Synthesis and Src Kinase Inhibitory Activity of 2-Phenyl- and 2-Thienyl-7-phenylaminothieno[3,2-*b*]pyridine-6-carbonitriles

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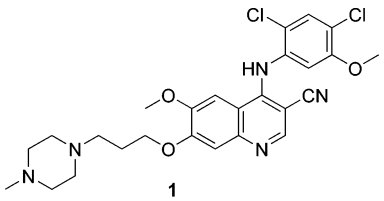
2-Phenyl-7-phenylaminothieno[3,2-*b*]pyridine-6-carbonitriles were recently reported to be inhibitors of Src kinase activity. In this study we present structure−activity relationships for additional thieno[3,2-*b*]pyridine-6-carbonitriles, modifying the substituents on the C-2 phenyl and C-7 phenylamino groups. Derivatives with various aminomethyl and aminoethyl substituents on the para position of the C-2 phenyl group retained the activity of the initial analogues. However, direct attachment of an amino group led to decreased activity. A 2,4-dichloro-5-methoxyphenylamino group at C-7 provided superior inhibition of Src enzymatic activity. Replacement of the C-2 phenyl group with a 3,5-substituted thiophene led to improved Src inhibitory activity compared to the parent compound, but other thiophene isomers were less active. One of the analogues reported here exhibited in vivo activity comparable to that of SKI-606, a related 3-quinolinecarbonitrile currently in clinical trials.

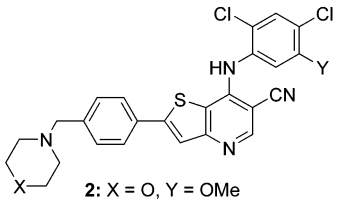
Introduction

The nonreceptor tyrosine kinase Src is the prototype member of the Src family of kinases (SFKs). The SFKs share a strong structural similarity but vary in their modes of expression. While Src, Yes, and Fyn are found in many cell types, other SFKs, including Lck and Lyn, have a more limited expression. Src acts in many cell signaling pathways,[1,2] and small molecule inhibitors of Src are being investigated as potential agents for the treatment of a variety of diseases. Early on, these efforts focused on cancer but soon expanded to additional therapeutic areas, including osteoporosis and stroke.[3−6] Recently, results supporting a critical role for Src in tumor metastasis have heightened interest in Src inhibitors.[7−11]

Wyeth has extensively studied 4-phenylamino-3-quinolinecarbonitriles as inhibitors of diverse kinases, including EGFR, HER-2, MEK, and Src.[12,13] Optimization of this class of compounds as Src inhibitors led to the identification of SKI-606, **1**, presently in clinical trials for the treatment of solid tumors.[14,15] We recently reported that replacement of the phenyl ring of the 3-quinolinecarbonitrile core with a thiophene ring to provide a thieno[3,2-*b*]pyridine-6-carbonitrile allowed for retention of Src kinase inhibitory activity.[16] Addition of a suitably substituted phenyl ring at C-2 of the thieno[3,2-*b*]pyridine ring was preferred over the same substitution at C-3. The limited initial structure−activity relationships for this new class of kinase inhibitor also showed that replacement of the morpholine group of **2** with a 1-methylpiperazine group increased the Src inhibitory activity, with **3** having an IC_{50} of 13 nM in a Src enzymatic assay, compared to an IC_{50} of 34 nM for **2**. Removal of the 5-methoxy substituent on the 7-phenylamino group of **3** decreased the activity, with **4** having an IC_{50} of 50 nM in the enzyme assay. It was also shown that para substitution on the C-2 phenyl ring was favored over meta or ortho substitution. We present here additional thieno[3,2-*b*]pyridine-6-carbonitrile analogues, varying the substituents on the phenyl group at C-2 and the phenylamino group at C-7. Some C-2 thiophene analogues were also evaluated.



1
SKI-606



2: X = O, Y = OMe
3: X = N-Me, Y = OMe
4: X = N-Me, Y = H

Chemistry

As depicted in Scheme 1, analogues of **3** with various substituted aminomethyl groups on the C-2 phenyl ring were prepared via reductive amination of the key aldehyde intermediate **5**[16] employing a variety of heterocyclic and alkylamines. Initially, this reaction was carried out in a solvent system consisting of dichloromethane and *N*,*N*-dimethylformamide. However, in some cases the presence of *N*,*N*-dimethylformamide resulted in the formation of small amounts of the dimethylamine analogue **11** along with the desired

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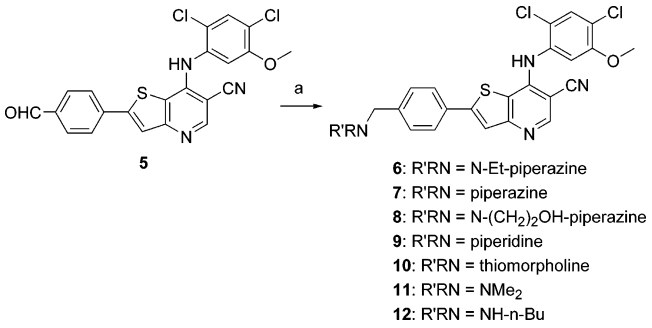
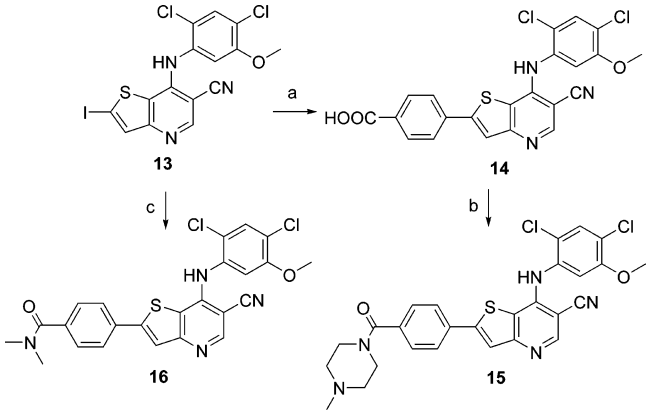
Scheme 1[a]



[a] Reagents: (a) R′RNH, $Na(OAc)_3BH$, CH_2Cl_2, NMP or DMF, HOAc.

Scheme 2[a]



[a] Reagents: (a) 4-carboxyphenylboronic acid, $(Ph_3P)_4Pd$, DME, sat. aq $NaHCO_3$; (b) (1) CDI, DMF; (2) 1-methylpiperazine; (c) 4-dimethylaminocarbonylphenylboronic acid, $(Ph_3P)_4Pd$, DMF, sat. aq $NaHCO_3$.

product. To avoid this side reaction, the later examples were prepared using 1-methyl-2-pyrrolidinone as a cosolvent.

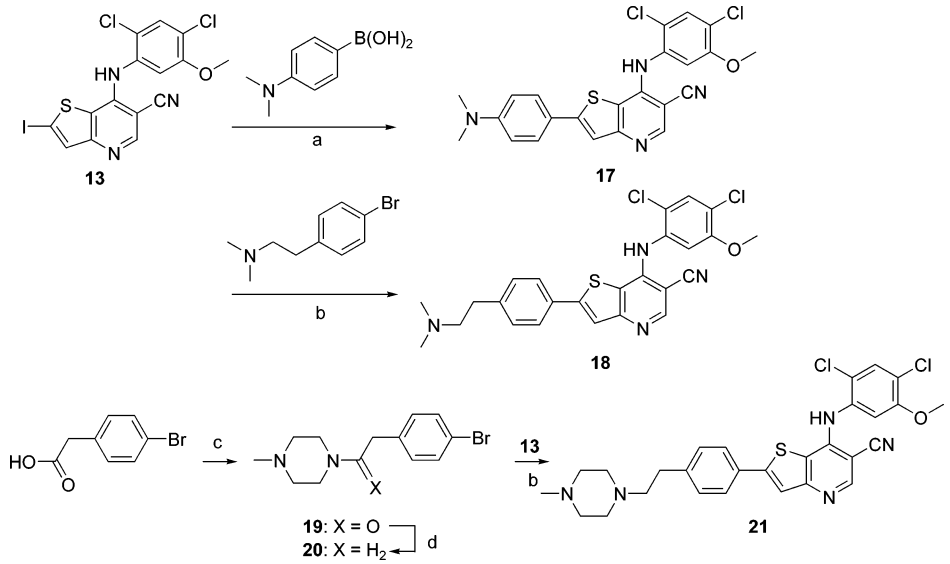
Compounds with an amide group on the C-2 phenyl ring were synthesized from the 2-iodo intermediate, **13**[16] (Scheme 2). Reaction of **13** with 4-carboxyphenylboronic acid in the presence of tetrakis(triphenylphosphine)-palladium(0) provided the acid derivative **14**. Treatment of **14** with 1,1′-carbonyldiimidazole followed by the addition of 1-methylpiperazine resulted in formation of the amide analogue **15**. The dimethyl amide analogue **16** was prepared by direct coupling of **13** with 4-(dimethylaminocarbonyl)phenylboronic acid.

Analogues of **11**, wherein the methylene linker on the C-2 phenyl group was replaced by either a bond or extended to an ethylene group, were prepared as shown in Scheme 3. Reaction of **13** with 4-(dimethylamino)-phenylboronic acid provided **17**, while reaction of **13** with the boronic acid generated from 4-bromo-*N*,*N*-dimethylphenethylamine[17] provided **18**. To prepare the ethylene analogue of **3**, it was necessary to first synthesize the bromo derivative **20**. Treatment of 4-bromophenylacetic acid with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide followed by reaction with 1-methylpiperazine provided the amide derivative **19**, which was reduced with borane−methyl sulfide complex to give the desired **20**. Subsequent coupling of **20** with **13**, following the conditions for the preparation of **18**, provided **21**.

Variation of the C-7 phenylamino group was carried out as depicted in Scheme 4. The 2-iodo-7-chloro[3,2-*b*]-thienopyridine **22**[16] was treated with 2,4-dichloro-5-ethoxyaniline in the presence of sodium hydride to provide **23**. Treatment of **22** with 4-phenoxyaniline in the presence of pyridine hydrochloride provided **24**. Coupling of **23** and **24** with 4-formylphenylboronic acid led to the aldehyde derivatives **25** and **26**. Subsequent reductive amination of **25** and **26** with 1-methylpiperazine gave **27** and **28**, the C-7 2,4-dichloro-5-ethoxy and 4-phenoxyphenylamino analogues of **3**.

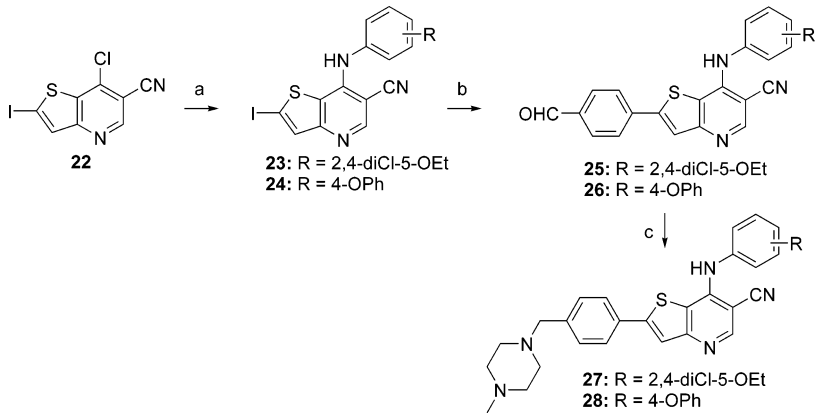
Scheme 5 shows the preparation of **30**, the 2,4-dichlorophenylamino analogue of **11**. Reductive amination of **29**[16] with dimethylamine provided **30**. This analogue was also prepared by an alternative route, in which the order of the steps was reversed. To this end, treatment of **22** with a slight excess of 4-formylphenylboronic acid resulted in displacement of the 2-iodo group to provide **31**. If an excess of the boronic acid was used, displacement of the 7-chloro group was also observed.

Scheme 3[a]



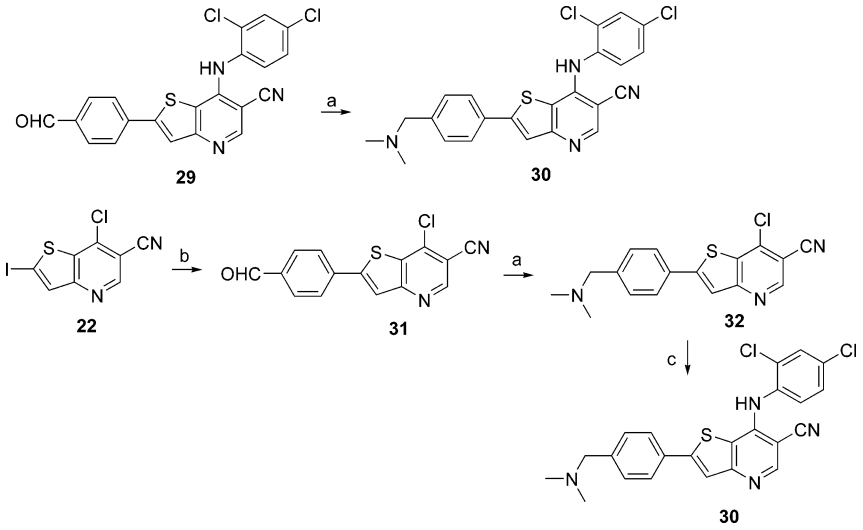
[a] Reagents: (a) $(Ph_3P)_4Pd$, DME, sat. aq $NaHCO_3$; (b) (1) n-BuLi, $B(OPr)_3$, THF; (2) $(Ph_3P)_4Pd$, DME, sat. aq $NaHCO_3$; (c) EDCI, DMAP, 1-methylpiperazine, CH_2Cl_2 (d) (1) BH_3-Me_2S, THF; (2) 1 N NaOH, EtOH.

Scheme 4[a]



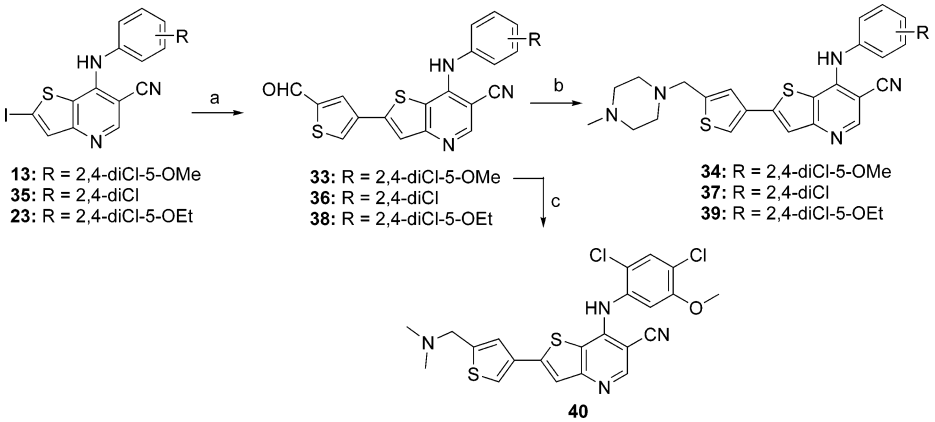
[a] Reagents: (a) aniline, NaH, THF or pyridine hydrochloride, 2-ethoxyethanol; (b) 4-formylphenylboronic acid, $(Ph_3P)_4Pd$, DME, sat. aq $NaHCO_3$; (c) 1-methylpiperazine, $Na(OAc)_3BH$, CH_2Cl_2, NMP or DMF, HOAc.

Scheme 5[a]



[a] Reagents: (a) dimethylamine, $Na(OAc)_3BH$, CH_2Cl_2, DMF, HOAc; (b) 4-formylphenylboronic acid, $(Ph_3P)_4Pd$, DME, sat. aq $NaHCO_3$; (c) 2,4-dichloroaniline, NaH, THF.

Scheme 6[a]

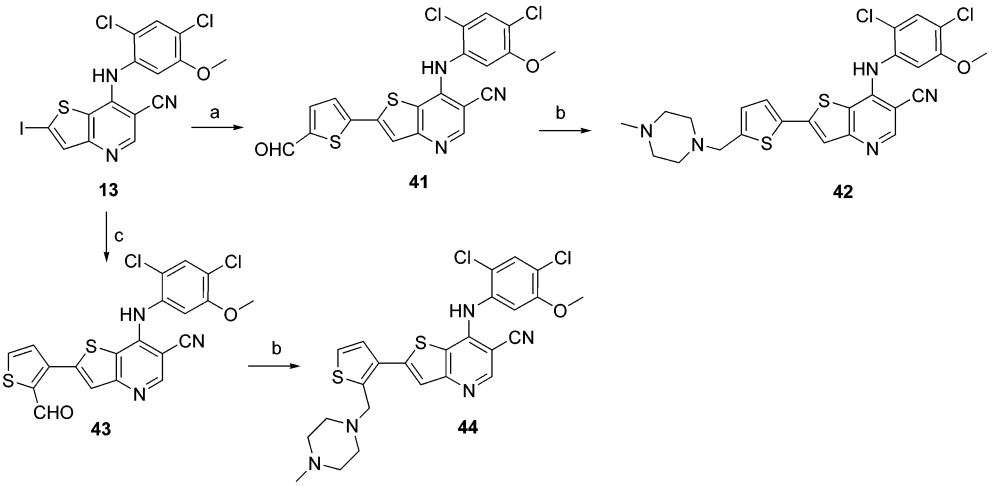


[a] Reagents: (a) (1) tributyl-(5-[1,3]dioxolan-2-ylthiophen-3-yl)stannane, $(Ph_3P)_2Cl_2Pd$, dioxane; (2) 1 N HCl, THF; (b) 1-methylpiperazine, $Na(OAc)_3BH$, CH_2Cl_2, NMP or DMF, HOAc; (c) dimethylamine, $Na(OAc)_3BH$, CH_2Cl_2, NMP, HOAc.

Reductive amination of **31** with dimethylamine provided the key intermediate **32**. Displacement of the 7-chloro group of **32** with 2,4-dichloroaniline resulted in **30**. While the first route to **30** allows for ready variation of the basic amine group on the phenyl ring at C-2, the later route allows for ready variation of the phenylamino group at C-7.

The preparation of analogues containing a 3,5-disubstituted thiophene group at C-2 is depicted in Scheme 6. The iodo intermediate **13** was treated with tributyl-(5-[1,3]dioxolan-2-ylthiophen-3-yl)stannane[18] in the presence of bis(triphenylphosphine)palladium(II) chloride. Hydrolysis of the intermediate acetal with aqueous hydrochloric acid provided the aldehyde **33**. Reductive

Scheme 7[a]



[a] Reagents: (a) (1) tributyl-(5-[1,3]dioxolan-2-ylthiophen-2-yl)stannane, $(Ph_3P)_2Cl_2Pd$, dioxane; (2) 1 N HCl, THF; (b) 1-methylpiperazine, $Na(OAc)_3BH$, CH_2Cl_2, NMP, HOAc; (c) 2-formyl-3-thiopheneboronic acid, $(Ph_3P)_4Pd$, DME, sat. aq $NaHCO_3$.

amination with 1-methylpiperazine under standard conditions gave **34**. In an analogous fashion, the iodo intermediates **35** and **23** were converted to the aldehydes **36** and **38** with subsequent reductive amination, leading to **37** and **39**. The dimethylamino analogue **40** was prepared via reductive amination of **33** with dimethylamine.

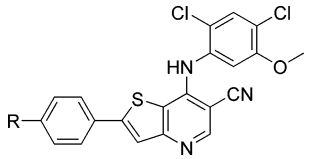
As shown in Scheme 7, **42**, the 2,5 isomer of the 3,5-disubstituted thiophene **34**, was prepared in an analogous fashion. Treatment of **13** with tributyl(5-[1,3]-dioxolan-2-ylthiophen-2-yl)stannane[18] followed by acid hydrolysis gave **41**, with subsequent reductive amination with 1-methylpiperazine providing **42**. En route to the 4,5 isomer **44**, **13** was reacted with commercially available 2-formyl-3-thiopheneboronic acid, resulting in the direct formation of aldehyde **43**. Reductive amination of **43** with 1-methylpiperazine gave **44**.

Results and Discussion

The ability of the compounds to inhibit Src activity was measured in both a LANCE format enzyme assay[19] and in a cell proliferation assay[20] utilizing c-Src transformed rat fibroblasts. As shown in Table 1, variation of the substituted aminomethyl group on the C-2 phenyl ring (analogues **6**−**12**) did not have a large effect on the inhibition of Src enzymatic activity. Most compounds had IC_{50}s within 2-fold of that of **3**, with the exception being the thiomorpholine analogue **10,** which was 4 times less active, having an IC_{50} of 52 nM. A similar loss of activity with this derivative was also seen in the Src cell assay, with **10** having an IC_{50} of only 3.0 μM. In general, a greater variation in activity upon changing the amine substituent was observed in the Src cell assay than in the enzyme assay. Improved Src cell activity compared to **3** was observed for the unsubstituted piperazine analogue **7**, the ethanol substituted piperazine analogue **8**, and the dimethylamine analogue **11**, which had IC_{50}s of 200, 420, and 350 nM, respectively.

Replacement of the methylene group of **3** with a carbonyl group, to provide **15**, did not affect the inhibition of Src enzyme activity but did increase the activity in the Src cell assay. Interestingly, while **15** and its dimethylamine analogue, **16**, had similar activities in the enzyme assays (IC_{50}s of 13 and 23 nM), the later compound was less active in the cell assay than was expected (IC_{50}s for **15** and **16** of 330 nM and 1.4 μM, respectively). Analogue **17**, wherein the methylene group of **11** was removed, had reduced enzyme and cell activity. However, extending the methylene group of both **11** and **3** to an ethylene group did not substantially change the activity, with **18** and **21** having similar IC_{50}s to those of their parent compounds.

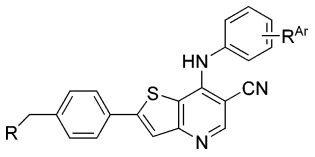
Table 1. Inhibition of Src Kinase Activity by 7-[(2,4-Dichloro-5-methoxyphenyl)amino]-2-phenylthieno[3,2-*b*]pyridine-6-carbonitriles



	R	IC_{50}, nM (SD)[36] Src enzyme	Src cells
1	SKI-606	3.8[19]	100[14]
2	morpholine	34[16]	1200[16]
3	CH_2-N-Me-piperazine	13[16]	720[16]
6	CH_2-N-Et-piperazine	8.2 (1.3)	630 (71)
7	CH_2-piperazine	7.2 (1.5)	200 (21)
8	CH_2-N-(CH_2CH_2OH)-piperazine	7.4 (1.3)	420 (120)
9	CH_2-piperidine	17 (2.1)	850 (200)
10	CH_2-thiomorpholine	52 (8.5)	3000 (180)
11	CH_2NMe_2	9.1 (2.1)	350 (96)
12	CH_2NH-*n*-Bu	11 (1.4)	890 (72)
15	C(O)-N-Me-piperazine	13 (2.8)	330 (81)
16	C(O)-NMe_2	23 (1.6)	1400 (350)
17	NMe_2	110 (18)	1300 (200)
18	$(CH_2)_2NMe_2$	8.8 (1.7)	390 (100)
21	$(CH_2)_2$N-Me-piperazine	15 (4.1)	630 (70)

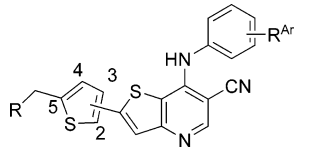
We had earlier reported that **4**, which has a 2,4-dichlorophenylamino group at C-7 was about 4 times less active than **3** in both the Src enzyme and cell assays, showing the importance of the 5-methoxy group (Table 2). This same effect was earlier seen in the 3-quinolinecarbonitrile series, where it was also observed that replacement of the 5-methoxy substituent of the 2,4-dichloro-5-methoxyphenylamino group at C-4 with a larger substituent, such as ethoxy or *n*-propoxy, led to decreased activity. This loss of activity was also seen in the thieno[3,2-*b*]pyridine series, with **27**, the 4-dichloro-5-ethoxyphenylamino analogue of **3**, having

Table 2. Inhibition of Src Kinase Activity by Variously Substituted 7-Phenylamino-2-phenylthieno[3,2-*b*]pyridine-6-carbonitriles



	R^{Ar}	R	IC_{50}, nM (SD)[36] Src enzyme	Src cells
3	2,4-diCl-5-OMe	N-Me-piperazine	13[16]	720[16]
4	2,4-diCl	N-Me-piperazine	50[16]	4000[16]
27	2,4-diCl-5-OEt	N-Me-piperazine	980 (54)	>10000
28	4-OPh	N-Me-piperazine	140 (4.2)	>10000
30	2,4-diCl	NMe_2	53 (4.9)	3300 (560)

Table 3. Inhibition of Src Kinase Activity by Variously Substituted 7-Phenylamino-2-thiophenylthieno[3,2-*b*]pyridine-6-carbonitriles



	R^{Ar}	isomer	R	IC_{50}, nM (SD)[36] Src enzyme	Src cells
34	2,4-diCl-5-OMe	3,5	N-Me-piperazine	7.2 (0.07)	430 (67)
37	2,4-diCl	3,5	N-Me-piperazine	67 (7.8)	1900 (150)
39	2,4-diCl-5-OEt	3,5	N-Me-piperazine	1300 (220)	6200 (1700)
40	2,4-diCl-5-OMe	3,5	NMe_2	14 (0.71)	690 (150)
42	2,4-diCl-5-OMe	2,5	N-Me-piperazine	25 (2.1)	2400 (71)
44	2,4-diCl-5-OMe	4,5	N-Me-piperazine	420 (140)	3100 (270)

an IC_{50} of 980 nM in the Src enzyme assay and an IC_{50} of greater than 10 μM in the Src cell assay. Reduced Src inhibition was also observed with **28**, which contains a 4-phenoxyphenylamino group at C-7. This result was anticipated, since it was earlier shown that 3-quinolinecarbonitriles with a 4-phenoxyphenylamino group at C-4 were potent inhibitors of MEK, not Src.[21] Variation of the amine group of **4** from 1-methylpiperazine to dimethylamine, **30**, did not have a large effect on the Src inhibitory activity. Therefore, the substitution on the aminomethyl group of the C-2 phenyl is not as crucial to the inhibition of Src activity as is the substitution on the C-7 phenylamino group.

It had been shown earlier in the 3-quinolinecarbonitrile series of Src inhibitors that 3,5- and 2,5-disubstituted thiophene[18] analogues at C-7 had improved Src inhibitory activity compared to the C-7 *p*-phenyl-substituted analogue.[22] In the thieno[3,2-*b*]pyridine series, the 3,5-disubstituted thiophene analogue, **34**, had improved Src enzyme and cell activity over the *p*-phenyl-substituted analogue **3** (data shown in Table 3). For this series of 3,5-disubstituted thiophene analogues at C-2, replacement of the 2,4-dichloro-5-methoxyphenylamino group of **34** with a 2,4-dichloro or a 2,4-dichloro-5-ethoxyphenylamino group led to a greater decrease in Src enzyme activity than was observed with the C-2 phenyl analogues. Analogues **37** and **39** had IC_{50}s in this assay of only 67 and 1300 nM, compared to an IC_{50} of 7.2 nM for **34**. The dimethylamine analogue of **34**, namely **40**, was less active than **34** in both the Src enzyme and cell assays. A greater decrease in activity was observed with **42**, the 2,5-substituted thiophene isomer of **34**, which had IC_{50}s of only 25 nM and 2.4 μM in the enzyme and cell assays, respectively. This result is in contrast to that observed in the 3-quinolinecarbonitrile series, where the 2,5-disubstituted thiophene analogue had comparable activity to the 3,5-disubstituted thiophene isomer. However, the loss of activity of the 4,5-disubstituted thiophene **44** did correlate with what was seen in the 3-quinolinecarbonitrile series. This isomer was less active than **34**, with **44** having IC_{50}s of only 420 nM and 3.1 μM in the Src enzyme and cell assays, respectively.

Table 4. Nude Mouse Plasma Levels (μg/mL) Following a 50 mg/kg Oral Dose

	plasma concentration			plasma concentration	
compd	4 h	24 h	compd	4 h	24 h
3	2.2	0.40	**11**	0.20	0.002
7	1.1	0.003	**15**	1.6	0.059
8	1.7	0.069	**34**	1.0	0.32

To select compounds for in vivo testing in nude mouse xenografts, plasma levels were obtained for **3**, **7**, **8**, **11**, and **15**. These compounds were chosen because they were among the most potent in the Src cell assay and also to include a variety of heterocyclic and dialkylamine and amide substituents. The C-2 thiophene derivative **34** was also selected for PK profiling. In this study, nude mice were administered a single 50 mg/kg oral dose of each compound, in a 0.5% methylcellulose/0.4% Tween 80 vehicle. Plasma samples were drawn at 4 and 24 h and analyzed for the amount of compound remaining. As shown in Table 4, the 1-methylpiperazine analogues **3** and **34** had superior blood levels at 24 h compared to the other four analogues. It should be noted that under these dosing conditions, SKI-606 had a 24 h plasma level of 0.04 μg/mL. Thus, although **3** and **34** were less effective Src inhibitors in vitro compared to SKI-606, they had higher exposure levels in the efficacy species and were therefore suitable candidates for in vivo testing.

Nude mice were implanted with HT29 cells, a human colon cancer line, and the tumors were staged to 200−300 mm^3. In the initial study, animals were treated with 150 mg/kg of **3** orally once a day for 14 days, with SKI-606 run as a control. As shown in Figure 1, these two compounds had similar efficacy in this model with **3** and SKI-606 providing T/Cs of 37 and 31%, respectively, when measured on the last day of dosing. Measurement of the tumors 7 days postdosing gave T/Cs of 45 and 37% for **3** and SKI-606, respectively. In this study (data not shown) animals were also dosed with 75 and 50 mg/kg of **3** orally, once a day. A dose response was observed, with these administered amounts providing T/Cs of 47 and 69%, respectively. In a second HT29 xenograft study, **34** was run side by side with SKI-606. Both compounds were dosed at 150 mg/kg orally once a day for 12 days with SKI-606 providing a T/C of 44% compared to a T/C of only 57% for **34**.

We previously reported that **3** did not inhibit several other kinases including KDR, CDK4, or MEK/Raf.[16] Further profiling showed that **3** also had no effect on additional kinases, including p38, IKK, and PDK1. However, when tested in an Lck cell assay, **3** had an IC_{50} of 71 nM. This cell proliferation assay uses rat

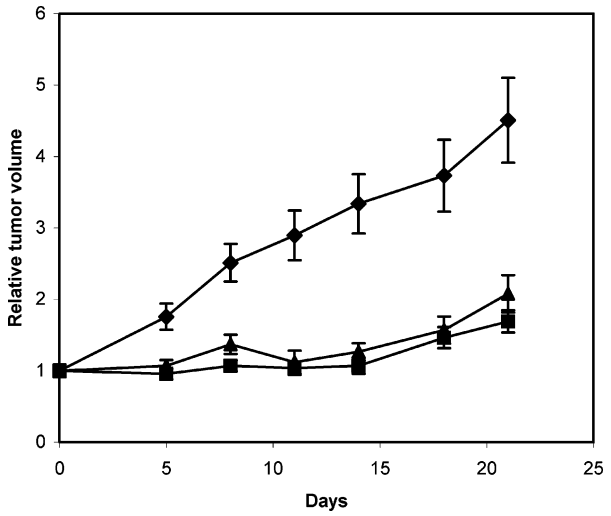


Figure 1. HT-29 xenograft study with **3** (▲) and SKI-606 (■) dosed at 150 mg/kg, po, qd, compared to vehicle (◆).

fibroblasts stably transfected with a plasmid expressing activated Lck. Since these Lck dependent cells were prepared in an identical manner to the Src dependent cells and the assays were run under the same conditions, these cell assays provide a reliable ratio of the activity of a compound against these two SFKs. The inhibition of Lck by **3** was predicted due to the extensive sequence conservation in the catalytic domains of Lck and Src. We had also previously reported that **3** inhibited the tyrosine kinase Abl, with an IC_{50} of 2.3 nM.[16] This result also was not surprising, since SKI-606 inhibited Abl kinase activity with an IC_{50} of 1.1 nM.[15,19] In a proliferation assay with K562 cells, a human chronic myelogenous leukemia (CML) cell line dependent on Abl kinase activity, **3** had an IC_{50} of 32 nM, similar to the IC_{50} of 20 nM observed for SKI-606.[19]

In addition to the 3-quinolinecarbonitriles, compounds from many other structurally diverse series that were initially reported to be Src kinase inhibitors were later found to also inhibit Abl.[23,24] The ability of these derivatives to inhibit both Src and Abl is attributed to the similar active conformations of the ATP binding sites of these two kinases. A chromosomal abnormality leads to the expression of Bcr/Abl, a constitutively active form of Abl. The presence of this kinase is the hallmark of CML. Although the Abl inhibitor imatinib is highly effective in treating the early stages of CML, patients often develop resistance, frequently as a result of mutations in the Abl kinase domain.[25,26] It has been documented that several dual Src/Abl inhibitors retain activity against many of these Abl mutations.[27−31] Furthermore, since SFKs, including Lyn and Hck, play a role in Abl signaling,[32,33] dual inhibitors of Abl and Src are being pursued as alternative therapies to imatinib.[23,24] One of these dual inhibitors, BMS-354825, is currently in clinical trials for the treatment of imatinib-resistant CML.[34,35]

Conclusion

Further SAR analysis of various 2-phenyl-7-phenylaminothieno[3,2-*b*]pyridine-6-carbonitriles has shown that the substitution on the phenylamino group at C-7 is crucial for potent Src inhibitory activity, while the solublizing amine substituent on the phenyl ring at C-2 is required for good bioavailability. These findings correlate with what was observed for the 3-quinolinecarbonitrile series, where the best headpiece for Src inhibition was also a 2,4-dichloro-5-methoxyphenylamino group and where a 1-methylpiperazine group also afforded the highest plasma levels. Replacement of the phenyl group at C-2 of the thieno[3,2-*b*]pyridine core with a 3,5-substituted thiophene also retains the Src inhibitory activity, provided that the 2,4-dichloro-5-methoxyphenylamino group at C-7 is preserved. One of the thieno[3,2-*b*]pyridines reported here, **3**, is a dual inhibitor of Src and Abl kinases, inhibits the proliferation of a CML line, and has in vivo activity in a colon tumor xenograft model. We are continuing to examine **3** in additional in vitro and in vivo models.

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 DRX-400 spectrometer. Chemical shifts (δ) are reported in parts per million referenced to Me_4Si. Electrospray (ES) mass spectra were recorded in either positive or negative mode on either a Micromass Platform or an LCT spectrometer. Electron impact (EI) and high-resolution mass spectra (HRMS) were obtained on a Finnigan MAT-90 spectrometer. HRMS were also obtained on a Bruker Apex II 9.4T FTMS.

Solvents and reagents that were obtained from commercial sources were used without purification, unless noted. The reported yields are for purified material and are not optimized. Reactions were carried out under an inert atmosphere of either nitrogen or argon. Flash chromatography was performed with Baker 40 μM silica gel. Preparative thin-layer chromatography was performed on silica gel GF/UV 254, 2000 μm, 20 × 20 cm plates from ANALTECH.

7-[(2,4-Dichloro-5-methoxyphenyl)amino]-2-{4-[(4-ethylpiperazin-1-yl)methyl]phenyl}thieno[3,2-*b*]pyridine-6-carbonitrile (6). 1-Ethylpiperazine (73 μL, 0.57 mmol) was added to a suspension of **5** (200 mg, 0.44 mmol) in 8 mL of dichloromethane and 2 mL of *N*,*N*-dimethylformamide. The reaction mixture was cooled to 0 °C and sodium triacetoxyborohydride (470 mg, 2.2 mmol) was added followed by 2 drops of acetic acid. The reaction mixture was warmed to room temperature for 6 h. Additional 1-ethylpiperazine (75 μL, 0.58 mmol), sodium triacetoxyborohydride (100 mg, 0.47 mmol), and 2 drops of acetic acid were added, and the reaction mixture was left at room temperature overnight. The reaction was quenched by the addition of ethyl acetate and water. The organic layer was separated, dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was triturated with hot diethyl ether, filtered, and dried. The resulting solid was suspended in hot water, filtered, and dried to give 47 mg of **6** as an off-white solid: mp 198−200 °C; ^{1}H NMR ($CDCl_3$) δ 1.13 (t, J = 7 Hz, 3H), 2.51 (br s, 10H), 3.56 (s, 2H), 3.81 (s, 3H), 6.81 (s, 1H), 6.90 (s, 1H), 7.38 (d, J = 8 Hz, 2H), 7.53 (d, J = 8 Hz, 2H), 7.56 (s, 1H), 7.64 (s, 1H), 8.66 (s, 1H); MS 552.0 $(M + H)^+$. Anal. ($C_{28}H_{27}Cl_2N_5OS\cdot1.4H_2O$) C, H, N.

7-[(2,4-Dichloro-5-methoxyphenyl)amino]-2-[4-(piperazin-1-ylmethyl)phenyl]thieno[3,2-*b*]pyridine-6-carbonitrile (7). Piperazine (1.14 g, 13 mmol) was added to a suspension of **5** (2.0 g, 4.4 mmol) in 40 mL of dichloromethane and 5 mL of 1-methyl-2-pyrrolidinone. The reaction mixture was cooled to 0 °C and sodium triacetoxyborohydride (4.7 g, 22 mmol), followed by 0.5 mL of acetic acid, was added. The reaction mixture was warmed to room temperature and stirred for 3 h. The reaction was quenched by the addition of saturated aqueous potassium carbonate and dichloromethane. The organic layer was separated and dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography eluting with a gradient of 2−5% methanol in dichloromethane followed by 10−20% methanol and 1% ammonium hydroxide in dichloromethane

to provide 1.10 g of **7** as a white solid: ^{1}H NMR (DMSO-d_6) δ 2.40 (br s, 4H), 2.84 (t, J = 4 Hz, 4H), 3.17 (s, 1H), 3.51 (s, 2H), 3.84 (s, 3H), 7.23 (s, 1H), 7.41 (d, J = 8 Hz, 2H), 7.67 (d, J = 8 Hz, 2H), 7.71 (s, 1H), 7.85 (s, 1H), 8.53 (s, 1H); MS 524.1 (M + H)$^+$. Anal. ($C_{26}H_{23}Cl_2N_5OS\cdot H_2O$) C, H, N.

7-[(2,4-Dichloro-5-methoxyphenyl)amino]-2-(4-{[4-(2-hydroxyethyl)piperazin-1-yl]methyl}phenyl)thieno[3,2-*b*]pyridine-6-carbonitrile (8). 1-(2-Hydroxyethyl)piperazine (1.72 g, 13.20 mmol) was added to a suspension of **5** (2.0 g, 4.40 mmol) in 48 mL of dichloromethane and 3.5 mL of 1-methyl-2-pyrrolidinone. The reaction mixture was cooled to 0 °C and sodium triacetoxyborohydride (4.66 g, 22.0 mmol) was added. After stirring at 0 °C for 5 min, 0.3 mL of acetic acid was added and the reaction mixture warmed to room temperature and stirred for 1.5 h. The reaction was quenched by the addition of water and then partitioned between aqueous sodium bicarbonate and dichloromethane. The organic layer was washed with saturated aqueous sodium chloride, dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography eluting with a gradient of 1% to 20% methanol in dichloromethane to provide 1.4 g of **8** as a white solid: mp 204−206 °C; ^{1}H NMR (DMSO-d_6) δ 2.38 (br s, 10H), 3.45−3.48 (m, 4H), 3.85 (s, 3H), 4.37 (br s, 1H), 7.37 (s, 1H), 7.40 (d, J = 8 Hz, 2H), 7.67 (d, J = 8 Hz, 2H), 7.76 (s, 1H), 7.89 (s, 1H), 8.60 (s, 1H), 9.74 (s, 1H); MS 568.0, 570.1 (M + H)$^+$. Anal. ($C_{28}H_{27}Cl_2N_5O_2S\cdot H_2O$) C, H, N.

7-[(2,4-Dichloro-5-methoxyphenyl)amino]-2-[4-(piperidin-1-ylmethyl)phenyl]thieno[3,2-*b*]pyridine-6-carbonitrile (9). Following the route used to prepare **7**, **9** was obtained as an off-white solid in 47% yield from **5**: ^{1}H NMR (DMSO-d_6, TFA) δ 1.34−1.37 (m, 1H), 1.55−1.66 (m, 3H), 1.79−1.83 (m, 2H), 2.84−2.92 (m, 2H), 3.30−3.48 (m, 2H), 3.86 (s, 1H), 4.33 (d, J = 4 Hz, 2H), 7.51 (s, 1H), 7.63 (d, J = 8 Hz, 2H), 7.80 (d, J = 8 Hz, 2H), 7.84 (s, 1H), 8.00 (s, 1H), 8.74 (s, 1H), 9.62 (brs, 1H); MS 523.0 (M + H)$^+$. Anal. ($C_{27}H_{24}Cl_2N_4OS\cdot 0.6H_2O$) C, H, N

7-[(2,4-Dichloro-5-methoxyphenyl)amino]-2-[4-(thiomorpholin-4-ylmethyl)phenyl]thieno[3,2-*b*]pyridine-6-carbonitrile (10). Following the route used to prepare **7**, **10** was obtained as an off-white solid in 55% yield from **5**: mp 198−201 °C; ^{1}H NMR (DMSO-d_6) δ 2.62 (br s, 8H), 3.53 (s, 2H), 3.85 (s, 3H), 7.37 (s, 1H), 7.41 (d, J = 8 Hz, 2H), 7.65 (d, J = 8 Hz, 2H), 7.77 (s, 1H), 7.90 (s, 1H), 8.60 (s, 1H), 9.63 (s, 1H); MS 541.0 (M + H)$^+$. Anal. ($C_{26}H_{22}Cl_2N_4OS_2\cdot H_2O$) C, H, N.

7-[(2,4-Dichloro-5-methoxyphenyl)amino]-2-{4-[(dimethylamino)methyl]phenyl}thieno[3,2-*b*]pyridine-6-carbonitrile (11). A solution of 2 M dimethylamine in tetrahydrofuran (1.73 mL, 3.45 mmol) was added to a suspension of **5** (312 mg, 0.69 mmol) in 7 mL of dichloromethane and 2 mL of *N*,*N*-dimethylformamide. The reaction mixture was cooled to 0 °C and sodium triacetoxyborohydride (877 mg, 4.14 mmol) was added. After stirring at 0 °C for 5 min, 0.2 mL of acetic acid was added and the reaction mixture was warmed to room temperature and stirred overnight. The reaction was quenched by the addition of water and then partitioned between aqueous sodium bicarbonate and dichloromethane. The organic layer was dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography eluting with a gradient of 1%−10% methanol in dichloromethane to provide 222 mg of **11** as a tan solid: mp 224−225 °C; ^{1}H NMR (DMSO-d_6) δ 2.16 (s, 6H), 3.44 (s, 2H), 3.86 (s, 3H), 7.37 (s, 1H), 7.40 (d, J = 8 Hz, 2H), 7.68 (d, J = 8 Hz, 2H), 7.76 (s, 1H), 7.90 (s, 1H), 8.60 (s, 1H), 9.73 (s, 1H); MS 483.1, 485.1 (M + H)$^+$. Anal. ($C_{24}H_{20}Cl_2N_4OS\cdot 0.5H_2O$) C, H, N.

2-{4-[(Butylamino)methyl]phenyl}-7-[(2,4-dichloro-5-methoxyphenyl)amino]thieno[3,2-*b*]pyridine-6-carbonitrile (12). Following the route used to prepare **7**, **12** was obtained as an off-white solid in 78% yield from **5**: mp 167−169 °C; ^{1}H NMR ($CDCl_3$) δ 0.91 (t, J = 7 Hz, 3H), 1.19−1.53 (m, 4H), 2.63 (t, J = 7 Hz, 2H), 3.83 (s, 2H), 3.85 (s, 3H), 6.81 (brs, 1H), 6.90 (s, 1H), 7.39 (d, J = 8 Hz, 2H), 7.54 (d, J = 8 Hz, 2H), 7.56 (s, 1H), 7.64 (s, 1H), 8.66 (s, 1H); MS 511.0 (M + H)$^+$. Anal. ($C_{26}H_{24}Cl_2N_4OS$) C, H, N.

4-{6-Cyano-7-[(2,4-dichloro-5-methoxyphenyl)amino]-thieno[3,2-*b*]pyridin-2-yl]benzoic Acid (14). A mixture of **13** (500 mg, 1.05 mmol), 4-carboxylphenylboronic acid (350 mg, 2.12 mmol), and 100 mg of tetrakis(triphenylphosphine)-palladium(0) in 50 mL of ethylene glycol dimethyl ether and 35 mL of saturated aqueous sodium bicarbonate was heated at reflux for 1 h. The reaction mixture was cooled to room temperature and partitioned between water and ethyl acetate. The organic layer was washed with saturated aqueous sodium chloride, dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography eluting with a gradient of chloroform to 10% methanol in chloroform to provide 165 mg of **14** as yellow crystals: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 3.86 (s, 3H), 7.40 (s, 1H), 7.78 (s, 1H), 7.86 (d, J = 8 Hz, 2H), 8.03 (d, J = 8 Hz, 2H), 8.03 (s, 1H), 8.08 (s, 1H), 8.64 (s, 1H), 9.80 (s, 1H), 13.14 (s, 1H); MS 470.2 (M + H)$^+$.

7-[(2,4-Dichloro-5-methoxyphenyl)amino]-2-{4-[(4-methylpiperazin-1-yl)carbonyl]phenyl}thieno[3,2-*b*]pyridine-6-carbonitrile (15). A mixture of **14** (327 mg, 0.70 mmol) and *N*,*N*-carbonyldiimidazole (260 mg, 1.60 mmol) in 25 mL of *N*,*N*-dimethylformamide was heated at 60 °C for 2 h. To the resulting solution was added 1-methylpiperazine (0.35 mL, 3.15 mmol) and the reaction mixture was heated at reflux for 20 min then concentrated in vacuo. The residue was purified by flash column chromatography eluting with a gradient of 1:4 methanol: ethyl acetate to 1:3 methanol:ethyl acetate. Further recrystallization from ethyl acetate provided 59 mg of **15** as a bright yellow solid: mp 240−243 °C; ^{1}H NMR ($CDCl_3$) δ 2.30−2.41 (br s, 2H), 2.33 (s, 3H), 2.43−2.58 (br s, 2H), 3.40−3.51 (br s, 2H), 3.76−3.90 (br s, 2H), 3.86 (s, 3H), 6.83 (s, 1H), 6.92 (s, 1H), 7.47 (d, J = 8 Hz, 2H), 7.58 (s, 1H), 7.63 (d, J = 8 Hz, 2H), 7.69 (s, 1H), 8.68 (s, 1H); MS 552.1, 554.1 (M + H)$^+$. Anal. ($C_{27}H_{23}Cl_2N_5O_2S$) C, H, N.

4-{6-Cyano-7-[(2,4-dichloro-5-methoxyphenyl)amino]-thieno[3,2-*b*]pyridin-2-yl}-*N*,*N*-dimethylbenzamide (16). To a solution of 4-bromo-*N*,*N*-dimethylbenzamide (400 mg, 1.75 mmol) in 20 mL of tetrahydrofuran at −78 °C was added triisopropyl borate (0.45 mL, 1.95 mol) followed by 1.1 mL of 2.6 M *n*-butyllithium in hexanes (2.86 mmol). The reaction mixture was stirred at −78 °C for 2 h then at room temperature overnight. The volatiles were removed by concentration in vacuo, and the residue was added to a mixture of **13** (400 mg, 0.89 mmol) and 50 mg of tetrakis(triphenylphosphine)-palladium(0) in 16 mL of *N*,*N*-dimethylformamide and 8 mL of saturated aqueous sodium bicarbonate. The reaction mixture was heated at reflux for 5 h and then cooled to room temperature and partitioned between ethyl acetate and saturated aqueous sodium bicarbonate. The organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography eluting with a gradient of 1:1 hexane:ethyl acetate to 20% methanol in ethyl acetate to provide 127 mg of **16** as an off-white solid: mp 246−249 °C; ^{1}H NMR (DMSO-d_6) δ 2.89 (s, 3H), 2.99 (s, 3H), 3.86 (s, 3H), 7.41 (s, 1H), 7.52 (d, J = 8 Hz, 2H), 7.77−7.80 (m, 3H), 8.02 (s, 1H), 8.64 (s, 1H), 9.79 (s, 1H); MS 497.1, 499.0 (M + H)$^+$. Anal. ($C_{24}H_{18}Cl_2N_4O_2S$) C, H, N.

7-[(2,4-Dichloro-5-methoxyphenyl)amino]-2-[4-(dimethylamino)phenyl]thieno[3,2-*b*]pyridine-6-carbonitrile (17). A mixture of **13** (200 mg, 0.42 mmol), 4-(dimethylamino)-phenylboronic acid (104 mg, 0.63 mmol), and 24 mg (0.021 mmol) of tetrakis(triphenylphosphine)palladium(0) in 5.5 mL of ethylene glycol dimethyl ether and 4.5 mL of saturated aqueous sodium bicarbonate was heated at reflux for 22 h. The reaction mixture was cooled to room temperature and partitioned between water and dichloromethane. The organic layer was washed with saturated aqueous sodium chloride, dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography eluting with a gradient of hexane to 60% ethyl acetate in hexane, followed by preparative thin-layer chromatography developing with 20% ethyl acetate in dichloromethane to provide 23 mg

of **17** as a yellow solid: mp 255−258 °C; ^{1}H NMR (DMSO-d_6) δ 2.97 (s, 6H), 3.85 (s, 3H), 6.77 (d, J = 9 Hz, 2H), 7.36 (s, 1H), 7.53 (d, J = 9 Hz, 2H), 7.66 (s, 1H), 7.76 (s, 1H), 8.54 (s, 1H), 9.57 (s, 1H); MS 469.0, 471.1 $(M + H)^+$. Anal. ($C_{23}H_{18}Cl_2N_4OS\cdot0.7H_2O$) C, H, N.

7-[(2,4-Dichloro-5-methoxyphenyl)amino]-2-{4-[2-(dimethylamino)ethyl]phenyl}thieno[3,2-*b*]pyridine-6-carbonitrile (18). To a solution of 4-bromo-*N*,*N*-dimethylphenethylamine [17] (242 mg, 1.06 mmol) in 8.0 mL of tetrahydrofuran at −78 °C was added tripropyl borate (0.26 mL, 1.17 mmol) followed by 0.96 mL of 2.5 M *n*-butyllithium in hexanes (2.4 mmol). The reaction mixture was stirred at −78 °C for 1 h and then at room temperature for 2 h. The volatiles were removed by concentration in vacuo, and to the residue was added **13** (250 mg, 0.53 mmol) and 46 mg of tetrakis(triphenylphosphine)palladium(0), 10 mL of ethylene glycol dimethyl ether and 6 mL of saturated aqueous sodium bicarbonate. The resulting mixture was heated at reflux for 2 h and then cooled to room temperature and partitioned between dichloromethane and saturated aqueous sodium bicarbonate. The organic layer was washed with saturated aqueous sodium chloride, dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography eluting with a gradient of dichloromethane to 15% methanol in dichloromethane to provide 173 mg of **18** as a tan solid: mp 196−197 °C; ^{1}H NMR (DMSO-d_6) δ 2.21 (s, 6H), 2.52 (t, J = 7 Hz, 2 H), 2.75 (t, J = 7 Hz, 2H), 3.85 (s, 3H), 7.35 (d, J = 8 Hz, 2H), 7.37 (s, 1H), 7.62 (d, J = 8 Hz, 2H), 7.76 (s, 1H), 7.87 (s, 1H), 8.59 (s, 1H), 9.74 (br s, 1H); MS 497.0, 499.0 $(M + H)^+$. Anal. ($C_{25}H_{22}Cl_2N_4OS\cdot0.3H_2O$) C, H, N.

1-[(4-Bromophenyl)acetyl]-4-methylpiperazine (19). A mixture of 4-bromophenylacetic acid (3.00 g, 14.0 mmol), 1-methylpiperazine (1.40 g, 14.0 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (2.95 g, 15.4 mmol), and 4-(dimethylamino)pyridine (9 mg, 0.075 mmol) in 30 mL of dichloromethane was stirred at room temperature for 19 h and then washed with water and extracted with 1 N aqueous HCl solution. The combined acidic aqueous phases were neutralized with saturated aqueous sodium carbonate and extracted with ethyl acetate. The organic phase was washed with saturated aqueous sodium chloride, dried over sodium sulfate, filtered, and concentrated in vacuo to give 3.20 g of **19** as a white solid: mp 81−83 °C; ^{1}H NMR ($CDCl_3$) 2.25 (t, J = 7 Hz, 2H), 2.27 (s, 3H), 2.36 (t, J = 7 Hz, 2H), 3.45 (t, J = 7 Hz, 2H), 3.66 (t, J = 7 Hz, 2H), 3.67 (s, 2H), 7.12 (d, J = 11 Hz, 2H), 7.45 (d, J = 11 Hz, 2H); MS 297.1, 299.1 $(M + H)^+$. Anal. ($C_{13}H_{17}BrN_2O$) C, H, N.

1-[2-(4-Bromophenyl)ethyl]-4-methylpiperazine (20). To a solution of **19** (1.5 g, 5.05 mmol) in 15 mL of tetrahydrofuran at room temperature was added dropwise a solution of 2 M borane−methyl sulfide complex in tetrahydrofuran (6.3 mL, 12.6 mmol). The resulting mixture was heated at reflux for 2 h, cooled to room temperature, and quenched with methanol. The volatiles were removed in vacuo, and 6 mL of ethanol and 12 mL of 1N aqueous sodium hydroxide solution were added to the residue. The reaction mixture was heated at reflux for 2 h, cooled to room temperature, and partitioned between ethyl acetate and water. The organic phase was washed with saturated aqueous sodium chloride, dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography eluting with a gradient of dichloromethane to 2% methanol in dichloromethane to provide 987 mg of **20** as a white solid: mp 88−90 °C; ^{1}H NMR ($CDCl_3$) δ 2.54−2.56 (m, 2H), 2.64 (s, 3H), 2.65−2.68 (m, 2H), 2.72−2.80 (m, 4H), 2.88−2.90 (m, 2H), 3.08−3.10 (m, 2H), 7.11 (d, J = 11 Hz, 2H), 7.45 (d, J = 11 Hz, 2H); MS 283.0, 285.1 $(M + H)^+$; HRMS (ESI) calcd for $C_{13}H_{19}BrN_2$ 283.0804, found 283.0800.

7-[(2,4-Dichloro-5-methoxyphenyl)amino]-2-{4-[2-(4-methylpiperazin-1-yl)ethyl]phenyl}thieno[3,2-*b*]pyridine-6-carbonitrile (21). To a solution of **20** (181 mg, 0.64 mmol) in 5 mL of tetrahydrofuran at −78 °C was added tripropyl borate (0.15 mL, 0.707 m mol) followed by 0.51 mL of 2.5 M *n*-butyllithium in hexanes (1.28 mmol). The reaction mixture was stirred at −78 °C for 30 min and then at room temperature for 1.5 h. The volatiles were removed by concentration in vacuo and to the residue was added **13** (256 mg, 0.54 mmol), 45 mg of tetrakis(triphenylphosphine)palladium(0), 6 mL of ethylene glycol dimethyl ether, and 4 mL of saturated aqueous sodium bicarbonate. The resulting mixture was heated at reflux overnight and then cooled to room temperature and partitioned between dichloromethane and saturated aqueous sodium bicarbonate. The organic layer was washed with saturated aqueous sodium chloride and dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography eluting with a gradient of dichloromethane to 15% methanol in dichloromethane and then preparative thin-layer chromatography developing with 10% methanol in dichloromethane to provide 43 mg of **21** as an off white solid: mp 211−213 °C; ^{1}H NMR (DMSO-d_6) δ 2.16 (s, 3H), 2.33 (br s, 4H), 2.44 (br s, 4H), 2.52 (t, J = 8 Hz, 2H), 2.75 (t, J = 8 Hz, 2H), 3.85 (s, 3H), 7.34 (d, J = 8 Hz, 2H), 7.37 (s, 1H), 7.62 (d, J = 8 Hz, 2H), 7.76 (s, 1H), 7.87 (s, 1H), 8.59 (s, 1H), 9.73 (br s, 1H); MS 552.1, 554.1 $(M + H)^+$. Anal. ($C_{28}H_{27}Cl_2N_5OS\cdot0.4H_2O$) C, H, N.

7-[(2,4-Dichloro-5-ethoxyphenyl))amino]-2-iodothieno-[3,2-*b*]pyridine-6-carbonitrile (23). A mixture of 2,4-dichloro-5-ethoxyaniline (2.50 g, 12.13 mmol) and 60% sodium hydride in mineral oil (480 mg, 12.0 mmol) in 60 mL of tetrahydrofuran was heated at reflux for 1 h. The solution was cooled and **22**[16] (1.94 g, 6.05 mmol) was added. The reaction mixture was heated at reflux for 2 h and then cooled and partitioned between ethyl acetate and saturated aqueous sodium bicarbonate. The organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was triturated with diethyl ether to provide 2.27 g of **23** as a white solid: mp 232−233 °C; ^{1}H NMR (DMSO-d_6) δ 1.34 (t, J = 5 Hz, 3H), 4.11 (q, J = 8 Hz, 2H), 7.36 (s, 1H), 7.77 (s, 1H), 7.78 (s, 1H), 8.55 (s, 1H), 9.74 (s, 1H); MS 489.9, 491.9 $(M + H)^+$. Anal. ($C_{16}H_{10}Cl_2IN_3OS\cdot0.3Et_2O$) C, H, N.

2-Iodo-7-[(4-phenoxyphenyl)amino]thieno[3,2-*b*]pyridine-6-carbonitrile (24). A mixture of 4-phenoxyaniline (1.05 g, 5.67 mmol), pyridine hydrochloride (100 mg, 0.76 mmol), and **22** (1.00 g, 3.12 mmol) in 50 mL of 2-ethoxyethanol was heated at reflux for 1.5 h. The solution was poured into saturated aqueous sodium bicarbonate and the resulting solid was collected by filtration, washed with water, and dried under reduced pressure. The solid was purified by flash column chromatography, eluting with chloroform. The fractions containing product were combined and concentrated. Diethyl ether was added and the insoluble material collected by filtration to provide 1.38 g of **24** as white crystals: mp 260−262 °C; ^{1}H NMR (DMSO-d_6) δ 7.09 (t, J = 8 Hz, 4H), 7.17 (t, J = 8 Hz, 1H), 7.36 (d, J = 8 Hz, 2H), 7.43 (t, J = 8 Hz, 2H), 7.73 (s, 1H), 8.53 (s, 1H), 9.56 (s, 1H); MS 468.0 $(M - H)^-$. Anal. ($C_{20}H_{12}IN_3OS\cdot0.10CHCl_3$) C, H, N.

7-[(2,4-Dichloro-5-ethoxyphenyl))amino]-2-(4-formylphenyl)thieno[3,2-*b*]pyridine-6-carbonitrile (25). A mixture of **23** (980 mg, 2.00 mmol), 4-formylphenylboronic acid (600 mg, 4.00 mmol), and 20 mg of tetrakis(triphenylphosphine)palladium(0) in 40 mL of ethylene glycol dimethyl ether and 32 mL of saturated aqueous sodium bicarbonate was heated at reflux for 3 h. An additional 60 mg of tetrakis-(triphenylphosphine)palladium(0) was added and the mixture was heated at reflux 3.5 h. The reaction mixture was cooled to room temperature and poured into saturated aqueous sodium bicarbonate. The aqueous mixture was extracted with ethyl acetate containing 10% methanol. The organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuo. The resultant solid was stirred with hot ethyl acetate and filtered to provide 224 mg of **25** as an off-white solid: mp >245 °C; ^{1}H NMR (DMSO-d_6) δ 1.35 (t, J = 7 Hz, 3H), 4.13 (q, J = 7 Hz, 2H), 7.39 (s, 1H), 7.78 (s, 1H), 7.85−8.08 (m, 4H), 8.14 (s, 1H), 8.64 (s, 1H), 9.82 (s, 1H), 10.05 (s, 1H); MS 468.1, 470.1 $(M + H)^+$. Anal. ($C_{23}H_{15}Cl_2N_3O_2S\cdot0.75H_2O$) C, H, N.

2-(4-Formylphenyl)-7-[(4-phenoxyphenyl)amino]thieno-[3,2-*b*]pyridine-6-carbonitrile (26). A mixture of **24** (600 mg, 1.28 mmol), 4-formylphenylboronic acid (380 mg, 2.53 mmol), and 75 mg of tetrakis(triphenylphosphine)palladium-(0) in 53 mL of ethylene glycol dimethyl ether and 40 mL of saturated aqueous sodium bicarbonate was heated at reflux for 1 h. The reaction mixture was cooled to room temperature and partitioned between water and ethyl acetate. The organic layer was washed with water, dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography eluting with 2:1 hexane:ethyl acetate to provide 536 mg of **26** as yellow crystals: mp 230−232 °C; ^{1}H NMR (DMSO-d_6) δ 7.06−7.20 (m, 5H), 7.36−7.45 (m, 4H), 7.92−8.05 (m, 4H), 8.11 (s, 1H), 8.63 (s, 1H), 9.67 (s, 1H), 10.05 (s, 1H); MS 446.2 $(M-H)^-$. Anal. ($C_{27}H_{17}N_3O_2S\cdot0.4H_2O$) C, H, N.

7-[(2,4-Dichloro-5-ethoxyphenyl))amino]-2-{4-[(4-methylpiperazin-1-yl)methyl]phenyl}thieno[3,2-*b*]pyridine-6-carbonitrile (27). To a 0 °C suspension of **25** (161 mg, 0.34 mmol) and 1-methylpiperazine (0.19 mL, 1.71 mmol) in 1.2 mL of 1-methyl-2-pyrrolidinone and 5 mL of dichloromethane was added sodium triacetoxyborohydride (437 mg, 2.06 mmol) followed by 120 μL of acetic acid. The mixture was stirred at 0 °C for 10 min and then at room temperature for 4 h. The reaction mixture was partitioned between dichloromethane and saturated aqueous sodium bicarbonate. The organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuo. Purification by flash column chromatography eluting with a gradient of 10% methanol in dichloromethane to 20% methanol in dichloromethane provided 116 mg of **27** as a white solid: mp 227−229 °C; ^{1}H NMR (DMSO-d_6) δ 1.34 (t, J = 7 Hz, 3H), 2.16 (s, 3H), 2.37 (br s, 8H), 3.49 (s, 2H), 4.13 (q, J = 7 Hz, 2H), 7.36 (s, 1H), 7.40 (d, J = 8 Hz, 2H), 7.67 (d, J = 8 Hz, 2H), 7.76 (s, 1H), 7.90 (s, 1H), 8.60 (s, 1H), 9.73 (s, 1H); MS 552.2, 554.2 $(M+H)^+$. Anal. ($C_{28}H_{27}Cl_2N_5OS\cdot0.5H_2O$) C, H, N.

2-[4-(4-Methylpiperazin-1-ylmethyl)phenyl]-7-[(4-phenoxyphenyl)amino]thieno[3,2-*b*]pyridine-6-carbonitrile (28). 1-Methylpiperazine (65 μL, 0.59 mmol) was added to a suspension of **26** (200 mg, 0.45 mmol) in 4 mL of dichloromethane and 1 mL of *N*,*N*-dimethylformamide. The reaction mixture was cooled to 0 °C and sodium triacetoxyborohydride (474 mg, 2.24 mmol) was added. After stirring at 0 °C for 10 min, 3 drops of acetic acid were added, and the reaction mixture was warmed to room temperature and stirred for 4 h. The reaction was quenched by the addition of water and then partitioned between saturated aqueous sodium bicarbonate and dichloromethane. The organic layer was washed with water, dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography eluting with 5% methanol in dichloromethane to provide 138 mg of **28** as white crystals: mp 199−201 °C; ^{1}H NMR (DMSO-d_6) δ 2.17 (s, 3H), 2.37 (br s, 8H), 3.50 (s, 2H), 7.05−7.18 (m, 5H), 7.35−7.44 (m, 6H), 7.66 (d, J = 8 Hz, 2H), 7.86 (s, 1H), 8.59 (s, 1H), 9.58 (s, 1H); MS 532.3 $(M+H)^+$. Anal. ($C_{32}H_{29}N_5OS$) C, H, N.

7-[(2,4-Dichlorophenyl)amino]-2-{4-[(dimethylamino)-methyl]phenyl}thieno[3,2-*b*]pyridine-6-carbonitrile (30). To a 0 °C suspension of **29** [16] (110 mg, 0.26 mmol) and 0.78 mL of 2 M dimethylamine in tetrahydrofuran (1.56 mmol) in 4 mL of dichloromethane and 1 mL of *N*,*N*-dimethylformamide was added sodium triacetoxyborohydride (370 mg, 1.75 mmol) followed by, after 10 min, a few drops of acetic acid. The reaction mixture was stirred at room temperature for 4 h and then partitioned between saturated aqueous sodium bicarbonate and ethyl acetate. The organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography eluting with a gradient of dichloromethane to 10% methanol in dichloromethane to provide 60 mg of **30** as a white solid: mp 231−233 °C; ^{1}H NMR (DMSO-d_6) δ 2.15 (s, 6H), 3.42 (s, 2H), 7.40 (d, J = 8 Hz, 2H), 7.50−7.61 (m, 2H), 7.67 (d, J = 8 Hz, 2H), 7.80 (s, 1H), 7.90 (s, 1H), 8.60 (s, 1H), 9.69 (s, 1H); MS 453.1, 455.1 $(M+H)^+$; HRMS (ESI) calcd for $C_{28}H_{18}Cl_2N_4OS$ 453.07020, found 453.06992. Anal. ($C_{28}H_{18}Cl_2N_4OS\cdot0.5H_2O$) C, N.

7-Chloro-2-(4-formylphenyl)thieno[3,2-*b*]pyridine-6-carbonitrile (31). A mixture of **22** (1.00 g, 3.12 mmol), 4-formylphenylboronic acid (936 mg, 6.24 mmol), and 108 mg of tetrakis(triphenylphosphine)palladium(0) in 30 mL of ethylene glycol dimethyl ether and 25 mL of saturated aqueous sodium bicarbonate was heated at reflux for 4 h. The mixture was cooled and the precipitate collected by filtration washing with ethyl acetate and diethyl ether to provide 818 mg of **31** as a yellow solid: mp 300−305 °C; ^{1}H NMR ($CDCl_3$) δ 7.93 (d, J = 8 Hz, 2H), 7.96 (s, 1H), 8.03 (d, J = 8 Hz, 2H), 8.87 (s, 1H), 10.1 (s, 1H); MS 298.0, 300.0 $(M-H)^-$.

7-Chloro-2-{4-[(dimethylamino)methyl]phenyl}thieno-[3,2-*b*]pyridine-6-carbonitrile (32). A mixture of **31** (700 mg, 2.34 mmol) and 6.0 mL of 2.0 M dimethylamine in tetrahydrofuran (12 mmol) in 30 mL of dichloromethane and 5 mL of *N*,*N*-dimethylformamide was cooled to 0−5 °C. Sodium triacetoxyborohydride (2.5 g, 11.7 mmol) was added in portions, and after 5 min, 0.10 mL of acetic acid was added. The mixture was stirred at room temperature for 2 h and then quenched by the addition of ice water and partitioned between dichloromethane and saturated aqueous sodium bicarbonate. The organic phase was dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography eluting with a gradient of dichloromethane to 10% methanol in dichloromethane to provide 496 mg (65%) of **32** as a yellow solid: mp 166−168 °C; ^{1}H NMR (DMSO-d_6) δ 2.21 (s, 6H), 3.52 (s, 2H), 7.48 (d, J = 8 Hz, 2H), 7.95 (d, J = 8 Hz, 2H), 8.28 (s, 1H), 9.11 (s, 1H); MS 328.1, 330.1 $(M+H)^+$. Anal. ($C_{17}H_{14}ClN_3S\cdot0.3H_2O$) C, H, N.

Alternative Route to 7-[(2,4-Dichlorophenyl)amino]-2-{4-[(dimethylamino)methyl]phenyl}thieno[3,2-*b*]pyridine-6-carbonitrile (30). A mixture of 2,4-dichloroaniline (126 mg, 0.78 mmol) and 60% sodium hydride in mineral oil (32 mg, 0.80 mmol) in 10 mL of tetrahydrofuran was heated at reflux for 1 h. The solution was cooled and **32** (126 mg, 0.39 mmol) was added. The reaction mixture was heated at reflux for 3 h and then cooled and partitioned between ethyl acetate and saturated aqueous sodium bicarbonate. The organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was triturated with diethyl ether to provide 62 mg of **30** as a light yellow solid.

7-[(2,4-Dichloro-5-methoxyphenyl)amino]-2-(5-formylthien-3-yl)thieno[3,2-*b*]pyridine-6-carbonitrile (33). A mixture of **13** (564 mg, 1.18 mmol), tributyl(5-[1,3]dioxolan-2-ylthiophen-3-yl)stannane (680 mg, 1.52 mmol) and a catalytic amount of bis(triphenylphosphine)palladium(II) chloride in 15 mL of dioxane was heated at reflux for 5 h. Additional bis-(triphenylphosphine)palladium(II) chloride was added and the reaction mixture was heated at reflux overnight. Additional bis(triphenylphosphine)palladium(II) chloride and 10 mL of dioxane were added, and the reaction was heated at reflux for 5 h. The reaction mixture was concentrated in vacuo and partitioned between water and chloroform. The organic layer was dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography eluting with 1:1 hexane:ethyl acetate to provide 447 mg of 7-[(2,4-dichloro-5-methoxyphenyl)amino]-2-[5-(1,3-dioxolan-2-yl)thien-3-yl]thieno[3,2-*b*]pyridine-6-carbonitrile as white crystals.

To a suspension of 7-[(2,4-dichloro-5-methoxyphenyl)amino]-2-[5-(1,3-dioxolan-2-yl)thien-3-yl]thieno[3,2-*b*]pyridine-6-carbonitrile (337 mg, 0.67 mmol) in 10 mL of tetrahydrofuran was added 5 mL of 2 N hydrochloric acid. The mixture was stirred at room temperature overnight. The mixture was slowly poured into 30 mL of saturated aqueous sodium bicarbonate and extracted with chloroform. The organic layer was washed with saturated aqueous sodium chloride, dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography eluting with 5% methanol in dichloromethane to provide 271 mg of **33** as yellow

crystals: mp 259−261 °C; ^{1}H NMR (DMSO-d_6) δ 3.85 (s, 3H), 7.38 (s, 1H), 7.76 (s, 1H), 7.93 (s, 1H), 8.42 (s, 1H), 8.50 (s, 1H), 8.63 (s, 1H), 9.76 (s, 1H), 9.98 (s, 1H); MS 458.1 (M − H)$^-$.

7-[(2,4-Dichloro-5-methoxyphenyl)amino]-2-{5-[(4-methylpiperazin-1-yl)methyl]thien-3-yl}thieno[3,2-*b*]pyridine-6-carbonitrile (34). 1-Methylpiperazine (70 μL, 0.63 mmol) was added to a suspension of **33** (225 mg, 0.49 mmol) in 4 mL of dichloromethane and 1 mL of *N*,*N*-dimethylformamide. The reaction mixture was cooled to 0 °C and sodium triacetoxyborohydride (520 mg, 2.45 mmol) was added. After stirring at 0 °C for 10 min, 2 drops of acetic acid were added, and the reaction mixture was warmed to room temperature and stirred for 4 h. The reaction was quenched by the addition of water and then partitioned between saturated aqueous sodium bicarbonate and dichloromethane. The organic layer was washed with water, dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography eluting with 5% methanol in dichloromethane to provide 133 mg of **34** as white crystals: mp 224−226 °C; ^{1}H NMR (DMSO-d_6) δ2.18 (s, 3H), 2.25−2.55 (m, 8H), 3.68 (s, 2H), 3.85 (s, 3H), 7.35 (s, 1H), 7.37 (s, 1H), 7.74 (s, 1H), 7.75 (s, 1H), 7.82 (s, 1H), 8.58 (s, 1H), 9.68 (s, 1H); MS 523.1, 544.2 (M + H)$^+$. Anal. ($C_{25}H_{23}Cl_2N_5OS_2\cdot0.5H_2O$) C, H, N.

7-[(2,4-Dichlorophenyl)amino]-2-(5-formylthien-3-yl)-thieno[3,2-*b*]pyridine-6-carbonitrile (36). Following the route used to prepare **33**, **36** was obtained as a yellow solid in 44% yield from **35**: ^{1}H NMR (DMSO-d_6) δ 7.55−7.69 (m, 2H), 7.81 (s, 1H), 7.94 (s, 1H), 8.42 (s, 1H), 8.64 (s, 1H), 9.71 (s, 1H), 9.98 (s, 1H); MS 429.9 (M + H)$^+$.

7-[(2,4-Dichlorophenyl)amino]-2-{5-[(4-methylpiperazin-1-yl)methyl]thien-3-yl}thieno[3,2-*b*]pyridine-6-carbonitrile (37). Following the route used to prepare **34**, **37** was obtained as an off-white solid in 48% yield from **36**: mp 226−228 °C; ^{1}H NMR (DMSO-d_6) δ2.16 (s, 3H), 2.27−2.53 (m, 8H), 3.67 (s, 2H), 7.36 (s, 1H), 7.54 (s, 2H), 7.74 (s, 1H), 7.79 (s, 1H), 7.82 (s, 1H), 8.58 (s, 1H), 9.64 (s, 1H); MS 514.1 (M + H)$^+$; HRMS (ESI) calcd for $C_{24}H_{21}Cl_2N_5S_2$ 514.06882, found 514.06975. Anal. ($C_{24}H_{21}Cl_2N_5S_2\cdot0.5H_2O$) C, N.

7-[(2,4-Dichloro-5-ethoxyphenyl)amino]-2-(5-formylthien-3-yl)thieno[3,2-*b*]pyridine-6-carbonitrile (38). Following the route used to prepare **33**, **38** was obtained as a yellow solid in 51% yield from **23**: ^{1}H NMR (DMSO-d_6) δ 1.35 (t, J = 7 Hz, 3H), 4.13 (q, J = 7 Hz, 2H), 7.37 (s, 1H), 7.76 (s, 1H), 7.93 (s, 1H), 8.42 (s, 1H), 8.48 (s, 1H), 8.63 (s, 1H), 9.74 (s, 1H), 9.98 (s, 1H); MS 473.9 (M + H)$^+$.

7-[(2,4-Dichloro-5-ethoxyphenyl)amino]-2-{5-[(4-methylpiperazin-1-yl)methyl]thien-3-yl}thieno[3,2-*b*]pyridine-6-carbonitrile (39). Following the route used to prepare **34**, **39** was obtained as a tan solid in 78% yield from **38**: mp 232−234 °C; ^{1}H NMR (DMSO-d_6) δ1.34 (t, J = 7 Hz, 3H), 2.16 (s, 3H), 2.26−2.52 (m, 8H), 3.68 (s, 2H), 4.12 (q, J = 7 Hz, 2H), 7.33 (s, 1H), 7.36 (s, 1H), 7.73 (s, 2H), 7.82 (s, 1H), 8.57 (s, 1H), 9.66 (s, 1H); MS 558.0 (M + H)$^+$; HRMS (ESI−FTMS) calcd for $C_{26}H_{25}Cl_2N_5OS_2$ 558.09504, found 558.09643. Anal. ($C_{26}H_{25}Cl_2N_5OS_2\cdot0.25H_2O$) C, N.

7-[(2,4-Dichloro-5-methoxyphenyl)amino]-2-{5-[(dimethylamino)methyl]thien-3-yl}thieno[3,2-*b*]pyridine-6-carbonitrile (40). To a 0 °C solution of **33** (270 mg, 0.59 mmol), 1.5 mL of 2 M dimethylamine in tetrahydrofuran (3.0 mmol), 7 mL of dichloromethane, and 1.5 mL of 1-methyl-2-pyrrolidinone was added sodium triacetoxyborohydride (630 mg, 2.97 mmol), followed by 100 μL of acetic acid. The reaction mixture was stirred at room temperature overnight. The reaction mixture was partitioned between saturated aqueous sodium bicarbonate and dichloromethane. The organic layer was washed with saturated aqueous sodium bicarbonate, dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography eluting with a gradient of 5% methanol in dichloromethane to 10% methanol in dichloromethane. Trituration with diethyl ether provided 41 mg of **40** as a white solid: mp 184−186 °C; ^{1}H NMR (DMSO-d_6) δ 2.20 (s, 6H), 3.61 (s, 2H), 3.85 (s, 3H), 7.35 (s, 1H), 7.37 (s, 1H), 7.75 (s, 2H), 7.83 (s, 1H), 8.58 (s, 1H), 9.68 (s, 1H); MS 488.9, 490.9 (M + H)$^+$. Anal. ($C_{22}H_{18}Cl_2N_4OS_2$) C, H, N.

7-[(2,4-Dichloro-5-methoxyphenyl)amino]-2-(5-formylthien-2-yl)thieno[3,2-*b*]pyridine-6-carbonitrile (41). A mixture of **13** (1.00 g, 2.10 mmol), tributyl(5-[1,3]dioxolan-2-ylthiophen-2-yl)stannane (1.62 mg, 3.64 mmol) and a catalytic amount of bis(triphenylphosphine)palladium(II) chloride in 25 mL of dioxane was heated at reflux for 2 h. The reaction mixture was partitioned between water and dichloromethane. The organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was triturated with diethyl ether to provide 811 mg of 7-[(2,4-dichloro-5-methoxyphenyl)amino]-2-[5-(1,3-dioxolan-2-yl)thien-2-yl]thieno[3,2-*b*]-pyridine-6-carbonitrile as a yellow solid. Concentration of the filtrate and trituration with diethyl ether provided an additional 125 mg of product.

To a suspension of 7-[(2,4-dichloro-5-methoxyphenyl)amino]-2-[5-(1,3-dioxolan-2-yl)thien-2-yl]thieno[3,2-*b*]pyridine-6-carbonitrile (879 mg, 1.74 mmol) in 20 mL of tetrahydrofuran was added 10 mL of 2 N hydrochloric acid. The reaction mixture was stirred at room temperature overnight and then slowly poured into saturated aqueous sodium bicarbonate and extracted into ethyl acetate. The organic layer was washed with saturated aqueous sodium bicarbonate, dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was triturated with diethyl ether to provide 242 mg of **41** as a yellow solid: mp >245 °C; ^{1}H NMR (DMSO-d_6) δ 3.86 (s, 3H), 7.42 (s, 1H), 7.72 (d, J = 4 Hz, 1H), 7.80 (s, 1H), 7.95 (s, 1H), 8.06 (d, J = 4 Hz, 1H), 8.65 (s, 1H), 9.87 (s, 1H), 9.95 (s, 1H); MS 459.9, 461.8 (M + H)$^+$. Anal. ($C_{20}H_{11}Cl_2N_3O_2S_2$) C, H, N.

Additional product was obtained by filtration of the aqueous layer. The collected solid was washed with water and dried to provide 362 mg of **41** as a yellow solid.

7-[(2,4-Dichloro-5-methoxyphenyl)amino]-2-{5-[(4-methylpiperazin-1-yl)methyl]thien-2-yl}thieno[3,2-*b*]pyridine-6-carbonitrile (42). To a 0 °C solution of **41** (200 mg, 0.43 mmol) and 1-methylpiperazine (0.24 mL, 2.16 mmol) in 5 mL of dichloromethane and 1 mL of 1-methyl-2-pyrrolidinone was added sodium triacetoxyborohydride (550 mg, 2.60 mmol) followed by 100 μL of acetic acid. The reaction mixture was stirred at room temperature overnight and then partitioned between saturated aqueous sodium bicarbonate and dichloromethane. The organic layer was washed with saturated aqueous sodium bicarbonate, dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography eluting with a gradient of 5% methanol in dichloromethane to 20% methanol in dichloromethane. Trituration with diethyl ether provided 120 mg of **42** as a pale yellow solid: mp 200−202 °C; ^{1}H NMR (DMSO-d_6) δ 2.17 (s, 3H), 2.26−2.51 (br d, 8H), 3.68 (s, 2H), 3.85 (s, 3H), 7.00 (d, J = 4 Hz, 1H), 7.34 (d, J = 4 Hz, 1H), 7.36 (s, 1H), 7.63 (s, 2H), 7.77 (s, 1H), 8.58 (s, 1H), 9.73 (s, 1H); MS 544.0, 546.0 (M + H)$^+$. Anal. ($C_{25}H_{23}Cl_2N_5OS_2\cdot H_2O$) C, H, N.

7-[(2,4-Dichloro-5-methoxyphenyl)amino]-2-(2-formylthien-3-yl)thieno[3,2-*b*]pyridine-6-carbonitrile (43). A mixture of **13** (1.00 g, 2.10 mmol), 2-formyl-3-thiopheneboronic acid (491 mg, 3.15 mmol), and 122 mg of tetrakis(triphenylphosphine)palladium(0) in 50 mL of ethylene glycol dimethyl ether and 30 mL of saturated aqueous sodium bicarbonate was heated at reflux overnight. The reaction mixture was cooled to room temperature and partitioned between water and ethyl acetate. The organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography eluting with a gradient of hexane to 3:1 hexane:ethyl acetate to provide 600 mg of a mixture of **43** and **13**. A second purification by flash column chromatography eluting with a gradient of dichloromethane to 0.5% methanol in dichloromethane provided 200 mg of **43** as a yellow solid: mp 128−130 °C; ^{1}H NMR (DMSO-d_6) δ 3.86 (s, 3H), 7.41 (s, 1H), 7.49 (d, J = 4 Hz, 1H), 7.78 (s, 1H), 8.01 (s, 1H), 8.23 (d, J = 4 Hz, 1H), 8.67 (s, 1H), 9.97 (s, 1H), 10.04 (s, 1H); MS 459.9 (M + H)$^+$. Anal. ($C_{20}H_{11}Cl_2N_3O_2S_2$) C, H, N.

7-[(2,4-Dichloro-5-methoxyphenyl)amino]-2-{2-[(4-methylpiperazin-1-yl)methyl]thien-3-yl}thieno[3,2-*b*]pyridine-6-carbonitrile (44). To a 0 °C solution of **43** (200 mg, 0.43 mmol) and 1-methylpiperazine (0.077 mL, 0.69 mmol) in 6 mL of dichloromethane and 2.5 mL of 1-methyl-2-pyrrolidinone was added sodium triacetoxyborohydride (456 mg, 2.15 mmol) followed by 150 μL of acetic acid. The reaction mixture was stirred at room temperature overnight and then poured into water. Sodium bicarbonate was added to reach a pH of 7. After stirring for 30 min, dichloromethane was added and the layers were separated. The organic layer was concentrated in vacuo, water was added, and the solid was collected by filtration. The residue was purified by flash column chromatography eluting with a gradient of 0.5% methanol in dichloromethane to 10% methanol in dichloromethane. Trituration with diethyl ether provided 130 mg of **44** as a yellow solid: mp 218−219 °C; ^{1}H NMR (DMSO-d_6) δ 2.21 (s, 3H), 2.28−2.52 (br s, 8H), 3.71 (s, 2H), 3.86 (s, 3H), 7.32 (d, J = 5 Hz, 1H), 7.39 (s, 1H), 7.56 (d, J = 5 Hz, 1H), 7.69 (s, 2H), 7.76 (s, 1H), 8.62 (s, 1H), 9.74 (s, 1H); MS 544.2 $(M + H)^+$. Anal. ($C_{25}H_{23}Cl_2N_5OS_2$) C, H, N.

HT29 Xenograft Assay. Human colon carcinoma HT29 cells (American Type Culture Collection, Rockville, Maryland #HTB-38) were grown in vitro. Athymic nu/nu female mice (Charles River, Wilmington, MA) were housed in a barrier-type facility under an approved Institutional Animal Care and Use Committee (IACUC) protocol. Animals were provided ad libitum access to both food and water. HT29 tumor cells were suspended to 50 million cells/mL, and 0.2 mL of the cell suspension was injected subcutaneously into a flank of 6−7-week-old female nude mice. Mice with tumors larger than 200 mm^3 after 1 week were administered vehicle or compound by oral gavage at the indicated doses in 0.2 mL of vehicle containing 0.5% methylcellulose and 0.4% polysorbate 80 (Tween 80).

Anchorage-Independent Lck-transformed Fibroblast Proliferation Assay. Rat2 fibroblasts stably transformed with a plasmid containing a CMV-promotor-controlled v-Src/Hu Lck fusion gene in which the catalytic domain of human Lck was inserted in place of the v-Src catalytic domain in the v-Src gene were used for the measurement of Lck-dependent suspension growth. Ultralow cluster plates (Costar # 3474) were seeded with 1000 cells per well on day 1 and compound was added in serial 2-fold dilutions from 10 to 0.009 μM on day 2. Cell Titer-Glo reagent (Promega) was added on day 5 and luminescence was measured. The results were analyzed using the LSW Data Analysis plug-in for Microsoft Excel to yield IC_{50}s for proliferation.

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Supporting Information Available: Elemental analysis data for compounds **6**−**12**, **15**−**19**, **21**, **23**−**28**, **30**, **32**, **34**, **37**, **39**−**44**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Boggon, T. J.; Eck, M. J. Structure and regulation of Src family kinases. *Oncogene* **2004**, *23*, 7918−7927.
(2) Parsons, S. J.; Parsons, J. T. Src family kinases, key regulators of signal transduction. *Oncogene* **2004**, *23*, 7906−7909.
(3) Irby, R. B.; Yeatman, T. J. Role of Src expression and activation in human cancer. *Oncogene* **2000**, *19*, 5636−5642.
(4) Frame, M. C. Src in cancer: Deregulation and consequences for cell behavior. *Biochim. Biophys. Acta* **2002**, *1602*, 114−130.
(5) Susva, M.; Missbach, M.; Green, J. Src inhibitors: Drugs for the treatment of osteoporosis, cancer or both? *Trends Pharmacol. Sci.* **2000**, *21*, 489−495.
(6) Paul, R.; Zhang, Z. G.; Eliceiri, B. P.; Jiang, Q.; Boccia, A. D.; Zhang, R. L.; Chopp, M.; Cheresh, D. A. Src deficiency or blockade of Src activity in mice provides cerebral protection following stroke. *Nat. Med.* **2001**, *7*, 222−227.
(7) Talamonti, M. S.; Roh, M. S.; Curley, S. A.; Gallick, G. E. Increase in activity and level of pp60c-src in progressive stages of human colorectal cancer. *J. Clin. Invest.* **1993**, *91*, 53−60.
(8) Ito, H.; Gardner-Thorpe, J.; Zinner, M. J.; Ashley, S. W.; Whang, E. E. Inhibition of tyrosine kinase Src suppresses pancreatic cancer invasiveness. *Surgery* **2003**, *134*, 221−226.
(9) Wiener, J. R.; Windham, T. C.; Estrella, V. C.; Parikh, N. U.; Thall, P. F.; Deavers, M. T.; Bast, R. C.; Mills, G. B.; Gallick, G. E. Activated SRC protein tyrosine kinase is overexpressed in late-stage human ovarian cancers. *Gynecol. Oncol.* **2003**, *88*, 73−79.
(10) Myoui, A.; Nishimura, R.; Williams, P. J.; Hiraga, T.; Tamura, D.; Michigami, T.; Mundy, G. R.; Yoneda, T. C−Src tyrosine kinase activity is associated with tumor colonization in bone and lung in an animal model of human breast cancer metastasis. *Cancer Res.* **2003**, *63*, 5028−5033.
(11) Matsumoto, T.; Jiang, J.; Kiguchi, K.; Ruffino, L.; Carbajal, S.; Beltran, L.; Bol, D. K.; Rosenberg, M. P.; DiGiovanni, J. Targeted expression of c-Src in epidermal basal cells leads to enhanced skin tumor promotion, malignant progression, and metastasis. *Cancer Res.* **2003**, *63*, 4819−4828.
(12) Boschelli, D. H. 4-Anilino-3-quinolinecarbonitriles: An emerging class of kinase inhibitors. *Curr. Top. Med. Chem.* **2002**, *2*, 1051−1063.
(13) Boschelli, D. H. 4-Anilino-3-quinolinecarbonitriles: An emerging class of kinase inhibitors—An update. *Med. Chem. Rev.-Online* **2004**, *1*, 457−463.
(14) 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.
(15) 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.
(16) Boschelli, D. H.; Wu, B.; Barrios Sosa, A. C.; Durutlic, H.; Ye, F.; Raifeld, Y.; Golas, J. M.; Boschelli, F. Identification of 7-phenyl-aminothieno-[3,2-b]pyridine-6-carbonitriles as a new class of Src kinase inhibitors. *J. Med. Chem.* **2004**, *47*, 6666−6668.
(17) Brown, S. D.; Armstrong, R. W. Parallel synthesis of Tamoxifen and derivatives on solid support via resin capture. *J. Org. Chem.* **1997**, *62*, 7076−7077.
(18) 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.
(19) 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.
(20) 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.
(21) Zhang, N.; Wu, B.; Powell, D.; Wissner, A.; Floyd, M. B.; Kovacs, E. D.; Toral-Barza, L.; Kohler, C. Synthesis and structure−activity relationships of 3-cyano-4-(phenoxyanilino)quinolines as MEK (MAPKK) inhibitors. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2825−2828.
(22) 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.
(23) Warmuth, M.; Damoiseaux, R.; Liu, Y.; Fabbro, D.; Gray, N. Src family kinases: Potential targets for the treatment of human cancer and leukemia. *Curr. Pharm. Des.* **2003**, *9*, 2043−2059.
(24) Boschelli, D. H. Dual inhibitors of Src and Abl tyrosine kinases. *Drug Des. Rev.-Online* **2004**, *1*, 203-214.
(25) Deininger, M. W. N.; Druker, B. J. SRCircumventing imatinib resistance. *Cancer Cell* **2004**, *6*, 108−110.
(26) Sawyers, C. L. Resistance to imatinib: More and more mutations. *Blood* **2003**, *102*, 4−5.
(27) La Rosee, P.; Corbin, A. S.; Stoffregen, E. P.; Deininger, M. W.; Druker, B. J. Activity of the Bcr-Abl kinase inhibitor PD180970 against clinically relevant Bcr-Abl isoforms that cause resistance to Imatinib mesylate (Gleevec, STI571). *Cancer Res.* **2002**, *62*, 7149−7153.

(28) Huron, D. R.; Gorre, M. E.; Kraker, A. J.; Sawyers, C. L.; Rosen, N.; Moasser, M. M. A novel pyridopyrimidine inhibitor of Abl kinase is a picomolar inhibitor of Bcr-Abl-driven K562 cells and is effective against STI571-resistant Bcr-Abl mutants. *Clin. Cancer Res.* **2003**, *9*, 1267−1273.
(29) von Bubnoff, N.; Veach, D. R.; Miller, W. T.; Li, W.; Saenger, J.; Peschel, C.; Bornmann, W. G.; Clarkson, B.; Duyster, J. Inhibition of wild-type and mutant Bcr-Abl by pyrido-pyrimidine-type small molecule kinase inhibitors. *Cancer Res.* **2003**, *63*, 6395−6404.
(30) Tipping, A. J.; Baluch, S.; Barnes, D. J.; Veach, D. R.; Clarkson, B. M.; Bornmann, W. G.; Mahon, F. X.; Goldman, J. M.; Melo, J. V. Efficacy of dual-specific Bcr-Abl and Src-family kinase inhibitors in cells sensitive and resistant to imatinib mesylate. *Leukemia* **2004**, *18*, 1352−1356.
(31) O'Hare, T.; Pollock, R.; Stoffregen, E. P.; Keats, J. A.; Abdullah, O. M.; Moseson, E. M.; Rivera, V. M.; Tang, H.; Metcalf, C. A., III.; Bohacek, R. S.; Wang, Y.; Sundaramoorthi, R.; Shakespeare, W. C.; Dalgarno, D.; Clackson, T.; Sawyer, T. K.; Deininger, M. W.; Druker, B. J. Inhibition of wild-type and mutant Bcr-Abl by AP23464, a potent ATP-based oncogenic protein kinase inhibitor: Implications for CML. *Blood* **2004**, *104*, 2532−2539.
(32) Roginskaya, V.; Zuo, S.; Caudell, E.; Nambudiri, G.; Kraker, A. J.; Corey, S. J. Therapeutic targeting of Src-kinase Lyn in myeloid leukemic cell crowth. *Leukemia* **1999**, *13*, 855−861.
(33) Warmuth, M.; Bergmann, M.; Priess, A.; Hauslmann, K.; Emmerich, B.; Hallek, M. The Src family kinase Hck interacts with Bcr-Abl by a kinase-independent mechanism and phosphorylates the Grb2-binding site of Bcr. *J. Biol. Chem.* **1997**, *272*, 33260−33270.
(34) 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*, 399−402.
(35) 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-methylphenyl)-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.
(36) Compounds were tested at least twice with the standard deviation reported within the brackets.

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