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Inhibition of Src kinase activity by 7-ethynyl-4-phenylamino-3-quinolinecarbonitriles: Identification of SKS-927

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Abstract—Of a series of 7-ethynyl-3-quinolinecarbonitriles, the most potent Src inhibitory activity was observed with 4-[(2,4-dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[4-(4-methylpiperazin-1-yl)but-1-ynyl]-3-quinolinecarbonitrile (SKS-927). Variation of the solubilizing amine tail or removal of the methoxy group from either C-6 of the quinoline core or C-5 of the aniline headpiece led to reduced activity.
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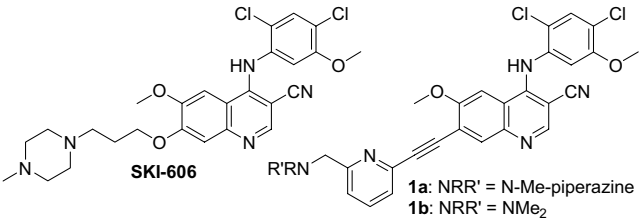
Src, a non-receptor tyrosine kinase, plays a key role in several cell signaling pathways. Studies with Src knock-out mice showed that Src is required for bone resorption,[1] and one of the early indications for small molecule Src inhibitors was the treatment of osteoporosis.[2,3] In addition, Src is over-expressed or upregulated in a variety of tumors and therefore Src inhibitors are also of interest for the treatment of cancer.[4–6]

Diverse templates have been used to develop small molecule Src inhibitors including pyrido[2,3-*d*]pyrimidines[7] pyrrolo[2,3-*d*]pyrimidines,[8] purines,[9] and quinazolines.[10,11] BMS-354825, dasatinib, a Src inhibitor with an aminothiazole core, was recently approved by the FDA for the treatment of Gleevec-resistant CML.[12,13] SKI-606, bosutinib, a Src inhibitor based on a 3-quinolinecarbonitrile template, is currently in oncology clinical trials.[14–16]

Another potential use of a small molecule Src inhibitor is for the treatment of stroke. Src regulates VEGF mediated vascular permeability[17] and both Src knock-out mice and mice treated with the Src kinase inhibitor PP1 demonstrated reduced brain injury in a model of acute stroke.[18] When SKI-606 was tested in a similar model in the rat, an iv dose of 10 mg/kg, as a solution in D5W/lactic acid at pH 4.5, provided a reduction in infarct volume along with decreased neurological deficits.[19,20]

Replacement of the 7-alkoxy group of SKI-606 with an ethynyl group, as exemplified by **1a**–**b**, retained the Src inhibitory activity of the lead compound.[21] Unfortunately these analogs had very poor solubility even at acidic pH. In the hope of increasing the water solubility, 7-ethynyl-3-quinolinecarbonitriles were targeted where the pyridine ring of **1a**–**b** was replaced with an alkyl chain.



The preparation of the analog of **1a** with a methylene linker is shown in Scheme 1. Coupling of **2**[22] with 1-(2-propynyl)-4-methylpiperazine[23] in the presence of tet-

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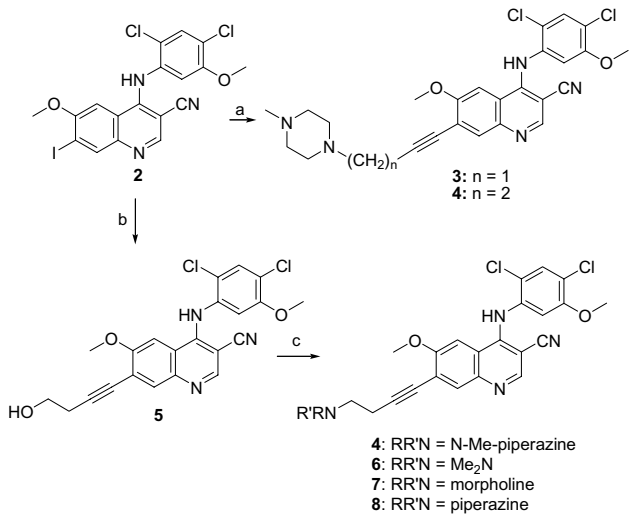


Scheme 1. Reagents and conditions: (a) for **3**: 1.5 equiv 1-(2-propynyl)-4-methylpiperazine, $(Ph_3P)_4Pd$, CuI, Et_3N, DMF, 100 °C, 3.5 h; for **4**: 3.5 equiv 1-(3-butynyl)-4-methylpiperazine, $(Ph_3P)_2PdCl_2$, CuI, Ph_3P, Et_3N, NMP, 70 °C, 4 h; (b) 1.7 equiv 3-butynol, $(Ph_3P)_4Pd$, CuI, Et_3N, dioxane, reflux, 1.5 h; (c) 1—3.0 equiv MsCl, 5.0 equiv Et_3N, DMF, THF, 0 °C to room temperature, 1.25 h; 2—4–12 equiv RR'NH, room temperature, 1–2 days.

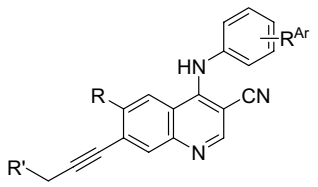
rakis(triphenylphosphine)palladium dichloride provided **3**. In an analogous fashion, treatment of **2** with 1-(3-butynyl)-4-methylpiperazine[24] gave the ethylene-linked analog **4**. When tested in a Src enzymatic assay **3** and **4** had IC_{50}s of 11 and 3.9 nM, respectively (see Table 1). A larger difference in activity was seen in a cell proliferation assay employing Src transformed rat fibroblasts. In this assay **3** had an IC_{50} of 740 nM, while that of **4** was 73 nM. Since the ethylene linker provided more potent Src inhibition than the methylene linker, additional ethylene analogs were targeted.

To prepare these analogs, a more flexible approach was investigated where 3-butynol was coupled to **2** to provide the alcohol **5**. In situ conversion of **5** to the corresponding mesylate, followed by addition of 1-methylpiperazine, provided **4**. This route was then used to prepare analogs **6**–**8** by substituting other amines for the 1-methylpiperazine in the reaction with **5**. As shown in Table 1, while these analogs had similar activity in the Src enzyme assay, **4** was the most potent by a factor of greater than 2 in the Src cell assay.

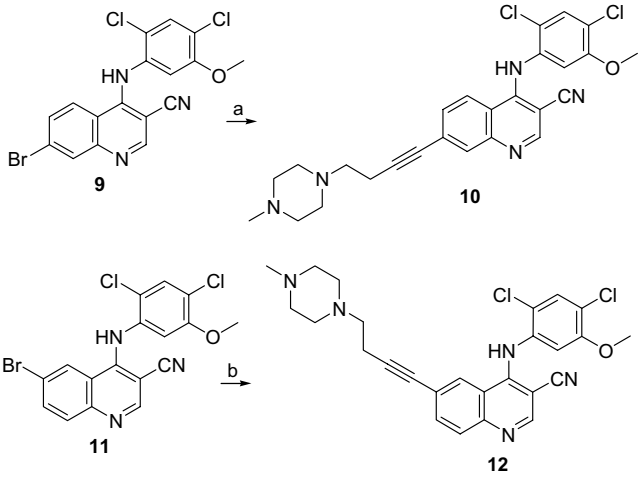
We previously reported examples where removal of the C-6 methoxy group from a series of 7-ethynyl-3-quinolinecarbonitriles led to reduced inhibition of Src activity.[25,26] The des 6-methoxy analog of **4**, namely **10**, was prepared as shown in Scheme 2. Reaction of **9**[27] with 3-butynol followed by mesylation and addition of 1-methylpiperazine provided **10**. As shown in Table 1, **10** was a weaker Src inhibitor than **4** having IC_{50}s of only 28 and 1700 nM in the enzyme and cell assays, respectively. The analog of **10** with the ethynyl tail at C-6 was prepared by coupling **11**[27] with 1-(3-butynyl)-4-methylpiperazine. Consistent with earlier findings where moving the ethynyl tail to C-6 resulted in decreased activity, **12** had reduced activity compared to **10**.[25]

The analog of **4** lacking the methoxy at C-5 of the aniline headpiece, namely **16**, was prepared as shown in Scheme 3. Reaction of 2,4-dichloroaniline with cyanoacetic acid and 1,2-diisopropylcarbodiimide provided the cyanoacetamide derivative **13**. Treatment of **13** with 3-iodo-4-methoxyaniline and triethylorthoformate gave **14** which upon treatment with phosphorus oxychloride cyclized to the 3-quinolinecarbonitrile **15**. Coupling of **15** with 1-(3-butynyl)-4-methylpiperazine under the standard conditions provided **16**. The finding that **16** was a weaker Src inhibitor than **4** was expected based on the reduced activity of related 3-quinolinecarbonitriles with a C-7

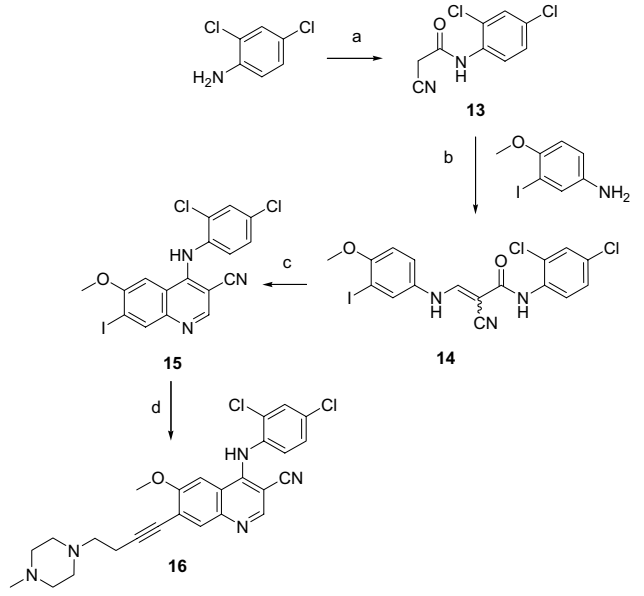
Table 1. Src enzyme and cell inhibitory activity of 7-ethynyl-3-quinolinecarbonitriles



Compound[32]	R′	R	R^{Ar}	Src enzyme IC_{50} (nM)	SD	Src cell IC_{50} (nM)	SD
SKI-606				3.8[33]		100[14]	
1a				4.2[21]		120[21]	
1b				4.5[21]		120[21]	
3	N–Me-piperazine	OMe	2,4-di-Cl, 5-OMe	11	2.9	740	230
4	CH_2–N–Me-piperazine	OMe	2,4-di-Cl, 5-OMe	3.9	0.50	73	3.8
6	CH_2–NMe_2	OMe	2,4-di-Cl, 5-OMe	4.9	0.21	250	8.4
7	CH_2-morpholine	OMe	2,4-di-Cl, 5-OMe	4.1	0.07	190	16
8	CH_2-piperazine	OMe	2,4-di-Cl, 5-OMe	5.4	0.78	520	120
10	CH_2–N–Me-piperazine	H	2,4-di-Cl, 5-OMe	28	4.2	1700	610
12	C-6 isomer of **10**			230	28	3900	370
16	CH_2–N–Me-piperazine	OMe	2,4-di-Cl	8.7	0.90	430	31
21	CH_2–N–Me-piperazine	OEt	2,4-di-Cl, 5-OMe	4.1	0.17	130	42
24	CH_2–N–Me-piperazine	OMe	3,4,5-tri-OMe	21	5.0	590	120
25	CH_2–N–Me-piperazine	H	3,4,5-tri-OMe	220	93	2100	28



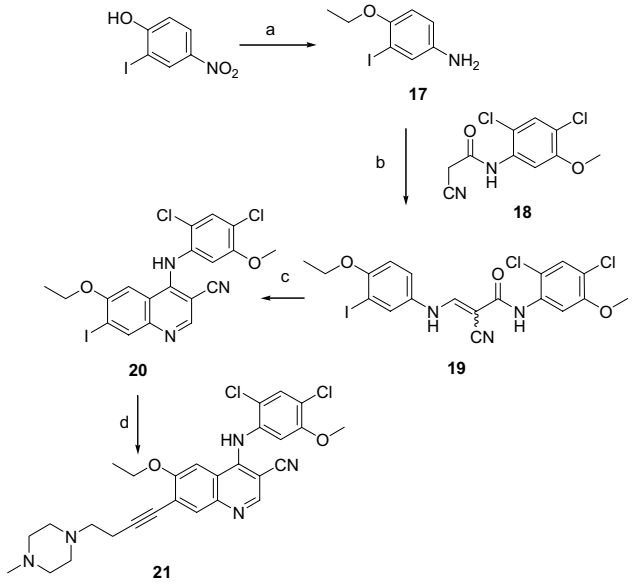
Scheme 2. Reagents and conditions: (a) 1—1.5 equiv 3-butynol, $(Ph_3P)_4P$, CuI, Et_3N, DMF, 98 °C, 3 h; 2—3.0 equiv MsCl, 5.0 equiv Et_3N, DMF, THF, 0 °C to room temperature, 1.25 h; 3—6.0 equiv 1-methylpiperazine, rt, overnight; (b) 4.0 equiv 1-(3-butynyl)-4-methylpiperazine $(Ph_3P)_2PdCl_2$, CuI, Ph_3P, Et_3N, NMP, 70 °C, 4 h.



Scheme 3. Reagents and conditions: (a) 1.0 equiv cyanoacetic acid, 1.0 equiv 1,2-diisopropylcarbodiimide, THF, reflux, 15 min; (b) 1.0 equiv 3-iodo-4-methoxyaniline, 1.0 equiv $(EtO)_3CH$, *i*-PrOH, reflux, overnight; (c) 7.0 equiv $POCl_3$, butyronitrile, reflux, overnight; (d) 3.6 equiv 1-(3-butynyl)-4-methylpiperazine, $(Ph_3P)_2PdCl_2$, CuI, Ph_3P, Et_3N, NMP, 70 °C, 4 h.

3-pyridinylethynyl substitutent upon removal of the C-5 methoxy group from the aniline.[26]

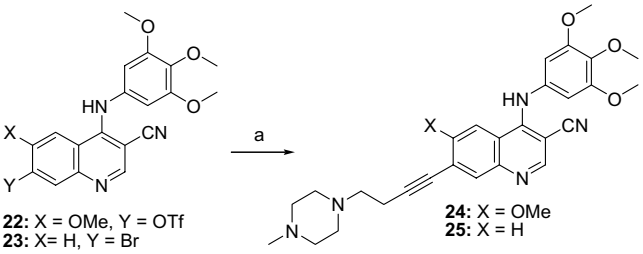
In a Src enzymatic assay, 4-[(2,4-dichlorophenyl)amino]-6,7-diethoxy-3-quinolinecarbonitrile was about threefold more potent than the corresponding 6,7-dimethoxy analog.[28] The C-6 ethoxy analog of **4** was therefore targeted. The prerequisite aniline **17** was prepared as shown in Scheme 4. Alkylation of 2-iodo-4-nitrophenol[29] with ethyl iodine in the presence of K_2CO_3, followed by reduction of the nitro group, provided **17**.



Scheme 4. Reagents and conditions: (a) 1—6.0 equiv EtI, 3.7 equiv K_2CO_3, DMF, 70 °C, 3 h; 2—3.5 equiv Fe, 5.0 equiv NH_4Cl, EtOH, H_2O, reflux, 1 h; (b) 2.6 equiv $(EtO)_3CH$, *i*-PrOH, reflux, overnight; (c) 6.0 equiv $POCl_3$, butyronitrile, reflux, overnight; (d) 1—1.5 equiv 3-butynol, $(Ph_3P)_4Pd$, CuI, Et_3N, dioxane, 95 °C, 3 h; 2—3.0 equiv MsCl, 5.0 equiv Et_3N, DMF, THF, 0 °C to room temperature, 1.25 h; 3—6.0 equiv 1-methylpiperazine, room temperature, overnight, followed by 45 °C, 7 h.

Reaction of **17** with 2-cyano-*N*-(2,4-dichloro-5-methoxyphenyl)acetamide, **18**,[22] and triethylorthoformate resulted in **19** with subsequent phosphorus oxychloride mediated ring closure leading to the formation of **20**. Coupling of **20** with 3-butynol, conversion to the mesylate, and displacement with 1-methylpiperazine provided **21**. Interestingly, although **21** had very similar activity to **4** in the Src enzyme assay, having an IC_{50} of 4.1 nM, this C-6 ethoxy analog was less potent than **4** in the Src cell assay, having an IC_{50} of 130 nM.

Replacement of the C-4 (2,4-dichloro-5-methoxyphenyl)amino group of **1b** with a (3,4,5-trimethoxyphenyl)amino group led to a sixfold reduction in Src enzymatic activity and a threefold reduction in Src cell activity.[21] As depicted in Scheme 5, coupling of the C-7 triflate derivative **22**[25] with 1-(3-butynyl)-4-methylpiperazine provided the (3,4,5-trimethoxyphenyl)amino analog of **4**, namely **24**.



Scheme 5. Reagents and conditions: (a) 3.0–4.0 equiv 1-(3-butynyl)-4-methylpiperazine, $(Ph_3P)_2PdCl_2$, CuI, Ph_3P, Et_3N, NMP, 70 °C, 4 h.

This change resulted in a fivefold reduction in Src enzymatic activity and an eightfold reduction in Src cell activity. The des C-6 methoxy analog of **24** was prepared by coupling of the C-7 bromo analog **23**[25] with 1-(3-butynyl)-4-methylpiperazine. This modification led to a further decrease in activity with **25** having IC_{50}s of only 220 and 2100 nM for the inhibition of Src enzymatic and cell activity, respectively.

When tested in a panel of kinases, **4** had an IC_{50} of 720 nM for the inhibition of EGFR and IC_{50}s of greater than 5 μM for the inhibition of AKT, CDK4, IGFR, KDR, and PDK1. Pharmaceutical profiling showed **4** had good permeability (PAMPA value of 6.8×10^{-6} cm/s) and while the solubility was low at neutral pH (5 μg/mL), the solubility increased dramatically at pH 4.5 to greater than 100 μg/mL. This increased solubility compared to that of **1b** allowed for successful iv formulation of **4** for testing in the rat stroke model. Preliminary results of these in vivo studies with **4** (SKS-927) were recently reported.[30,31]

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