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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 3993–3997

7-(Aryl/heteroaryl-2-ylethynyl)-4-phenylamino-3-quinolinecarbonitriles as new Src kinase inhibitors: Addition of water solubilizing groups

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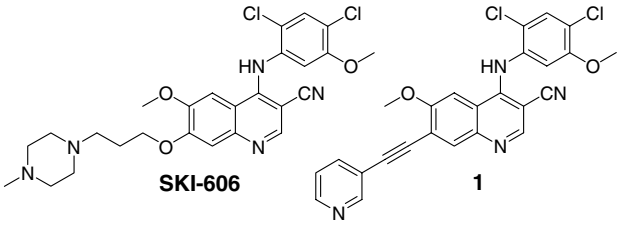
Received 29 March 2006; revised 3 May 2006; accepted 4 May 2006
Available online 2 June 2006

Abstract—New 4-phenylamino-3-quinolinecarbonitriles with a 7-ethynyl group substituted by a pyridine, phenyl or thiophene ring containing basic water solubilizing groups were prepared and evaluated as Src kinase inhibitors. Of these new analogs, potent activity was observed with compounds having a (2,4-dichloro-5-methoxyphenyl)amino group at C-4, a methoxy or ethoxy group at C-6, and a pyridyl group bearing a dimethylamine or *N*-methylpiperazine on the ethynyl group at C-7.

Src, a non-receptor tyrosine kinase, has a central role in signaling pathways controlling cell proliferation and migration.[1] Over-expression or over-activation of Src has been implicated in several diseases including cancer, osteoporosis, stroke, and myocardial infarction.[2a–e] As a result, Src has been recognized as a significant therapeutic target and various classes of small molecule Src inhibitors have been synthesized.[3a–f]



In earlier work, we identified 7-alkoxy-4-phenylamino-3-quinolinecarbonitriles as potent Src inhibitors, with the optimal compound being **SKI-606**.[4a–d] It was recently shown that analogs where the 7-alkoxy group of **SKI-606** was replaced by a 7-ethynyl heteroaryl/aryl group, as exemplified by **1**, retained activity against Src.[5a,b] Based on these findings, new 4-phenylamino-7-ethynyl-3-quinolinecarbonitriles containing basic water solubilizing groups were designed and synthesized.

The synthesis of **7a–e** and **9** is shown in Scheme 1. Treatment of the allylic bromo group of **2**[6a,6b,6c] with dimethylamine or *N*-methylpiperazine led to the aminomethyl-substituted pyridines **3a–f**. Reaction of **3a–e** with trimethylsilylacetylene followed by desilylation provided **4a–e**. Intermediates **3b–c** were also prepared via reductive amination of 2-bromo formylpyridines **5**. Sonogashira coupling of the 3-quinolinecarbonitriles **6a–c**[7a,7b] with **4a–e** resulted in the analogs **7a–e**. The 2,3-isomer of **7a**, compound **9**, was prepared by an alternative route. Introduction of trimethylsilylacetylene onto C-7 of **6b**[7a] yielded **8** and subsequent coupling of **8** with **3f** under Sonogashira conditions with microwave heating gave **9**.

As shown in Table 1, positional variation of the dimethylaminomethyl group on the pyridine ring had a large effect on Src inhibition. Moving the dimethylaminomethyl group from C-6 (**7c**) to C-5 (**7b**) to C-4 (**7a**) to C-3 (**9**) of the pyridine decreased Src-inhibitory activity. Of these analogs, the 2,6-pyridine isomer **7c** was the most potent inhibitor, having an IC_{50} of 4.5 nM in the Src enzyme assay and an IC_{50} of 120 nM in a Src-dependent cell proliferation assay. The 2,3-pyridine isomer **9**

Keywords: Src; 3-Quinolinecarbonitrile.
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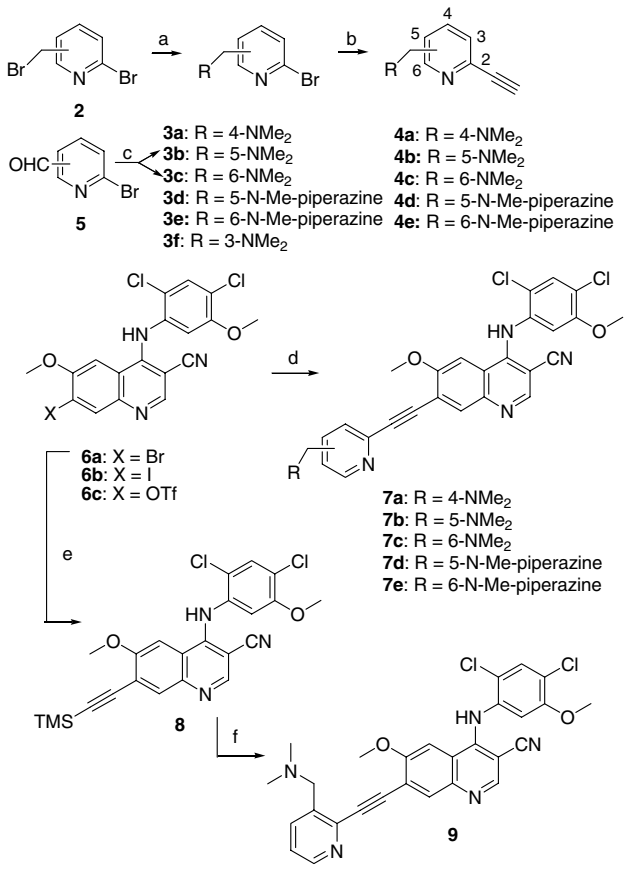
doi:10.1016/j.bmcl.2006.05.015



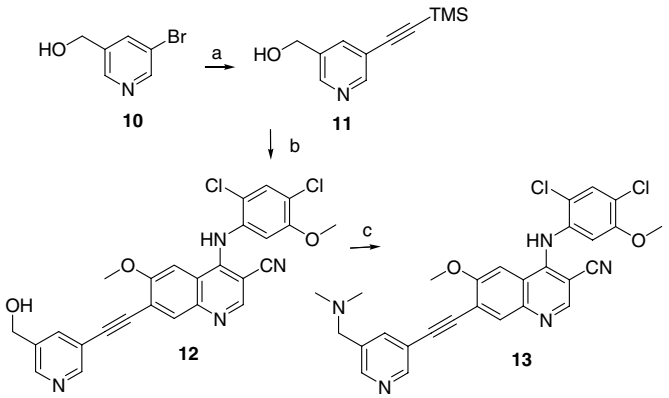
Scheme 1. Reagents and conditions: (a) Me_2NH or *N*-Me-piperazine, Hűnig's base, MeCN; (b) (1) TMS–acetylene, $Pd(PPh_3)_2Cl_2$, CuI, diisopropylamine, THF; (2) 1 N aq K_2CO_3, MeOH; (c) Me_2NH, $Na(OAc)_3BH$, AcOH, CH_2Cl_2; (d) **4a**–**e**, $Pd(PPh_3)_4$, CuI, Et_3N, dioxane; (e) TMS–acetylene, $Pd(PPh_3)_2Cl_2$, CuI, diisopropylamine, THF; (f) **3f**, $Pd(PPh_3)_2Cl_2$, CuI, PPh_3, K_2CO_3, DMF, CH_3OH, microwave.

was a much less active inhibitor, having an IC_{50} of only 2700 nM in the Src enzyme assay. It was observed that replacement of the dimethylamino group of **7b** with *N*-methylpiperazine to provide **7d** led to a 2-fold decrease in activity in the Src enzyme and cell assays. However, **7e** bearing a 6-methyl-*N*-methylpiperazine group had similar potency to the dimethylamino-substituted analog **7c**. In general, the analogs **7c** and **7e** with a C-6 group were more active than the analogs **7b** and **7d** with a C-5 group in the Src cell assay.

An isomer of **7c** where there is a 3,5-substituted pyridine on the ethynyl group at C-7 was prepared by the route shown in Scheme 2. Treatment of **10**[8] with trimethylsilylacetylene gave **11**. Coupling of **11** with **6c**[7b] resulted in the formation of **12**. Mesylation of **12** with MsCl followed by addition of dimethylamine provided **13**. The 3,5-pyridine isomer **13** had a similar activity to the 2,6-pyridine isomer **7c**, with **13** having IC_{50}s of 3.6 and 150 nM in the enzyme and cell assays, respectively.

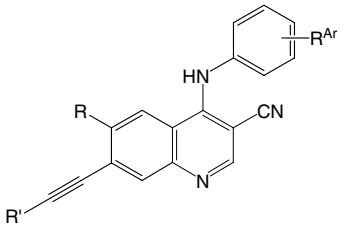
In order to investigate the effect of lengthening the chain, analogs with an aminoethyl group at C-6 of the 2-pyridine were prepared (Scheme 3). Reduction of **14**[9] with DIBAL-H yielded **15**. Sonogashira coupling of **15** with **8** under microwave heating gave **16**. Treatment of **16** with MsCl followed by addition of dimethylamine or *N*-methylpiperazine provided **17a** and **17b**. Compared to **7c**, these analogs had similar Src enzyme activity and showed slightly improved Src cell activity.

We next looked at the effect of replacing the pyridine with a thiophene or phenyl group on Src-inhibitory activity. The preparation of these analogs is depicted in Scheme 4. Reductive amination of 5-bromo-2-thiophene-carboxaldehyde **18** with dimethylamine gave **19**. Treatment of **19** with trimethylsilylacetylene followed by desilylation provided **20**. Coupling of **20** with **6b** led to the thiophene analog **21**. The phenyl analogs **24a** and **24b** were prepared by using the same conditions as those used to prepare **9**. Replacement of the allylic bromo group of **22a** or **22b** with dimethylamine yielded **23a** or **23b**. Subsequent reaction of **23a** or **23b** with **8** provided the two isomers **24a** or **24b**, respectively. The 2-thiophene analog **21** was about three times less active than **7c** in both the enzyme and cell assays. The 4-phenyl isomer **24b** was more potent than the 3-phenyl isomer **24a**, having an IC_{50} of 5.3 nM in the enzyme assay and an IC_{50} of 190 nM in the cell assay.

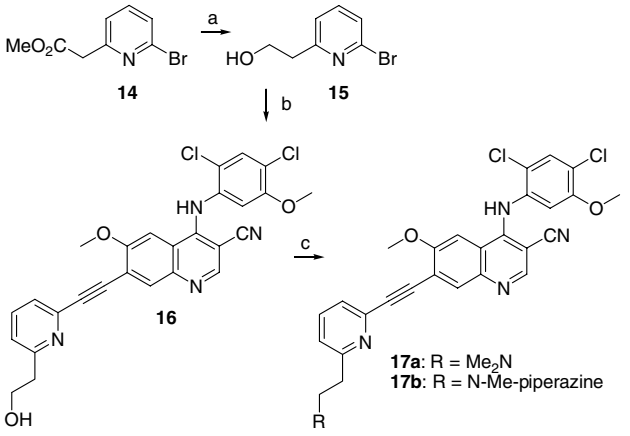


Scheme 2. Reagents and conditions: (a) TMS–acetylene, $Pd(PPh_3)_2Cl_2$, CuI, diisopropylamine, THF; (b) **6c**, $Pd(PPh_3)_2Cl_2$, CuI, PPh_3, K_2CO_3, THF, CH_3OH; (c) (1) MsCl, TEA, THF, DMF; (2) Me_2NH.

Table 1. Src enzyme and cell-inhibitory activity of 7-ethynyl-3-quinolinecarbonitriles



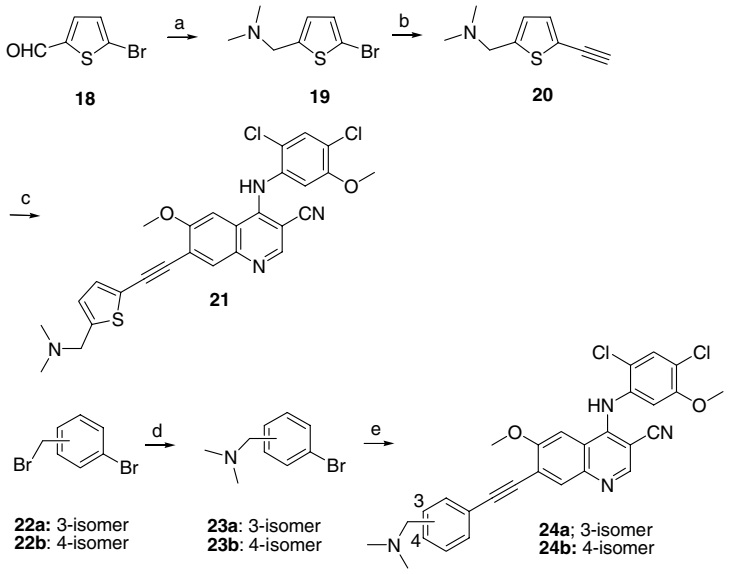
Compound[12]	R^{Ar}	R′	R	Src enzyme IC_{50} (nM)[4e,13]	Src cell IC_{50} (nM)[4a,13]
SKI-606				3.8[4e]	100[4b]
1	2,4-DiCl-5-OMe	3-Pyridine	OMe	12[5b]	270[5b]
7a	2,4-DiCl-5-OMe	2-Pyridyl-4-CH_2NMe_2	OMe	18	580
7b	2,4-DiCl-5-OMe	2-Pyridyl-5-CH_2NMe_2	OMe	4.9	240
7c	2,4-DiCl-5-OMe	2-Pyridyl-6-CH_2NMe_2	OMe	4.5	120
7d	2,4-DiCl-5-OMe	2-Pyridyl-5-CH_2-*N*-Me-piperazine	OMe	9.0	390
7e	2,4-DiCl-5-OMe	2-Pyridyl-6-CH_2-*N*-Me-piperazine	OMe	4.2	120
9	2,4-DiCl-5-OMe	2-Pyridyl-3-CH_2NMe_2	OMe	2700	NT
13	2,4-DiCl-5-OMe	3-Pyridyl-5-CH_2NMe_2	OMe	3.6	150
17a	2,4-DiCl-5-OMe	2-Pyridyl-6-$(CH_2)_2NMe_2$	OMe	5.3	87
17b	2,4-DiCl-5-OMe	2-Pyridyl-6-$(CH_2)_2$*N*-Me-piperazine	OMe	4.3	81
21	2,4-DiCl-5-OMe	2-Thienyl-5-CH_2NMe_2	OMe	15	390
24a	2,4-DiCl-5-OMe	Phenyl-3-CH_2NMe_2	OMe	9.9	230
24b	2,4-DiCl-5-OMe	Phenyl-4-CH_2NMe_2	OMe	5.3	190
26a	2,4-DiCl-5-OMe	2-Pyridyl-6-CH_2NMe_2	OEt	3.6	86
26b	2,4-DiCl-5-OMe	2-Pyridyl-6-CH_2NMe_2	H	22	470
28a	3,4,5-Tri-OMe	2-Pyridyl-6-CH_2NMe_2	OMe	29	350
28b	3,4,5-Tri-OMe	2-Pyridyl-6-CH_2NMe_2	H	120	2200



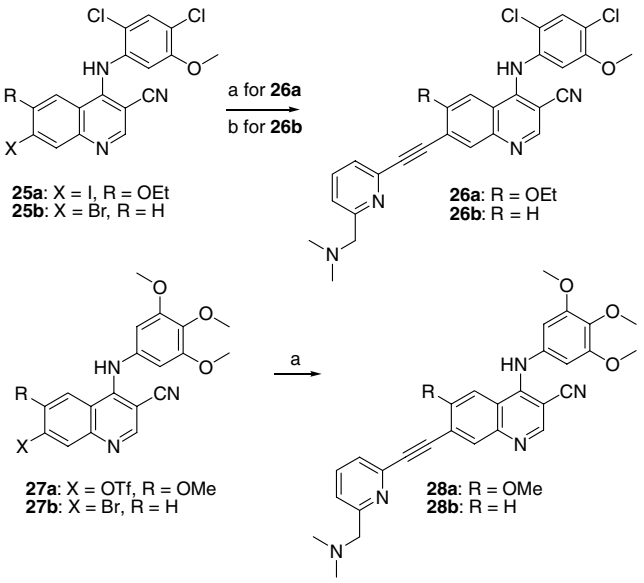
Scheme 3. Reagents and conditions: (a) DIBAL-H, THF; (b) **8**, $Pd(PPh_3)_2Cl_2$, CuI, PPh_3, K_2CO_3, DMF, CH_3OH, microwave; (c) (1) MsCl, TEA, THF, DMF; (2) Me_2NH or *N*-Me-piperazine.

Finally, the effect of variation of the C-6 group and the C-4 headpiece on Src inhibition was investigated. Scheme 5 shows the preparation of 7-[2-(2-pyridyl)-ethynyl]-3-quinolinecarbonitriles with different groups at C-6 and C-4 of the core structure. Coupling of **25a**[7a], **25b**[10], **27a**[5b] and **27b**[5b] with **4d** afforded **26a**, **26b**, **28a** and **28b**, respectively. Compound **26a** with an ethoxy substituent at C-6 had a moderate increase in activity in both the Src enzyme and cell assays compared to **7c**. A large difference in potency was seen between the 6-methoxy analog **7c** and its 6-hydrogen analog **26b**. Compound **7c** was about 5-fold more potent than **26b** in the enzyme assay and 4-fold more potent in the cell assay. We had previously reported[11,5b] that a 3,4,5-trimethoxyanilino group at C-4 of 3-quinolinecarbonitriles also provided a series of Src inhibitors. However, a decrease in Src inhibition was observed in both the Src enzyme and cell assays when the 2,4-dichloro-5-methoxyanilino group at C-4 of **7c** was replaced with a 3,4,5-trimethoxyanilino group. **28a**, the 3,4,5-trimethoxyanilino analog of **7c**, had an IC_{50} of 29 nM in the enzyme assay and an IC_{50} of 350 nM in the cell assay. As expected, **28b**, the 6-hydrogen analog of **28a**, showed greatly reduced Src-inhibitory activity.

Several of these 7-ethynyl analogs had comparable activity to **SKI-606** and **7c** was chosen for a pharmacokinetic study. Twenty-four hours after administration of a single oral 50 mg/kg dose to nude mice, the plasma levels of **7c** were about a third those of **SKI-606** under the same conditions. Studies with nude mouse liver microsomes showed that the metabolic stability of **7c** was lower than that of **SKI-606**. In an in vivo HT29 xenograft study, **7c** had only moderate activity, possibly as a result of its lower plasma level and shorter half-life compared to these same parameters for **SKI-606**. We are continuing to investigate other 4-[(2,4-dichloro-5-methoxyphenyl)amino]-3-quinolinecarbonitriles with various groups at C-7.



Scheme 4. Reagents and conditions: (a) Me_2NH, $Na(OAc)_3BH$, AcOH, CH_2Cl_2; (b) (1) TMS–acetylene, $Pd(PPh_3)_2Cl_2$, CuI, diisopropylamine, THF; (2) 1 N aq K_2CO_3, MeOH; (c) **6b**, $Pd(PPh_3)_4$, CuI, Et_3N, dioxane; (d) Me_2NH, Hűnig's base, MeCN; (e) **8**, $Pd(PPh_3)_2Cl_2$, CuI, PPh_3, K_2CO_3, MeOH, DMF, microwave.



Scheme 5. Reagents and conditions: (a) for **26a**, **28a**, and **28b**: **4d**, $Pd(PPh_3)_4$, CuI, Et_3N, dioxane; (b) for **26b**: **4d**, $Pd(PPh_3)_2Cl_2$, CuI, PPh_3, K_2CO_3, DMF, CH_3OH, microwave.

Acknowledgments

We thank the members of the Wyeth Chemical Technology Department for their services, Judy Lucas for the in vivo studies, and the Drug Safety and Metabolism Department for the metabolic stability and plasma level determinations. We also thank Drs. Janis Upeslacis and Tarek Mansour for advice and support.

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12. All compounds were characterized by MS, NMR, and CHN combustion analyses.
13. Assay: IC_{50} values represent means of at least two separate determinations.