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Inhibition of Src kinase activity by 7-[(2,4-dichloro-5-methoxyphenyl)-amino]-2-heteroaryl-thieno[3,2-*b*]pyridine-6-carbonitriles

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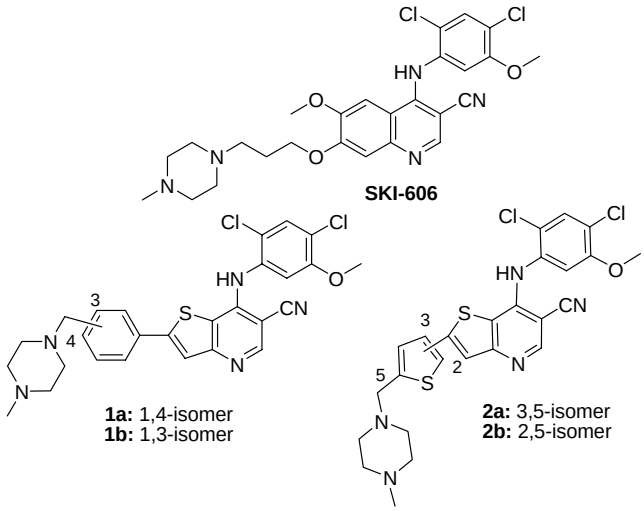
Abstract—7-[(2,4-Dichloro-5-methoxyphenyl)amino]thieno[3,2-*b*]pyridine-6-carbonitriles with various heteroaryl groups at C-2 are inhibitors of Src kinase activity. Of these new analogs, compounds substituted at C-2 by a 3,5-furan or a 2,5-pyridine had the best activity in the Src enzyme and cell assays.

The non-receptor tyrosine kinase Src is a member of a family of highly homologous kinases known as the SFKs (Src family kinases). Since overexpression or over-activation of Src is implicated in various diseases, small molecule Src inhibitors are being pursued for several therapeutic indications.[1,2] Initial efforts were focused on new agents for the treatment of cancer and osteoporosis, and later diversified to incorporate ischemic diseases, such as stroke and myocardial infarction.[3–6]

4-Phenylamino-3-quinolinecarbonitriles have been extensively studied as kinase inhibitors,[7,8] with a compound from this class, SKI-606, being a potent inhibitor of Src kinase.[9,10] It was recently reported that this activity is shared by the structurally related 7-(phenylamino)thieno[3,2-*b*]pyridine-6-carbonitriles.[11] The C-2 phenyl thieno[3,2-*b*]pyridine-6-carbonitrile **1a** had an IC_{50} of 13 nM for the inhibition of Src kinase activity. This 1,4 substituted derivative was a more potent Src inhibitor than the 1,3 substituted isomer **1b**, while the 1,2 substituted isomer had greatly reduced activity. A difference in activity was also observed with the C-2 thiophene analogs, where **2a** was a more potent Src inhibitor than **2b**.[12] To further study the effect of the substituent at C-2, additional analogs were prepared varying the heteroaryl ring at this position.



The preparation of two C-2 furan analogs is shown in Scheme 1. Treatment of the previously reported 2-iodo intermediate **3**[11] with commercially available 2-formyl-4-furanboronic acid provided the aldehyde derivative **4**. Reaction of **3** with tributyl[5-(1,3-dioxolan-2-yl)-2-furanyl]stannane[13], followed by acid hydrolysis of the

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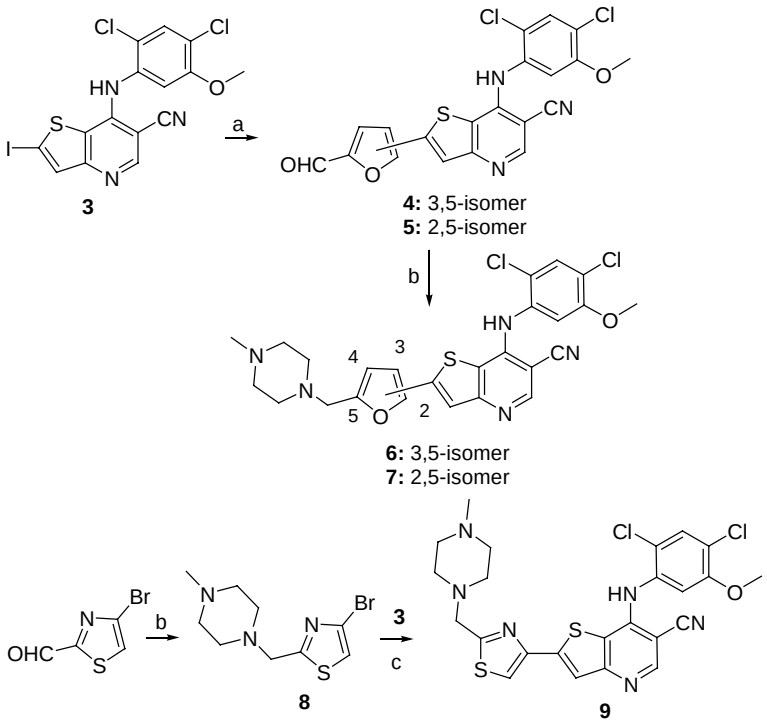
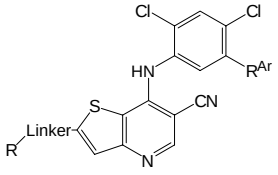


Scheme 1. Reagents: (a) for **4**: 2-formyl-4-furanboronic acid, $(Ph_3P)_4Pd$, DME, satd aq $NaHCO_3$; for **5**: (1) tributyl[5-(1,3-dioxolan-2-yl)-2-furanyl]stannane, $(Ph_3P)_2PdCl_2$, dioxane, (2) 2N HCl, THF; (b) 1-methylpiperazine, $Na(OAc)_3BH$, CH_2Cl_2, NMP, HOAc; (c) (1) $(Me_3Sn)_2$, $(Ph_3P)_4Pd$, dioxane, (2) $(Ph_3P)_4Pd$, dioxane.

intermediate acetal, provided aldehyde **5**. Reductive amination of **4** and **5** with 1-methylpiperazine in the presence of sodium triacetoxyborohydride led to the desired derivatives **6** and **7**.

As shown in Table 1, **6**, the 3,5-furan isomer, was more potent than **7**, the 2,5-furan isomer, with **6** having an IC_{50} of 13 nM in the Src enzyme assay and an IC_{50} of 260 nM in the Src cell assay. These results correspond to those observed with the thiophene isomers **2a** and **2b**. Since the C-2 3,5-thiophene substituent showed good Src inhibitory activity, the C-2 4,2-thiazole **9** was targeted. This compound has a N in place of the CH group at C-4 of the thiophene ring of **2a**.

Table 1. Inhibition od Src kinase activity



Compound	Linker	R	R^{Ar}	Src Enzyme IC_{50} nM[18]	Src Cell IC_{50} nM[18]
SKI-606				3.8[19]	100[20]
1a	1,4-Phenyl	CH_2-N-Me-piperazine	OMe	13	720
1b	1,3-Phenyl	CH_2-N-Me-piperazine	OMe	21	1200
2a	3,5-Thiophene	CH_2-N-Me-piperazine	OMe	7.2	430
2b	2,5-Thiophene	CH_2-N-Me-piperazine	OMe	25	2400
6	3,5-Furan	CH_2-N-Me-piperazine	OMe	13	260
7	2,5-Furan	CH_2-N-Me-piperazine	OMe	52	3100
9	4,2-Thiazole	CH_2-N-Me-piperazine	OMe	56	2900
12	2,5-Pyridine	CH_2-N-Me-piperazine	OMe	13	420
13	2,6-Pyridine	CH_2-N-Me-piperazine	OMe	51	1900
14	Alkyne	CH_2-N-Me-piperazine	OMe	300	>5000
16	2,5-Pyridine	CH_2NMe_2	OMe	13	650
18	2,5-Pyridine	CO-N-Me-piperazine	OMe	14	360
20	2,5-Pyridine	CH_2-N-Me-piperazine	H	47	>5000

Known 4-bromo-1,3-thiazole-2-carbaldehyde[14] was converted to **8** via reductive amination with 1-methylpiperazine. In situ conversion of **8** to the tributylstannane derivative, followed by coupling with **3**, provided **9**. Surprisingly, **9** was much less potent than **2a**, having an IC_{50} in the Src enzyme assay of only 56 nM. If replacement of the CH of the thiophene with a N led to reduced activity, would the same replacement in the phenyl series also lead to reduced activity?

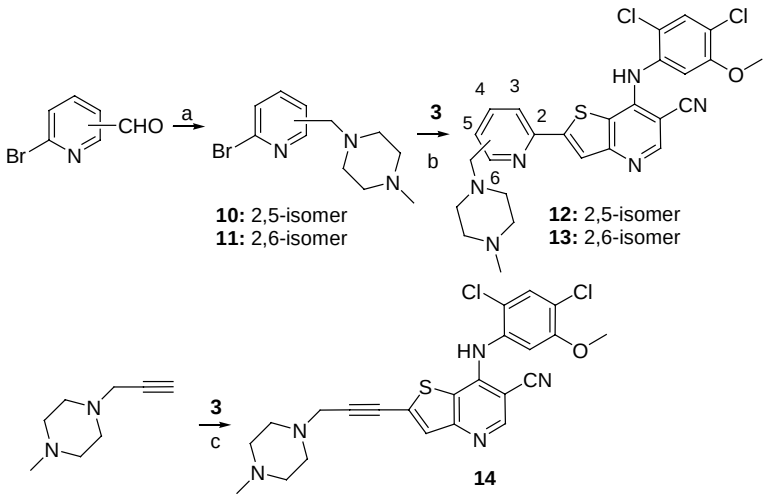
To this end, Scheme 2 depicts the preparation of the C-2 2-pyridine analogs of **1a** and **1b**. The commercially available 6-bromo 2- and 3-pyridinecarboxaldehydes were converted to **10** and **11** via reductive amination with 1-methylpiperazine. Preparation of the tributylstannane derivatives of **10** and **11**, followed by coupling with **3**, resulted in the formation of **12** and **13**. As shown in Table 1, **12** was as potent in the Src enzyme assay as **1a** and was more potent than **1a** in the Src cell assay. Therefore, as opposed to what was observed with the thiophene to thiazole pair of analogs, replacement of the CH group at C-2 of the phenyl ring of **1a** with a N did not lead to a reduction in activity. Corresponding to the reduced activity of **1b** compared to **1a**, the 2,6-isomer **13** was about 5-fold less potent than **12**. The compounds with greater Src inhibitory activity can be viewed as being more linear than the less active compounds. Conceivably, the most linear analog possible would contain a triple bond at C-2 of the thieno[3,2-*b*]pyridine ring. The highly rigid ethynyl analog **14** was prepared by reaction of **3** with 1-methyl-4-(2-propynyl)piperazine.[15] This compound was a weak Src inhibitor having an IC_{50} of only 300 nM in the enzyme assay and an IC_{50} of greater than 5 μM in the cell assay. Therefore, to inhibit Src activity it may be necessary that a molecule has a greater degree of flexibility at C-2 than that conferred by the triple bond.

Additional analogs of **12** were prepared, as shown in Scheme 3. The dimethylamine analog **16** was obtained by coupling of **3** with the tributylstannane derivative of **15**, which was prepared by reductive amination of 5-bromo-2-pyridinecarboxaldehyde with dimethylamine. To prepare the amide analog, the methyl group of 2-bromo-5-methylpyridine was first oxidized to the corresponding acid with potassium permanganate. Subsequent amide formation with *N*,*N*-carbonyldiimidazole and 1-methylpiperazine provided **17**. The trimethylstannane derivative of **17** was generated and coupled with **3** to provide **18**.

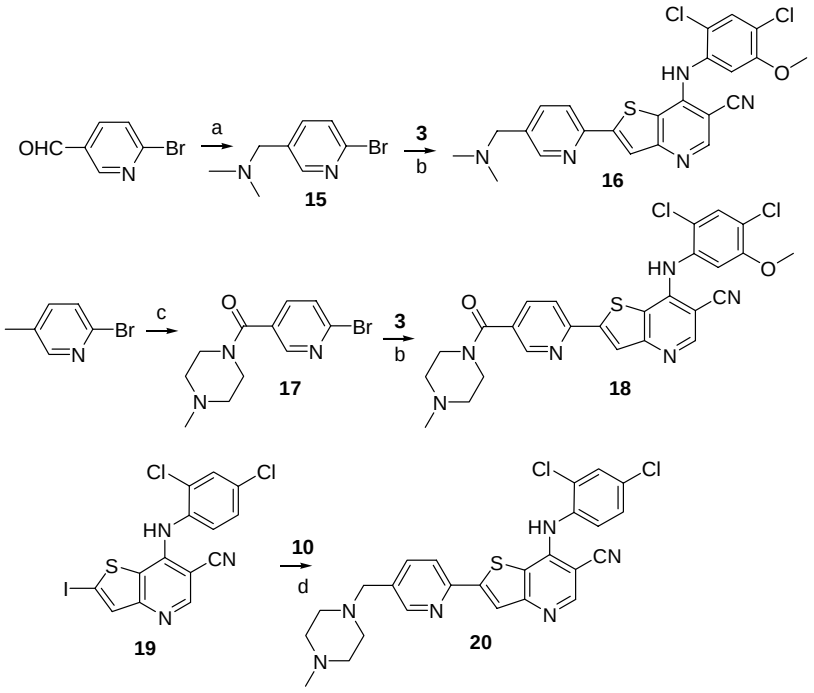
We had previously observed that **1a** and **2a** were more potent Src inhibitors than the analogs without a 5-OMe group on the aniline at C-7.[11,12] The C-7 (2,4-dichlorophenyl)amino analog of **12** was readily prepared from the known **19**[11] using the same reaction conditions used to convert **3** to **12**. As shown in Table 1, while the dimethylamine analog **16** and the amide analog **18** had comparable activity to that of **12** in the enzyme assay, and values within 2 fold in the cell assay, the C-7 (2,4-dichlorophenyl)amino analog **20** had greatly reduced activity especially in the Src cell assay.

Of these analogs, compound **12** was selected for further evaluation. When tested against a panel of kinases, **12** had IC_{50}s of 1.6 μM for the inhibition of Raf/MEK and 2.0 μM for the inhibition of EGFR. IC_{50}s of greater than 10 μM were obtained for the inhibition of CDK4, IKK, AKT, and PDK1. When tested for inhibition of Abl kinase, **12** had an IC_{50} of 1.3 nM, making it a comparable Abl inhibitor to **1a** and SKI-606, which had IC_{50}s of 2.3 and 1.0 nM, respectively. Due to similarly active conformations of the ATP binding sites of these two kinases, many compounds first identified as Src inhibitors were later found to also inhibit Abl.[16,17]

Testing of **12** at 3 μM, resulted in less than 30% inhibition of CYPs 3A4, 2D6, and 2C9. When incubated with nude mouse liver microsomes, **12** had a half-life of greater than 30 min. In addition, 24 h after administration of a 50 mg/kg oral dose of **12** to nude mice, a plasma level



Scheme 2. Reagents: (a) 1-Methylpiperazine, $Na(OAc)_3BH$, CH_2Cl_2, NMP, HOAc; (b) (1) *n*-BuLi, Bu_3SnCl, THF, (2) $(Ph_3P)_2PdCl_2$, dioxane; (c) $(Ph_3P)_4Pd$, CuI, Et_3N, benzene.



Scheme 3. Reagents: (a) Dimethylamine, $Na(OAc)_3BH$, CH_2Cl_2, NMP, HOAc; (b) $(Me_3Sn)_2$, $(Ph_3P)_4Pd$, dioxane; (c) (1) $KMnO_4$, H_2O, Aliquat 336, (2) CDI, THF, (3) 1-methylpiperazine; (d) (1) *n*-BuLi, Bu_3SnCl, THF, (2) $(Ph_3P)_2PdCl_2$, dioxane.

of 160 ng/mL was observed. In comparison, in a similar PK study **1a** had a plasma level of 400 ng/mL and was subsequently found to have good activity in an HT-29 xenograft model.[11] Based on these data, **12** is an acceptable candidate for in vivo testing in xenograft models.

Acknowledgments

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