Identification of 7-Phenylaminothieno-[3,2-*b*]pyridine-6-carbonitriles as a New Class of Src Kinase Inhibitors

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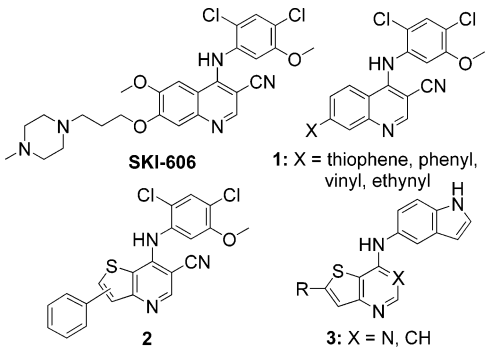
Abstract: We disclose here a new class of kinase inhibitors, obtained by replacing the phenyl ring of a 3-quinolinecarbonitrile system with a thiophene ring. When suitably substituted, the resultant 7-phenylaminothieno[3,2-*b*]pyridine-6-carbonitrile analogues show potent inhibition of Src kinase activity.

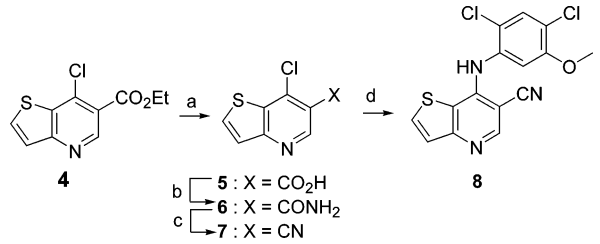
Src, the prototype member of a family of highly related nonreceptor tyrosine kinases,[1] is over-expressed and/or activated in several types of cancer and also plays a key role in tumor progression and metastases.[2−4] Increased levels of activated Src were first observed in metastatic colorectal cancer[5] and more recently in late-stage pancreatic[6] and ovarian cancers.[7] Small molecule Src kinase inhibitors may therefore prove useful in the treatment of the more aggressive forms of cancer, including bone metastases in breast cancer patients.[8] Since Src plays a role in additional signaling pathways, Src inhibitors are also being pursued for the treatment of other diseases including osteoporosis and stroke.[9,10]

We have reported that 7-alkoxy-4-[(2,4-dichloro-5-methoxyphenyl)amino]-3-quinolinecarbonitriles are potent Src inhibitors, exemplified by the lead compound SKI-606.[11−15] Replacement of the C-7 alkoxy group of these original analogues with a thiophene, phenyl, vinyl, or ethynyl group resulted in compounds of structure **1**, which retained activity against Src.[16−18] We report here the preparation of compounds with the core structure of **2**, where the phenyl ring of the quinoline system is replaced by a thiophene and show that with suitable substitution these analogues can effectively inhibit Src kinase activity. During the course of this work, Pfizer reported that related thienopyrimidines and thienopyridines (**3**) are VEGFR kinase inhibitors.[19]



Ethyl 7-chlorothieno[3,2-*b*]pyridine-6-carboxylate, **4**, was prepared as reported in the literature.[20] As shown in Scheme 1, hydrolysis of the ethyl ester of **4** provided the corresponding acid **5**. Subsequent reaction of **5** with thionyl chloride followed by addition of ammonium hydroxide gave the primary amide **6** which was dehydrated with cyanuric chloride to afford the key 6-carbonitrile intermediate **7**. Displacement of the 7-chloro group of **7** with 2,4-dichloro-5-methoxyaniline provided **8**.

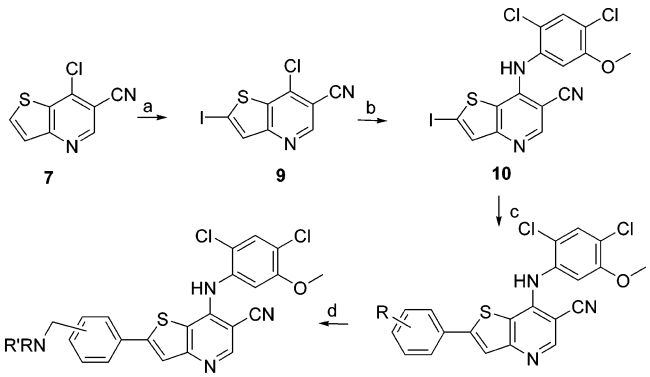
Scheme 1[a]



[a] Reagents: (a) NaOH (1N aq), EtOH; (b) (1) $SOCl_2$; (2) NH_4OH (aq); (c) cyanuric chloride, DMF; (d) 2,4-diCl-5-OMe aniline, NaH, THF.

To prepare C-2-functionalized analogues, **7** was first treated with lithium diisopropylamine followed by addition of iodine to provide the 2-iodo derivative **9** as shown in Scheme 2. Addition of 2,4-dichloro-5-methoxyaniline to **9** gave **10**. Reaction of **10** with phenylboronic acid under Suzuki conditions provided **11**, the C-2 phenyl analogue of **8**. Analogously, coupling of **10** with 4-formylphenylboronic acid provided **12**. Reductive amination of the aldehyde group of **12** with morpholine and *N*-methylpiperazine resulted in **13** and **14**, respectively. Alternatively **10** was coupled with 3-formylphenylboronic acid followed by reductive amination of **15** with *N*-methylpiperazine to provide **16**, the meta isomer of **14**. The ortho isomer of **14**, namely **18**, was prepared in a similar fashion via the aldehyde intermediate **17**.

Scheme 2[a]



[a] Reagents: (a) (1) LDA, heptane, THF, ethylbenzene; (2) I_2, THF; (b) 2,4-diCl-5-OMe aniline, NaH, THF; (c) boronic acid, $(Ph_3P)_4Pd$, DME, saturated aqueous $NaHCO_3$; (d) R′RNH, $Na(OAc)_3BH$, CH_2Cl_2, NMP, HOAc.

Analogues functionalized at C-3 were prepared as shown in Scheme 3. Treatment of **7** with *N*-bromosuc-

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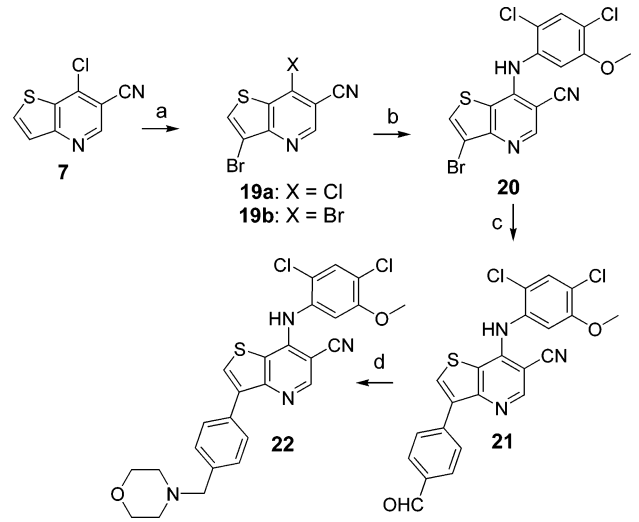
cinimide in acetic acid provided a mixture of 3-bromo analogues **19a** and **19b**. While it was possible to obtain only **19a** by performing the bromination in DMF, the yield was low and the purification difficult. Since both the 7-chloro group of **19a** and the 7-bromo group of **19b** were readily displaced, the mixture was converted to the 7-phenylamino analogue **20**. Suzuki reaction of **20** with 4-formylphenylboronic acid and subsequent reductive amination of **21** with morpholine provided **22**, the C-3 isomer of **13**.

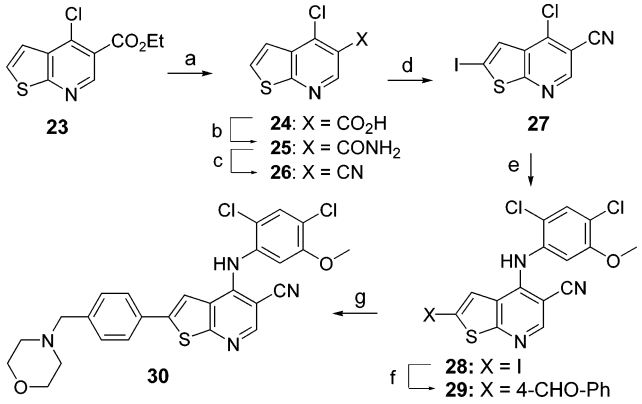
Scheme 3[a]



[a] Reagents: (a) NBS, HOAc; (b) 2,4-diCl-5-OMe aniline, NaH, THF; (c) 4-formylphenylboronic acid, $(Ph_3P)_4Pd$, DME, saturated aqueous $NaHCO_3$; (d) morpholine, $Na(OAc)_3BH$, CH_2Cl_2, NMP, HOAc.

The thieno[2,3-*b*]pyridine isomer of **13** was obtained as shown in Scheme 4. The known ethyl 4-chlorothieno-[2,3-*b*]pyridine-5-carboxylate **23** was prepared according to the literature procedure.[21] Following the same sequence of reactions used to convert **4** to **13**, **23** was converted into **30**, the [2,3-*b*] isomer of **13**.

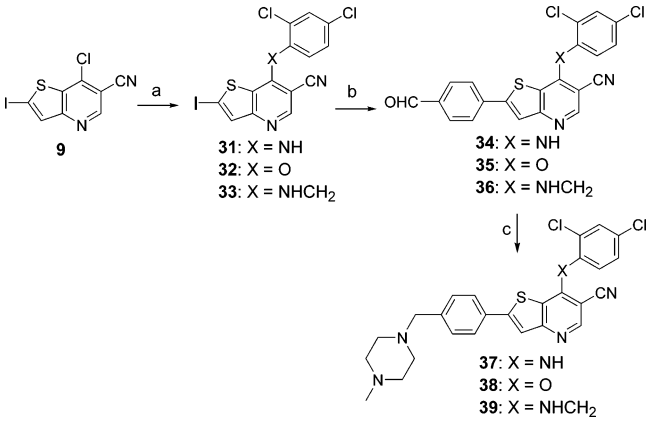
Scheme 4[a]



[a] Reagents: (a) NaOH (1N aq), EtOH; (b) (1) $SOCl_2$; (2) NH_4OH (aq); (c) cyanuric chloride, DMF; (d)(1) LDA, heptane, THF, ethylbenzene; (2) I_2, THF; (e) 2,4-diCl-5-OMe aniline, NaH, THF; (f) 4-formylphenylboronic acid, $(Ph_3P)_4Pd$, DME, saturated aqueous $NaHCO_3$; (g) morpholine, $Na(OAc)_3BH$, CH_2Cl_2, NMP, HOAc.

We had shown in the 3-quinolinecarbonitrile series that a phenylamino group at C-4 provided more potent Src inhibition than a phenoxy or benzylamino substitutent at this position. [11,18] To determine if this same effect was seen in this new series, thieno[3,2-*b*]pyridines with different linkers at C-7 were prepared as shown in Scheme 5. Treatment of **9** with 2,4-dichloroaniline under the standard conditions provided the 7-phenylamino analogue **31**. Treatment of **9** with 2,4-dichlorophenol in the presence of potassium carbonate provided the 7-phenoxy analogue **32**, while treatment of **9** with 2,4-dichlorobenzylamine in the presence of Hunig's base provided the 7-benzylamino analogue **33**. Suzuki reaction of **31**−**33** with 4-formylphenylboronic acid gave **34**−**36**; subsequent reductive amination with *N*-methylpiperazine afforded **37**−**39**.

Scheme 5[a]



[a] Reagents: (a) **31**: 2,4-diCl aniline, NaH, THF; **32**: 2,4-diCl phenol, K_2CO_3, DMF; **33**: 2,4-diCl benzylamine, Hunig's base, THF; (b) 4-formylphenylboronic acid, $(Ph_3P)_4Pd$, DME, saturated aqueous $NaHCO_3$; (c) N-Me-piperazine, $Na(OAc)_3BH$, CH_2Cl_2, NMP, HOAc.

Compounds were tested in the LANCE format Src enzyme assay[15] and the Src-dependent cell proliferation assay[11] as previously reported. While the unsubstituted thieno[3,2-*b*]pyridine **8** was a weak Src kinase inhibitor, the addition of a phenyl group at C-2 increased the activity in the Src enzyme assay by 10-fold, with **11** having an IC_{50} of 250 nM (Table 1). Another large

Table 1. Inhibition of Src Kinase Activity

compound	Src enzyme, IC_{50} nM (SD)[22]	Src cell, IC_{50} nM (SD)[22]
1	3.8[15]	100[12]
8	2500 (410)	
11	250 (37)	>10000
13	34 (10)	1200 (80)
14	13 (3)	720 (120)
16	21 (7)	1200 (370)
18	>10000	
22	>10000	
30	830 (190)	
37	50 (2)	4000 (580)
38	580 (100)	>10000
39	240 (57)	>10000

increase in activity was observed with the addition of a morpholinomethyl substitutent at the para position of the phenyl ring of **11** to provide **13**. Improved activity was seen with **14**, the *N*-methylpiperazine analogue of **13**, which had an IC_{50} of 13 nM in the Src enzyme assay and an IC_{50} of 720 nM in the Src cell assay. The meta isomer of **14**, namely **16**, had slightly reduced activity in both assays, while the ortho isomer, **18**, had greatly reduced activity. This precipitous loss of Src inhibition was also seen in the 7-phenyl-3-quinolinecarbonitrile

series where an analogue with a morpholinomethyl group at the ortho position was about 3 log orders less potent than the para isomer.[17]

A dramatic difference in activity was also observed between **13**, the C-2 substituted thieno[2,3-*b*]pyridine, and **22**, its C-3 isomer. While **13** had an IC_{50} of 34 nM in the Src enzyme assay, **22** had an IC_{50} of greater than 10 μM. A more modest decrease in potency was observed with **30**, the thieno[2,3-*b*]pyridine analogue of **13**, which had an IC_{50} of 830 nM in the Src enzyme assay.

As was observed in the 3-quinolinecarbonitrile series, the 7-phenylamino analogue **37** was a more potent Src inhibitor than the corresponding 7-phenoxy **38** or 7-benzylamino **39** analogues. Furthermore, as was seen previously, **37**, which lacks the 5-OMe group on the aniline, was about 1/4 as active as **14**, which contains the preferred 2,4-dichloro-5-methoxyaniline group.

Compound **14** was tested against a panel of kinases. While IC_{50}s of greater than 1 μM were observed against KDR, CDK4, and raf/MEK, **14** was a potent inhibitor of Abl kinase, having an IC_{50} of 2.3 nM. This inhibition of Abl activity by **14** was not unexpected since several 3-quinolinecarbonitriles are also dual inhibitors of Src and Abl, with SKI-606 having an IC_{50} of 1.1 nM for Abl inhibition.[14,15]

We are continuing to investigate the biological properties of **14** and expanding the SAR of this new core for Src kinase inhibitors.

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Supporting Information Available: Experimental details, ^{1}H NMR, HRMS, and analytical data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(22) Compounds were tested at least twice with the standard deviation reported within the brackets.

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