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Further studies on ethenyl and ethynyl-4-phenylamino-3-quinolinecarbonitriles: identification of a subnanomolar Src kinase inhibitor

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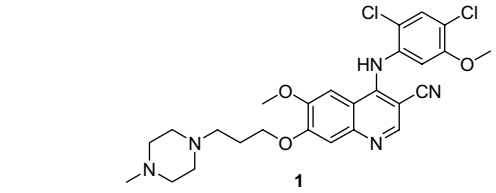
Abstract—Several new ethynyl- and ethenyl-4-phenylamino-3-quinolinecarbonitriles were synthesized and tested for Src inhibition. Derivatives bearing an ethenyl or ethynyl substituent at C-6 showed decreased Src inhibitory activity. Incorporation of an ethenylpyridine *N*-oxide group at C-7 provided **20b**, a 0.6 nM inhibitor of Src enzymatic activity with excellent cellular potency.

Src is a member of a group of structurally similar non-receptor tyrosine kinases. This group of enzymes, which have highly homologous ATP binding regions, is referred to as the Src family of kinases. Src plays a pivotal role in many cell signalling pathways and elevated Src activity/expression has been implicated in various disease states including cancer and osteoporosis.[1]

In earlier work, the 4-phenylamino-6,7-dialkoxy-3-quinolinecarbonitrile **1** (SKI-606) was identified as a potent inhibitor of Src kinase activity.[2a,b] This compound is characterized by the presence of a 2,4-diCl, 5-OMe aniline group at C-4, a 3-(4-methyl-1-piperazinyl)-propoxy group at C-7 and a methoxy substituent at C-6. Although extensive synthetic work has been reported on the 6,7-dialkoxy-3-quinolinecarbonitrile series,[3] groups other than alkoxy are tolerated at C-7.[4a,b] The Src inhibitors **2a** and **3a**, which bear a 7-ethenyl and a 7-ethynyl group, respectively, constitute the most recent examples.[5] As part of these ongoing efforts, the synthesis and Src inhibitory activity of new derivatives of this class are hereby reported.

Earlier studies on 6,7-dialkoxy-3-quinoline-carbonitriles showed that a 2,4-diCl, 5-OMe aniline group at C-4 provided potent inhibition of Src kinase.[2a,3a–d] Although removing the 5-OMe group of **2b** decreased Src inhibition (**2c**),[5] other variations in the aniline group had not yet been investigated in the 7-ethenyl and 7-ethynyl series. For this reason, intermediates **5a–d** were synthesized from 7-bromo-4-chloro-3-quinolinecarbonitrile (**4**) using known methods.[4a] Coupling of **5a–d** with 4-vinylpyridine under Heck conditions gave derivatives **6a–d**.[5] Similarly, Sonogashira reaction of **5d** with 3-ethynylpyridine yielded analog **7** (Scheme 1).[5]



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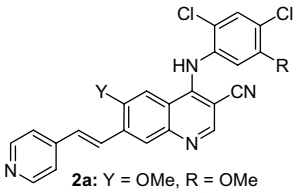
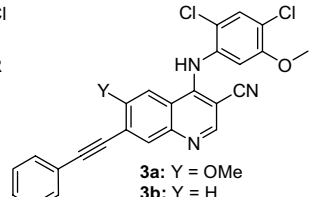


2a: Y = OMe, R = OMe
2b: Y = H, R = OMe
2c: Y = R = H



3a: Y = OMe
3b: Y = H

Keywords: Src; Kinase; 3-Quinolinecarbonitriles.
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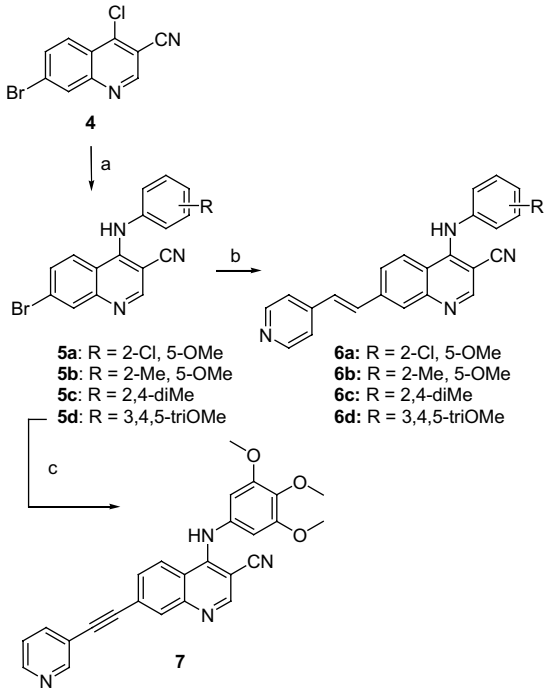
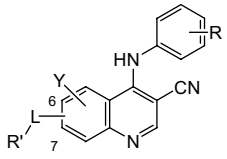


Scheme 1. Reagents and conditions: (a) aniline, pyridine hydrochloride, 2-ethoxyethanol or aniline, NaH, THF; (b) 4-vinylpyridine, $Pd(OAc)_2$, P(*o*-Tol)$_3$, DMF, TEA; (c) 3-ethynylpyridine, $Pd(Ph_3P)_4$, CuI, DMF, TEA.

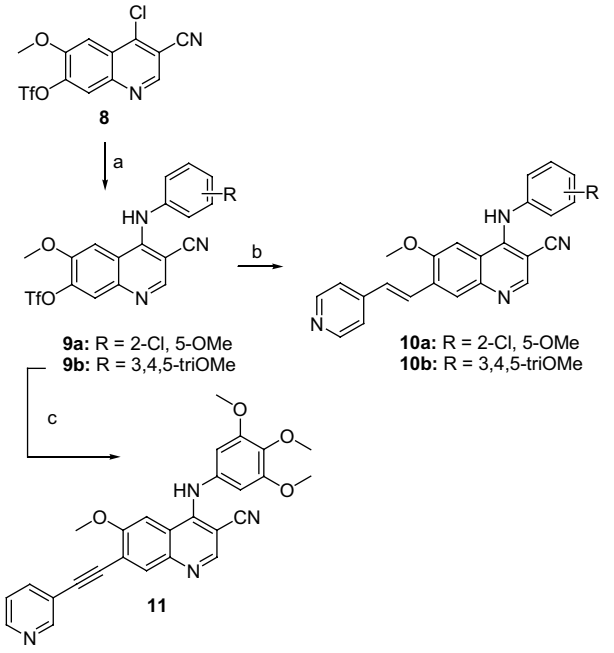
As shown in Table 1, variation of the aniline substituents of **2b** from 2,4-diCl, 5-OMe to 2-Cl, 5-OMe (**6a**) and 2-Me, 5-OMe (**6b**) decreased the Src enzyme inhibition from 9.9 nM to 16 and 21 nM, respectively. Analog **6c**, with a 2,4-diMe aniline group at C-4, was 4-fold less potent than **2b** with an IC_{50} value of 46 nM. Analog **6d**, bearing a 3,4,5-triOMe aniline group at C-4, exhibited an IC_{50} value of 19 nM in the Src enzyme assay, which represents a 2-fold decrease in inhibition over **2b**. A similar effect was observed for the corresponding 7-ethynyl derivative **7**, which was almost 2-fold less potent than the original lead **3b**. Similar decreases in Src inhibitory activity upon variation of the substituents on the 4-aniline group were reported for the 6,7-dialkoxy-4-phenylamino-3-quinolinecarbonitrile series.[3a–3d]

Derivatives with a methoxy substituent at C-6 were then synthesized to investigate if this change would result in an increase in Src inhibitory activity as was previously observed for **2b** and **3b** (Scheme 2). Intermediate **8**[4b] was reacted with 2-Cl, 5-OMe, and 3,4,5-triOMe aniline to yield the 7-trifluoro-methylsulfonate intermediates **9a** and **9b**, which were treated with 4-vinylpyridine to yield **10a** and **10b**. Analog **11**, the 6-OMe derivative of alkyne **7**, was also synthesized. As shown in Table 1, derivative **10a**, with a 2-Cl, 5-OMe aniline group at C-4, was 4-fold more potent than its 6-H analog **6a**. In addition, **10a** showed comparable Src enzyme inhibition to the 6-

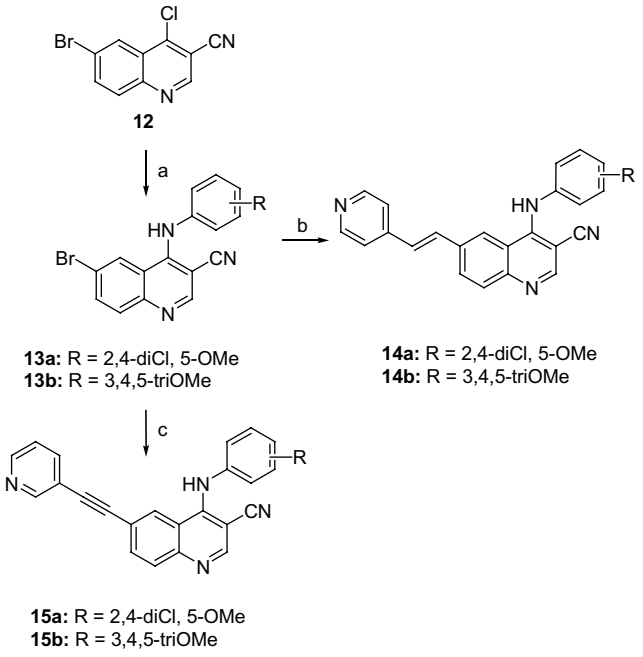
Table 1. Src enzyme inhibitory activity of ethenyl- and ethynyl-3-quinolinecarbonitriles



Compound[7]	R′	L	Y	R	Src enzyme[8] IC_{50} nM
1	—	—	—	—	3.6[5]
2a	4-Pyridyl	7-Ethenyl	6-OMe	2,4-DiCl, 5-OMe	4.2[5]
2b	4-Pyridyl	7-Ethenyl		2,4-DiCl, 5-OMe	9.9[5]
2c	4-Pyridyl	7-Ethenyl		2,4-DiCl	42[5]
3a	3-Pyridyl	7-Ethynyl	6-OMe	2,4-DiCl, 5-OMe	12[5]
3b	3-Pyridyl	7-Ethynyl		2,4-DiCl, 5-OMe	47[5]
6a	4-Pyridyl	7-Ethenyl		2-Cl, 5-OMe	16 (2.1)
6b	4-Pyridyl	7-Ethenyl		2-Me, 5-OMe	21 (1.4)
6c	4-Pyridyl	7-Ethenyl		2,4-DiMe	46 (15)
6d	4-Pyridyl	7-Ethenyl		3,4,5-TriOMe	19 (4.8)
7	3-Pyridyl	7-Ethynyl		3,4,5-TriOMe	89 (28)
10a	4-Pyridyl	7-Ethenyl	6-OMe	2-Cl, 5-OMe	4.7 (0.3)
10b	4-Pyridyl	7-Ethenyl	6-OMe	3,4,5-TriOMe	17 (1.9)
11	3-Pyridyl	7-Ethynyl	6-OMe	3,4,5-TriOMe	34 (2.4)
14a	4-Pyridyl	6-Ethenyl		2,4-DiCl, 5-OMe	610 (59)
14b	4-Pyridyl	6-Ethenyl		3,4,5-TriOMe	740 (270)
15a	3-Pyridyl	6-Ethynyl		2,4-DiCl, 5-OMe	440 (93)
15b	3-Pyridyl	6-Ethynyl		3,4,5-TriOMe	910 (230)
18a	2-Pyrazinyl	7-Ethenyl		2,4-DiCl, 5-OMe	37 (7.1)
18b	2-Pyrazinyl	7-Ethenyl	6-OMe	2,4-DiCl, 5-OMe	10 (3.7)
19a	1-Imidazolyl	7-Ethenyl		2,4-DiCl, 5-OMe	6.6 (0.1)
19b	1-Imidazolyl	7-Ethenyl	6-OMe	2,4-DiCl, 5-OMe	2.0 (0.8)
20a	4-Pyridyl *N*-oxide	7-Ethenyl		2,4-DiCl, 5-OMe	4.2 (0.8)
20b	4-Pyridyl *N*-oxide	7-Ethenyl	6-OMe	2,4-DiCl, 5-OMe	0.6 (0.1)
20c	4-Pyridyl *N*-oxide	7-Ethenyl	6-OMe	3,4,5-TriOMe	11 (1.7)



Scheme 2. Reagents and conditions: (a) 2-Cl, 5-OMe aniline HCl, 2-ethoxyethanol; 3,4,5-triOMe aniline, pyridine HCl, 2-ethoxyethanol; (b) 4-vinylpyridine, $Pd(OAc)_2$, $P(o\text{-}Tol)_3$, DMF, TEA; (c) 3-ethynylpyridine, $Pd(Ph_3P)_4$, CuI, DMF, TEA.



Scheme 3. Reagents and conditions: (a) 2-Cl, 5-OMe aniline HCl, 2-ethoxyethanol; 3,4,5-triOMe aniline, pyridine HCl, 2-ethoxyethanol; (b) 4-vinylpyridine, $Pd(OAc)_2$, $P(o\text{-}Tol)_3$, DMF, TEA; (c) 3-ethynylpyridine, $Pd(Ph_3P)_4$, CuI, DMF, TEA.

OMe analog **2a**. However, this was not the case in a Src-dependent cell proliferation assay, where **2a** was twice as potent (Table 2). Interestingly, analog **10b**, with a 3,4,5-triOMe aniline group at C-4, showed comparable activity to its 6-H analog **6d**. However, in the 7-ethynyl series, 3,4,5-triOMe aniline derivative **11** showed a 2-fold increase in Src enzyme inhibition over its 6-H analog **7**.

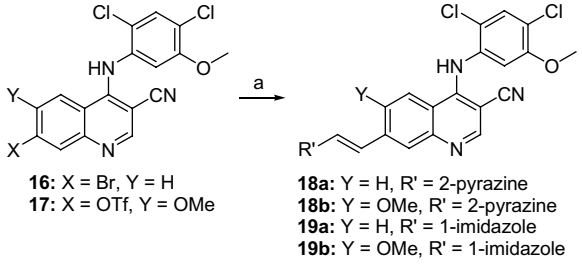
In earlier work on 6,7-dialkoxy-4-phenylamino-3-quinolinecarbonitriles it was observed that placing a large alkoxy group at C-6 rather than at C-7 was generally detrimental to the Src inhibitory activity.[3c] A similar result was obtained for the 7-thienyl-4-phenylamino-3-quinolinecarbonitrile series when the thienyl substituent was placed at C-6.[4a] To verify if the same was true for the 7-ethenyl and ethynyl series, the 6-ethenyl derivatives of **2b** and **6d** (**14a** and **14b**) and the 6-ethynyl analogs of **3b** and **7** (**15a** and **15b**) were synthesized from known 6-bromo-4-chloro-3-quinolinecarbonitrile (**12**)[4a] using the same approach applied for the synthesis of the corresponding C-7 isomers (Scheme 3). As shown in Table 1, 6-ethenyl-3-quinolinecarbonitriles **14a** and **14b** showed a significant decrease in potency in a Src enzyme assay over their corresponding 7-isomers **2b** and **6d**, with IC_{50} values of 610 and 740 nM, respectively. A decrease in enzyme inhibitory activity was also observed for the 6-ethynyl derivatives **15a** and **15b** over their 7-isomers **3b** and **7**.

We previously reported that the analog of **2a** where the pyridine ring was replaced by a phenyl was less potent than **2a**.[5] Since the addition of the nitrogen atom led to an increase in Src activity, other nitrogen containing heterocycles were investigated. The 7-ethenylpyrazine derivatives **18a** and **18b** and the 7-ethenylimidazole analogs **19a** and **19b** were synthesized from the known intermediates **16**[4a] and **17**[4b] (Scheme 4).

Although the pyrazine analogs **18a** and **18b** were 3 and 2-fold less potent in the Src enzyme assay than the ethenylpyridine derivatives **2b** and **2a**, respectively, ethenylimidazoles **19a** and **19b** showed increased Src inhibition. In addition, as previously observed with other members of this class, analogs **18b** and **19b** showed

Table 2. Inhibition of Src activity in a Src-dependent cell proliferation assay

Compound[7]	Src cell[8] IC_{50} nM
1	100[2a]
2a	80[5]
10a	160 (43)
19b	39 (0)
20a	56 (16)
20b	16 (3)



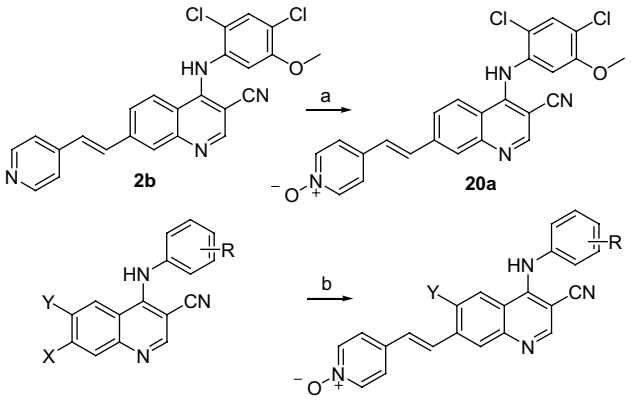
Scheme 4. Reagents and conditions: (a) 2-vinylpyrazine or 1-vinylimidazole, $Pd(OAc)_2$, $P(o\text{-}Tol)_3$, DMF, TEA.

increased Src inhibitory activity over their 6-H analogs **18a** and **19a**. Moreover, 7-ethenylimidazole **19b** exhibited an IC_{50} value of 39 nM in a Src dependent cell proliferation assay, which represents a 2-fold increase in potency over the 7-ethenylpyridine **2a** (Table 2).

Finally, the synthesis of 1-oxidopyridine analogs was investigated to determine if oxidation of the pyridine nitrogen would have an effect on the ability of the compound to inhibit Src. As shown in Scheme 5, 4-vinylpyridine **2b** was treated with *m*CPBA at room temperature to provide derivative **20a**, which exhibited a 2-fold increase in inhibitory activity, both in the enzyme and cell assays, compared to its parent compound **2b** (Tables 1 and 2). Since the 6-OMe analog of **20a** was expected to show improved potency, the synthesis of *N*-oxide **20b** was pursued next. In this case, intermediate **17** was treated with 4-vinylpyridine *N*-oxide[6] to give the desired product. This route, which circumvents the formation of over oxidized products, was also used for the synthesis of analog **20c**, which bears a 3,4,5-triOMe aniline group at C-4.

N-oxide **20b** exhibited a subnanomolar IC_{50} value in the enzyme assay (IC_{50} = 0.6 nM), making it the most potent 3-quinolinecarbonitrile Src inhibitor reported to date. In addition, analog **20b** showed an IC_{50} value of 16 nM in the Src dependent cell proliferation assay, which represents more than a 4-fold increase in potency compared to its unoxidized analog **2a**. Analog **20c**, on the other hand, was approximately 20-fold less potent than **20b** in the enzyme assay. However, this analog showed improved enzyme inhibitory activity compared to its unoxidized derivative **10b**.

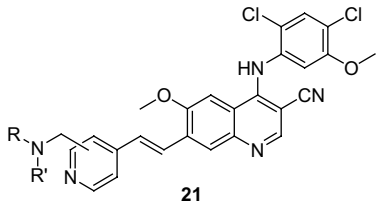
Derivatives with the best potency, namely **2a**, **19b** and **20b**, were selected for a PK study. Twenty-four hours after administration of a single oral 50 mg/kg dose to nude mice, plasma concentrations of 19 and 20 ng/mL were measured for **2a** and **20b**, respectively. These values are approximately half the plasma concentration determined for **1** (40 ng/mL) under the same conditions. In addition, the plasma levels of **19b** were below the quantification limit of 5 ng/mL. In order to improve the bioavailability of this class of Src inhibitors, future efforts will focus on the synthesis of analogs where water solubilizing amine groups have been introduced onto the pyridine ring, as in the case of **21**. Our findings with these new derivatives will be reported in due course.



17: X = OTf, Y = OMe, R = 2,4-diCl, 5-OMe
5d: X = Br, Y = H, R = 3,4,5-triOMe

20b: Y = OMe, R = 2,4-diCl, 5-OMe
20c: Y = H, R = 3,4,5-triOMe

Scheme 5. Reagents and conditions: (a) *m*CPBA, dichloromethane, AcOH; (b) 4-vinylpyridine *N*-oxide, $Pd(OAc)_2$, P(*o*-Tol)$_3$, DMF, TEA.



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Acknowledgements

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References and notes

1. (a) Yeatman, T. J. *Nat. Rev. Cancer* **2004**, *4*, 470; (b) Frame, M. C. *Biochem. Biophys. Acta* **2002**, *1602*, 114; (c) Metcalf, C. A., III; van Schravendijk, M. R.; Dalgarno, D. C.; Sawyer, T. K. *Curr. Pharm. Des.* **2002**, *8*, 2049; (d) Courtneidge, S. A. *Biochem. Soc. Trans.* **2002**, *30*, 11; (e) Susa, M.; Missbach, M.; Green, J. *Trends Pharmacol. Sci.* **2000**, *21*, 489.
2. (a) Boschelli, D. H.; Ye, F.; Wang, Y. D.; Dutia, M.; Johnson, S.; Wu, B.; Miller, K.; Powell, D. W.; Arndt, K.; Discafani, C.; Etienne, C.; Gibbons, J.; Grod, J.; Lucas, J.; Weber, J. M.; Boschelli, F. *J. Med. Chem.* **2001**, *44*, 3965; (b) Golas, J. M.; Arndt, K.; Etienne, C.; Lucas, J.; Nardin, D.; Gibbons, J.; Frost, P.; Ye, F.; Boschelli, D. H.; Boschelli, F. *Cancer Res.* **2003**, *63*, 375.
3. (a) Boschelli, D. H.; Wang, Y. D.; Johnson, S.; Wu, B.; Ye, F.; Barrios Sosa, A. C.; Golas, J. M.; Boschelli, F. *J. Med. Chem.* **2004**, *47*, 1599; (b) Boschelli, D. H.; Ye, F.; Wu, B.; Wang, Y. D.; Barrios Sosa, A. C.; Yaczko, D.; Powell, D.; Golas, J. M.; Lucas, J.; Boschelli, F. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3797; (c) 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. *J. Med. Chem.* **2001**, *44*, 822; (d) Wang, Y. D.; Miller, K.; Boschelli, D. H.; Ye, F.; Wu, B.; Floyd, M. B.; Powell, D. W.; Wissner, A.; Weber, J. M.; Boschelli, F. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2477.
4. (a) Boschelli, D. H.; Wang, D. Y.; Ye, F.; Yamashita, A.; Zhang, N.; Powell, D. W.; Weber, J. M.; Boschelli, F. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2011; (b) Berger, D.; Dutia, M.; Powell, D.; Wissner, A.; DeMorin, F.; Raifeld, Y.; Weber, J.; Boschelli, F. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2989.
5. Barrios Sosa, A. C.; Boschelli, D. H.; Ye, F.; Golas, J. M.; Boschelli, F. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2155.

6. Vinylpyridine *N*-oxide was synthesized by reaction of 4-vinylpyridine with *m*CPBA.
7. All compounds were characterized by MS, NMR, and CHN combustion analyses.
8. The IC_{50} values reported represent the means of at least 2 determinations. The standard deviations (SD) are shown in the brackets. Compounds were tested according to the Src cellular assay[2a] and Src enzyme assay[5] reported previously.