M.; Jeschke, M.; Feyen, J.; Muller, K.; Glatt, M.; Green, J.; Susa, M. A novel inhibitor of the tyrosine kinase Src suppresses phosphorylation of its major cellular substrates and reduces bone resorption in vitro and in rodent models in vivo. *Bone* **1999**, *24*, 437−449. (b) Missbach, M.; Altmann, E.; Widler, L.; Susa, M.; Buchdunger, E.; Mett, H.; Meyer, T.; Green, J. Substituted 5,7-diphenylpyrrolo[2,3-*d*]pyrimidines: potent inhibitors of the tyrosine kinase c-Src. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 945−949. (c) Widler, L.; Green, J.; Missbach, M.; Susa, M.; Altmann, E. 7-Alkyl- and 7-cycloalkyl-5-arylpyrrolo[2,3-*d*]-pyrimidines, potent inhibitors of the tyrosine kinase c-src. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 849−852. (d) Altmann, E.; Missbach, M.; Green, J.; Susa, M.; Wagenknecht, H.-A.; Widler, L. 7-Pyrrolidinyl- and 7-piperidinyl-5-arylpyrrolo[2,3-*d*]pyrimidines, potent inhibitors of the tyrosine kinase c-src. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 853−856. (e) Sundaramoorthi, R.; Shakespeare, W. C.; Keenan, T. P.; Metcalf, C. A., III; Wang, Y.; Mani, U.; Taylor, M.; Liu, S.; Bohacek, R. S.; Narula, S. S.; Dalgarno, D. C.; van Schravandijk, M. R.; Violette, S. M.; Liou, S.; Adams, S.; Ram, M. K.; Keats, J. A.; Weigele, M.; Sawyer, T. K. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3063−3066.

(10) Hanke, J. H.; Gardner, J. P.; Dow, R. L.; Changelian, P. S.; Brissette, W. H.; Weringer, E. J.; Pollok, B. A.; Connelly, P. A. Discovery of a novel, potent, and Src family-selective tyrosine kinase inhibitor. Study of Lck- and FynT-dependent T cell activation. *J. Biol. Chem.* **1996**, *271*, 695−701.

(11) Guan, H.; Laird, A. D.; Blake, R. A.; Tang, C.; Liang, C. Design and synthesis of aminopropyl tetrahydroindole-based indolin-2-ones as selective and potent inhibitors of Src and Yes tyrosine kinase. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 187−190.

(12) (a) Shah, N. P.; Tran, C.; Lee, F. Y.; Chen, P.; Norris, D.; Sawyers, C. L. Overriding imatinib resistance with a novel Abl kinase inhibitor. *Science* **2004**, *305*, 399−401. (b) Lombardo, L. J.; Lee, F. Y.; Chen, P.; Norris, D.; Barrish, J. C.; Behnia, K.; Castaneda, S.; Cornelius, L. A. M.; Das, J.; Doweyko, A. M.; Fairchild, C.; Hunt, J. T.; Inigo, I.; Johnston, K.; Kamath, A.; Kan, D.; Klei, H.; Marathe, P.; Pang, S.; Peterson, R.; Pitt, S.; Schieven, G. L.; Schmidt, R. J.; Tokarski, J.; Wen, M.-L.; Wityak, J.; Borzilleri, R. M. Discovery of *N*-(2-chloro-6-methyl-phenyl)-2-(6-(4-(2-hydroxyethyl)-piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide (BMS-354825), a dual Src/Abl kinase inhibitor with potent antitumor activity in preclinical assays. *J. Med. Chem.* **2004**, *47*, 6658−6661.

(13) Boschelli, D. H.; Powell, D.; Golas, J. M.; Boschelli, F. Inhibition of Src kinase activity by 4-anilino-5,10-dihydropyrimido[4,5-*b*]-quinolines. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2977−2980.

(14) Ple, P. A.; Green, T. P.; Hennequin, L. F.; Curwen, J.; Fennell, M.l; Allen, J.; Lambert-van der Brempt, C.; Costello, G. Discovery of a new class of anilinoquinazoline inhibitors with high affinity and specificity for the tyrosine kinase domain of c-Src. *J. Med. Chem.* **2004**, *47*, 871−887.

(15) (a) Wang, Y. D.; Miller, K.; Boschelli, D. H.; Ye, F.; Wu, B.; Floyd, M. B.; Powell, D. W.; Wissner, A.; Weber, J. M.; Boschelli, F. Inhibitors of Src tyrosine kinase: the preparation and structure−activity relationship of 4-anilino-3-cyanoquinolines and 4-anilinoquinazolines. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2477−2480. (b) Boschelli, D. H.; Wang, Y. D.; Ye, F.; Wu, B.; Zhang, N.; Dutia, M.; Powell, D. W.; Wissner, A.; Arndt, K.; Weber, J. M.; Boschelli, F. Synthesis and Src kinase inhibitory activity of a series of 4-phenylamino-3-quinolinecarbonitriles. *J. Med. Chem.* **2001**, *44*, 822−833. (c) Boschelli, D. H.; Ye, F.; Wang, Y. D.; Dutia, M.; Johnson, S. L.; Wu, B.; Miller, K.; Powell, D. W.; Yaczko, D.; Young, M.; Tischler, M.; Arndt, K.; Discafani, C.; Etienne, C.; Gibbons, J.; Grod, J.; Lucas, J.; Weber, J. M.; Boschelli, F. Optimization of 4-phenylamino-3-quinolinecarbonitriles as potent inhibitors of Src kinase activity. *J. Med. Chem.* **2001**, *44*, 3965−3977. (d) Boschelli, D. H.; Wang, D. Y.; Ye, F.; Yamashita, A.; Zhang, N.; Powell, D.; Weber, J.; Boschelli, F. Inhibition of Src kinase activity by 4-anilino-7-thienyl-3-quinolinecarbonitriles. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2011−2014. (e) Berger, D.; Dutia, M.; Powell, D.; Wissner, A.; DeMorin, F.; Raifeld, Y.; Weber, J.; Boschelli, F. Substituted 4-anilino-7-phenyl-3-quinolinecarbonitriles as Src kinase inhibitors. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2989−2992. (f) Boschelli, D. H.; Ye, F.; Wu, B.; Wang, Y. D.; Barrios Sosa, A. C.; Yaczko, D.; Powell, D.; Golas, J. M.; Lucas, J.; Boschelli, F. Investigation of the effect of varying the 4-anilino and 7-alkoxy groups of 3-quinolinecarbonitriles on the inhibition of Src kinase activity. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3797−3800. (g) Golas, J. M.; Arndt, K.; Etienne, C.; Lucas, J.; Nardin, D.; Gibbons, J.; Frost, P.; Ye, F.; Boschelli, D. H.; Boschelli, F. SKI-606, a 4-anilino-3-quinolinecarbonitrile dual inhibitor of Src and Abl kinases, is a potent antiproliferative agent against chronic myelogenous leukemia cells in culture and causes regression of K562 xenografts in nude mice. *Cancer Res.* **2003**, *63*, 375−381. (h) Barrios Sosa, A. C.; Boschelli, D. H.; Ye, F.; Golas, J. M.; Boschelli, F. Synthesis and inhibition of Src kinase activity by 7-ethenyl and 7-ethynyl-4-anilino-3-quinolinecarbonitriles. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2155−2158. (i) Boschelli, D. H.; Wang, Y. D.; Johnson, S.; Wu, B.; Ye, F.; Barrios Sosa, A. C.; Golas, J. M.; Boschelli, F. 7-Alkoxy-4-phenylamino-3-quinolinecarbonitriles as dual inhibitors of Src and Abl kinases. *J. Med. Chem.* **2004**, *47*, 1599−1601. (j) Barrios

Sosa, A. C.; Boschelli, D. H.; Wu, B.; Wang, Y.; Golas, J. M. Further studies on ethenyl and ethynyl-4-phenylamino-3-quinolinecarbonitriles: identification of a subnanomolar Src kinase inhibitor. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1743−1747.

(16) Berger, D.; Dutia, M.; Powell, D.; Wu, B.; Wissner, A.; DeMorin, F.; Weber, J.; Boschelli, F. 8-Anilinoimidazo[4,5-*g*]quinoline-7-carbonitriles as Src kinase inhibitors. *Bioorg. Med. Chem.* Lett. **2002**, *12*, 2761−2765.

(17) Boschelli, D. H.; Ye, F. Preparation of a series of benzothieno-[3,2-*b*]pyridine-3-carbonitriles and benzofuro[3,2-*b*]pyridine-3-carbonitriles. *J. Heterocycl. Chem.* **2002**, *39*, 783−788.

(18) Wang, Y. D.; Boschelli, D. H.; Johnson, S.; Honores, E. A facile one-pot synthesis of 2-substituted-3-aminoquinolines: preparation of benzo[*b*]naphthyridine-3-carbonitriles. *Tetrahedron* **2004**, *60*, 2937−2942.

(19) Zhang, N.; Wu, B.; Wissner, A.; Powell, D. W.; Rabindran, S. K.; Kohler, C.; Boschelli, F. 4_aniline-3-cyanobenzo[*g*]quinolines as kinase inhibitors. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 423−425.

(20) (a) Boschelli, D. H.; Wu, B.; Barrios Sosa, A. C.; Durutlic, H.; Ye, F.; Raifeld, Y.; Golas, J. M.; Boschelli, F. Identification of 7-phenylaminothieno[3,2-*b*]pyridine-6-carbonitriles as a new class of Src kinase inhibitors. *J. Med. Chem.* **2004**, *47*, 6666−6668. (b) Boschelli, D. H.; Wu, B.; Barrios Sosa, A. C.; Durutlic, H.; Chen, J. J.; Wang, Y.; Golas, J. M.; Lucas, J.; Boschelli, F. Synthesis and Src kinase activity of 2-phenyl and 2-thienyl-7-phenylaminothieno[3,2-*b*]pyridine-6-carbonitriles. *J. Med. Chem.* **2005**, *48*, 3891−3902.

(21) (a) Boschelli, F.; Weber, J. M.; Lucas, J.; Etienne, C.; Arndt, K.; Boschelli, D. H.; Dutia, M.; Powell, D.; Wu, B.; Yazcko, D.; Ye, F. Characterization of a Novel Class of Src Kinase Inhibitors. *Proc. Am. Assoc. Cancer Res.* **2002**, *43*, 849 (Abstract 4206). (b) Donato, N. J.; Wu, J. Y.; Talpaz, M.; Dutia, M.; Ye, F.; D Boschelli, D.; Boschelli. F. Novel tyrosine kinase inhibitors suppress BCR-ABL signaling and induce apoptosis in STI-571 sensitive and resistant CML cells. Presented at the 44th Annual Meeting of the American Society of Hematology, Philadelphia, PA, December, 2002; Abstract 1434. (c) Dutia, M.; Birnberg, G. H.; Wang, Y. D.; DeMorin, F.; Boschelli, D. H.; Powell, D. W.; Golas, J. M.; Boschelli, F. Synthesis and SAR of 7,8-dialkoxy-4-anilinobenzo[*g*]quinoline-3-carbonitriles as potent Src kinase inhibitors. *Abstracts of Papers*, 228th National Meeting of the American Chemical Society, Philadelphia, PA, August 22−26, 2004; American Chemical Society: Washington, DC, 2004; MEDI-228.

(22) Kienzle, F. Die Reaktion von Phthalaldehyden mit 3-Nitropropionsäureestern. Ein einfacher Zugang zu 3-Nitro-2-naphthoesäuren. (The reaction of phthalaldehydes with 3-nitropropionic esters. An easy access to 3-nitro-2-naphthoic acids). *Helv. Chim. Acta* **1980**, *63*, 2364−2369.

(23) Berger, D. M.; Birnberg, G.; DeMorin, F.; Dutia, M.; Powell, D.; Wang, Y. D. Regioselective synthesis of a potent Src kinase inhibitor: 4-(2,4-dichloro-5-methoxyphenylamino)-7-methoxy-8-(2-morpholin-4-yl-ethoxy)benzo[*g*]quinoline-3-carbonitrile. *Synthesis* **2003**, 1712−1716.

(24) Boschelli, F.; Golas, J. M. Unpublished observations.

(25) Staley, C. A.; Parikh, N. U.; Gallick, G. E. Decreased tumorigenicity of a human colon adenocarcinoma cell line by an antisense expression vector specific for c-Src. *Cell Growth Differ.* **1997**, *8*, 269−274.

(26) Rush, T. S., III; Manas, E.; Tawa, G.; Alvarez, J. Solvation-based scoring for high throughput docking. In *Virtual Screening in Drug Discovery*; Alvarez, J., Shoichet, B., Eds.: CRC Press: Boca Raton, FL, 2005; pp 249−277.

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