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Synthesis and inhibition of Src kinase activity by 7-ethenyl and 7-ethynyl-4-anilino-3-quinolinecarbonitriles

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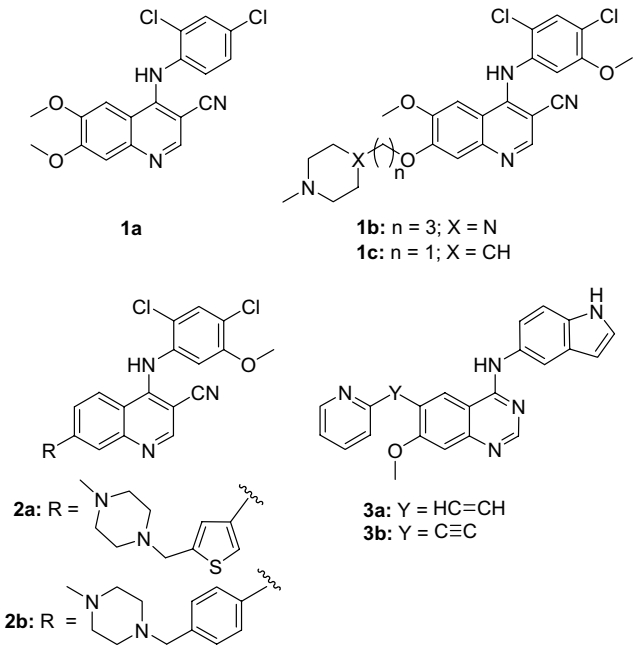
Abstract—A series of 7-ethynyl and 7-ethenyl-4-anilino-3-quinolinecarbonitriles were synthesized and tested for Src inhibition. Derivatives bearing a C-6 methoxy group and 2,4-dichloro-5-methoxyaniline at C-4 showed optimal inhibition of Src enzymatic and cellular activity. The ethenyl and ethynyl groups were incorporated at C-7 utilizing a Stille, Heck, or Sonogashira coupling reaction.

Protein tyrosine kinases constitute approximately 20% of known and predicted protein kinases. These enzymes play a central role in signal transduction and cellular mechanisms.[1] Src, a nonreceptor tyrosine kinase, is the founding member of a group of structurally homologous proteins referred to as the Src family of kinases (SFKs). Elevated Src activity/expression has been associated with several disease states including cancer and osteoporosis.[2] As a result, Src has been recognized as an important therapeutic target and several classes of inhibitors have been synthesized.[3]

In earlier work we identified 4-anilino-6,7-dialkoxy-3-quinolinecarbonitrile **1a** as an ATP competitive inhibitor of Src kinase activity, with no significant inhibition against other kinases, including EGFr, FGFr, and Cdk4.[4] After optimization of the substituents at C-4 and C-7, superior Src inhibitory activity was seen with derivatives **1b**[5] and **1c**,[6] which bear a 2,4-dichloro-5-methoxy aniline group at C-4 and a 3-(4-methyl-1-piperazinyl)-propoxy or 1-methylpiperidinemethoxy substituent at C-7, respectively.

We have also shown that groups other than alkoxy are tolerated at C-7.[7] For example, 7-thienyl and 7-phenyl derivatives **2a** and **2b** constitute potent Src inhibitors.



Interestingly, related 4-heteroaryl-6-ethenyl quinazolines and their 6-ethynyl derivatives (**3a**, **3b**) have been

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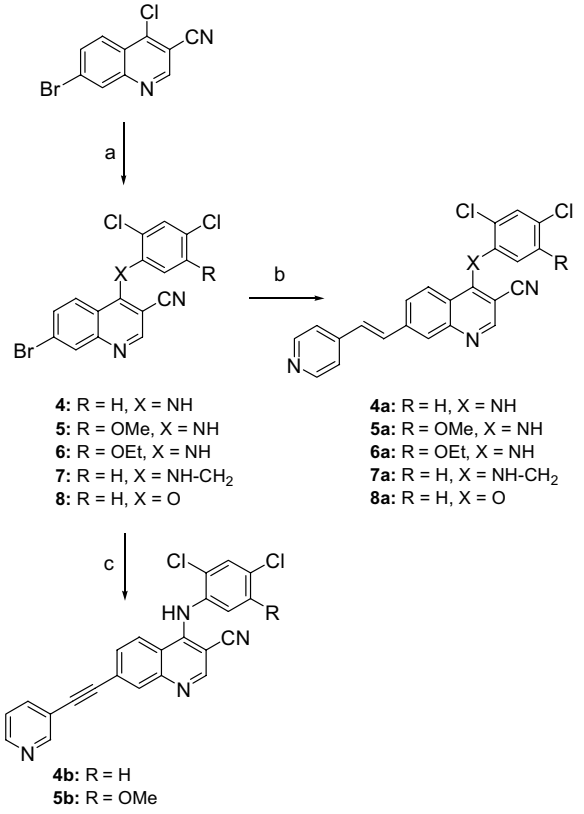
claimed to be EGFr inhibitors.[8] As part of our ongoing investigations, we sought to introduce an ethenyl or ethynyl group at C-7 of the 3-quinolinecarbonitrile.

We began our synthetic studies by reacting the previously reported intermediate 7-bromo-4-chloro-3-quinolinecarbonitrile[7a] with 2,4- and 2,4,5-substituted anilines, 2,4-dichlorobenzylamine, and 2,4-dichlorophenol to give the 4-substituted derivatives **4**–**8** (Scheme 1). Subsequent treatment of **4**–**8** with 4-vinylpyridine under Heck coupling conditions provided C-7 ethenyl derivatives **4a**–**8a**. Similarly, treatment of intermediates **4** and **5**[7a] with 3-ethynylpyridine under Sonogashira coupling conditions yielded C-7 ethynyl derivatives **4b** and **5b**.

As previously observed with the 7-alkoxy, 7-thienyl, and 7-phenyl series, 2,4-dichloro-5-methoxy aniline (**5a**) was found to be the preferred aniline for Src inhibition (Table 1). Although removal of the aniline C-5 methoxy substituent (**4a**) resulted in a decrease in inhibitory activity, replacement of this group with an ethoxy substituent (**6a**) was more detrimental. Similarly, extending the C-4 NH linker of **4a** with a methylene group (**7a**) or replacing it with an oxygen (**8a**) decreased the Src inhibitory activity 24- and 9-fold, respectively, in the enzyme assay. Loss of activity was also observed in the Src-dependent cell proliferation assay.



Scheme 1. Reagents and conditions: (a) aniline, pyridine hydrochloride, or NaH; benzylamine, Hünigs base/DMF; phenol, K_2CO_3/DMF; (b) 4-vinylpyridine, $Pd(OAc)_2$, P(*o*-Tol)$_3$, DMF, TEA; (c) 3-ethynylpyridine, $Pd(Ph_3P)_4$, CuI, DMF, TEA.

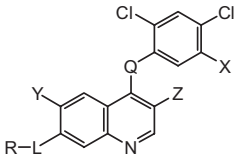
We then studied the effect of replacing the 3-cyano substituent with an ester (**12**) or acid group (**13**). For the synthesis of these derivatives, ethyl 7-bromo-1,4-dihydro-4-oxo-3-quinolinecarboxylate **9**, synthesized by a known route,[9] was treated with phosphorous oxychloride to give ethyl 7-bromo-4-chloro-3-quinolinecarboxylate **10** (Scheme 2). Reaction of **10** with 2,4-dichloroaniline using pyridine hydrochloride gave intermediate **11**. Heck coupling reaction of **11** with 4-vinylpyridine provided ethyl 3-quinolinecarboxylate **12**, which was hydrolyzed to give carboxylic acid **13**. Derivatives **12** and **13** showed no significant Src inhibitory activity, implying that the 3-cyano group is required for good Src inhibition.

Following the synthesis of derivatives with varying groups at C-3, we proceeded to replace the pyridine ring linked to C-7, with other aryl or heteroaryl groups. As shown in Scheme 3, intermediate **5**[7a] was reacted with several ethenyl[10] and ethynyl reagents under Heck and Sonogashira coupling conditions, respectively. For the synthesis of the unsubstituted vinyl derivative **14**, **5** was reacted with tributyl(vinyl)tin under Suzuki conditions. Ethyl derivative **24** was synthesized by treatment of **23** with Pd/C under a hydrogen atmosphere.

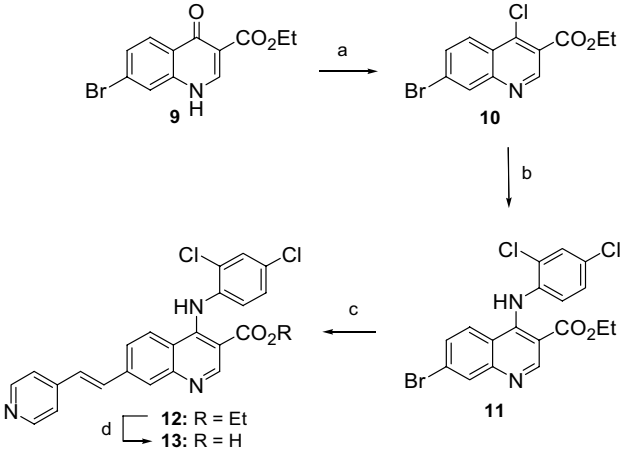
Of the different aryl and heteroaryl groups tested in the 7-ethenyl series, 3- and 4-ethenylpyridine derivatives **19** and **5a** showed superior Src inhibitory activity. Although the IC_{50} value obtained for 2-ethenylpyridine **18** in an enzyme assay did not differ significantly from the values found for **19** and **5a**, compound **18** was much less potent in a cell proliferation assay. In contrast to the ethenyl series, in the C-7 ethynyl series, 3-ethynylpyridine **5b** was found to inhibit Src-dependent cell proliferation with an IC_{50} value of 0.65 μM, while the 4-ethynylpyridine **23** had an IC_{50} of only 2.7 μM and the IC_{50} for the 2-ethynylpyridine **22** was >10 μM. The IC_{50} values obtained from the enzyme assay showed a similar correlation. Saturation of the ethynyl linker (**24**) resulted in a large decrease in Src inhibition.

Finally, we investigated the importance of having an alkoxy substituent at C-6. Previous studies on 6,7-dialkoxy-3-quinolinecarbonitries showed that the presence of a small alkoxy group at C-6, like OMe, generally improved Src inhibitory activity.[6] Interestingly, the opposite is true for the 7-phenyl series,[7b] where the best Src inhibition was observed when C-6 was unsubstituted. As shown in Scheme 4, known intermediate 4-chloro-3-cyano-6-methoxy-7-quinolinyl-trifluoromethanesulfonate was prepared as previously reported.[7b] 6-Methoxy-7-ethenyl derivatives **25**–**27** and 6-methoxy-7-ethynyl derivatives **28** and **29** were prepared as previously described. As observed for the 6,7-dialkoxy-3-quinolinecarbonitrile series, the presence of a methoxy group at C-6 improved Src inhibition, in most cases, more than 2-fold. 4-Ethenylpyridine derivative **27** exhibited an improved IC_{50} value of 0.08 μM in the Src dependent cell assay and of 4.2 nM in the enzyme assay, compared to its C-6 unsubstituted derivative **5a**, which exhibited IC_{50} values of 0.23 μM and 9.9 nM, respectively.

Table 1.

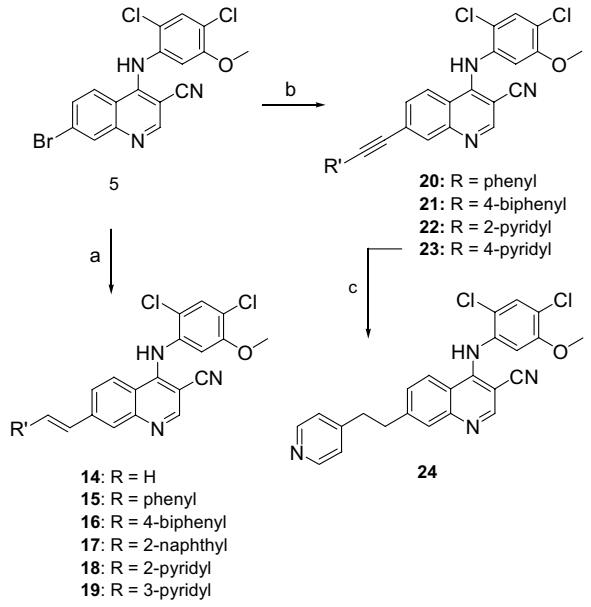


Compound[11]	R	L	Y	X	Q	Z	Src enzyme[12] IC_{50} nM (SD)	Src cells[13] IC_{50} μM (SD)
1b	—	—	—	—	—	—	3.6	0.10
4a	4-Pyridyl	Ethenyl	H	H	NH	CN	42 (6.0)	1.2 (0.13)
4b	3-Pyridyl	Ethynyl	H	H	NH	CN	200 (49)	3.9 (0.049)
5a	4-Pyridyl	Ethenyl	H	OMe	NH	CN	9.9 (2.9)	0.23 (0.10)
5b	3-Pyridyl	Ethynyl	H	OMe	NH	CN	47 (11)	0.65 (0.093)
6a	4-Pyridyl	Ethenyl	H	OEt	NH	CN	3100 (610)	>10 ($n = 2$)
7a	4-Pyridyl	Ethenyl	H	H	$NHCH_2$	CN	1000 (180)	>10 ($n = 2$)
8a	4-Pyridyl	Ethenyl	H	H	O	CN	380 (23)	7.2 (2.3)
12	4-Pyridyl	Ethenyl	H	H	NH	CO_2Et	12,000 (4300)	>10 ($n = 2$)
13	4-Pyridyl	Ethenyl	H	H	NH	CO_2H	1600 (200)	>10 ($n = 2$)
14	H	Ethenyl	H	OMe	NH	CN	97 (28)	9.3 (3.2)
15	Phenyl	Ethenyl	H	OMe	NH	CN	180 (9.8)	2.7 (0.98)
16	4-Biphenyl	Ethenyl	H	OMe	NH	CN	2300 (500)	>10 ($n = 2$)
17	2-Naphthyl	Ethenyl	H	OMe	NH	CN	320 (86)	>10 ($n = 2$)
18	2-Pyridyl	Ethenyl	H	OMe	NH	CN	19 (2.8)	50% inhibition at 10 μM (22%)
19	3-Pyridyl	Ethenyl	H	OMe	NH	CN	15 (1.6)	0.23 (0.003)
20	Phenyl	Ethynyl	H	OMe	NH	CN	320 (48)	>10 ($n = 2$)
21	4-Biphenyl	Ethynyl	H	OMe	NH	CN	420 (31)	>10 ($n = 2$)
22	2-Pyridyl	Ethynyl	H	OMe	NH	CN	530 (81)	>10 ($n = 2$)
23	4-Pyridyl	Ethynyl	H	OMe	NH	CN	83 (12)	2.7 (1.0)
24	4-Pyridyl	Ethylene	H	OMe	NH	CN	440 (53)	>10 ($n = 2$)
25	Phenyl	Ethenyl	OMe	OMe	NH	CN	65 (20)	1.5 (0.16)
26	3-Pyridyl	Ethenyl	OMe	OMe	NH	CN	8.4 (2.6)	0.14 (0.051)
27	4-Pyridyl	Ethenyl	OMe	OMe	NH	CN	4.2 (0.7)	0.08 (0.006)
28	Phenyl	Ethynyl	OMe	OMe	NH	CN	57 (14)	3.0 (0.87)
29	3-Pyridyl	Ethynyl	OMe	OMe	NH	CN	12 (4.2)	0.27 (0.033)

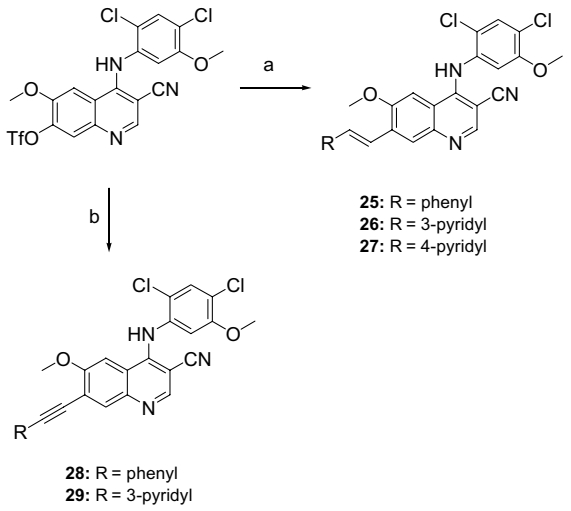


Scheme 2. Reagents and conditions: (a) phosphorous oxychloride, DMF; (b) 2,4-dichloroaniline, pyridine hydrochloride; (c) 4-vinylpyridine, $Pd(OAc)_2$, P(*o*-Tol)$_3$, DMF, TEA; (d) NaOH.

With these studies we have shown that 3-quinolinecarbonitriles bearing ethynylpyridine and ethenylpyridine groups at C-7 represent potent Src inhibitors. Analogous to the results observed with the 6,7-dialkoxy-3-quinolinecarbonitrile series, the preferred group at C-4 was



Scheme 3. Reagents and conditions: (a) alkene, $Pd(OAc)_2$, P(*o*-Tol)$_3$, DMF, TEA; (b) alkyne, $Pd(Ph_3P)_4$, CuI, DMF, TEA; (c) Pd/C, MeOH, H_2.



Scheme 4. Reagents and conditions: (a) alkene, $Pd(OAc)_2$, P(*o*-Tol)$_3$, DMF, TEA; (b) alkyne, $Pd(Ph_3P)_4$, CuI, DMF, TEA.

2,4-dichloro-5-methoxyaniline. Substituting C-6 with a methoxy group resulted in an increase in Src inhibition. Derivative **27** was the most potent inhibitor in this series, showing comparable activity to **1b** in both the Src enzyme and Src-dependent cell proliferation assays. We are continuing to investigate the physicochemical and biological properties of these analogs and other related compounds. The results will be reported in due time.

Acknowledgements

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References and notes

1. (a) Cohen, P. *Nature Rev. Drug Discovery* **2002**, *1*, 309; (b) Blume-Jensen, P.; Hunter, T. *Nature* **2001**, *411*, 355.
2. (a) Frame, M. C. *Biochem. Biophys. Acta* **2002**, *1602*, 114; (b) Metcalf, C. A., III; van Schravendijk, M. R.; Dalgarno, D. C.; Sawyer, T. K. *Curr. Pharm. Des.* **2002**, *8*, 2049; (c) Courtneidge, S. A. *Biochem. Soc. Trans.* **2002**, *30*, 11; (d) Susa, M.; Missbach, M.; Green, J. *TiPS* **2000**, *21*, 489.
3. (a) Missbach, M.; Jeschke, M.; Feyen, J.; Muller, K.; Glatt, M.; Green, J.; Susa, M. *Bone* **1999**, *24*, 437; (b) Kraker, A. J.; Hartl, B. G.; Amar, A. M.; Barvian, M. R.; Showalter, H. D. H.; Moore, C. W. *Biochem. Pharm.* **2000**, *60*, 885; (c) Blake, R. A.; Broome, M. A.; Liu, X.; Wu, J.; Gishizky, M.; Sun, L.; Courtneidge, S. A. *Mol. Cell Biochem.* **2000**, *20*, 9018.
4. 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.
5. 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.
6. Boschelli, D. H.; Ye, F.; Wu, B.; Wang, Y. D.; Barrios Sosa, A. C.; Yaczko, D.; Powell, D. W.; Golas, J. M.; Lucas, J.; Boschelli, F. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3797.
7. (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.
8. Sobolov-Jaynes, S. B.; Arnold, L. D. 4-Aminoquinazoline Derivatives. US Patent 6,225,318, 2001.
9. Lin, A. J.; Loo, T. L. *J. Med. Chem.* **1978**, *21*, 268.
10. Noncommercial 3-vinylpyridine was synthesized by reaction of 3-iodopyridine with tributyl(vinyl)tin under Suzuki conditions.
11. All compounds were characterized by MS, NMR, and CHN combustion analysis.
12. The IC_{50} values reported represent the means of at least 2 determinations. The standard deviations (SD) are shown in the brackets. *Src kinase assay*: Recombinant human Src enzyme was obtained from PanVera (P3044). Biotinylated peptide corresponding to residues 6–20 of Cdk1 was used as a substrate (Biotin-KVEKIGEGTYGVVYK-COOH). Homogeneous fluorescence resonance energy transfer kinase assays were performed using the europium/APC detection format (LANCE, Perkin Elmer). Src enzyme (10 ng) was mixed with biotinylated peptide (final concentration 2 μM), 50 mM Hepes (pH 7.5), 10 mM $MgCl_2$, 20 μg/mL BSA, 0.001% Brij-35 (Sigma), 100 μM ATP, 1% DMSO. The kinase reaction was incubated for 70 min at 37 °C. The reaction was stopped with EDTA at a final concentration of 30 mM EDTA/25 mM Hepes (pH 7.5)/10 μg/mL BSA. The mixture was combined with Eu-labeled anti-phosphotyrosine antibody PT66 (Perkin Elmer, AD0068) and Streptavidin Surelight-APC (Perkin Elmer, CR130-100) in 50 mM Hepes (pH 7.5)/20 μg/mL BSA, and incubated for 30 min according to manufacturer's specifications. Fluorescence intensity at 665 nM was used to monitor the extent of the kinase reaction.
It should be noted that the Src enzyme assay used here is different from the ELISA assay used in our previous publications (Refs. 4–7).
13. Compounds were tested according to the Src cellular assay reported previously.[4]