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Optimization of 7-alkene-3-quinolinecarbonitriles as Src kinase inhibitors

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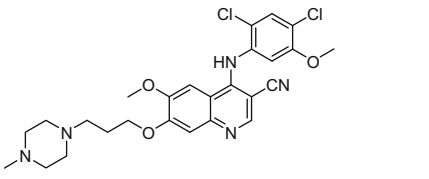
ABSTRACT

The 7-alkene-3-quinolinecarbonitrile **20**, a potent inhibitor of Src enzymatic and cellular activity with IC_{50} values of 2.1 and 58 nM, respectively, had comparable efficacy to bosutinib in a colon tumor xenograft study.

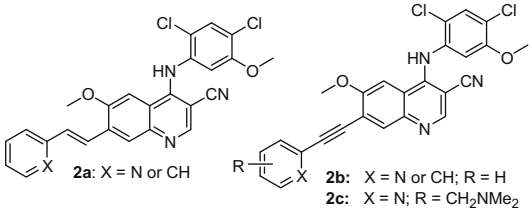
Next year will mark the 100th anniversary of Peyton Rous's discovery that a virus can act as a transmissible agent and induce sarcomas in healthy animals.[1] It was subsequently determined that a single gene contained in this virus was responsible for the tumor formation.[2] Additional studies established that this gene, which was the first known oncogene, encoded a tyrosine kinase, v-Src, which has a normal cellular counterpart, c-Src (Src). Activation of Src causes aberrant cell signaling that can contribute to cancer. Over the years considerable efforts have been expended to identify small molecule inhibitors of Src kinase activity as potential anticancer therapeutics.[3–5] Three ATP competitive Src inhibitors are currently in clinical trials for the treatment of solid tumors[6]: dasatinib (BMS-354825),[7,8] saracatinib (AZD-0530),[9,10] and bosutinib (SKI-606).[11,12]

We previously reported that replacement of the C-7 alkoxy substituent on the 3-quinolinecarbonitrile core of bosutinib with certain alkenylaryl, **2a**, or alkynylaryl, **2b**, groups preserved the Src kinase inhibitory activity.[13,14] We also demonstrated that attachment of a water solubilizing (dimethylamino)methyl group to the aryl ring of the alkynylaryl analogs, as in **2c**, provided potent Src kinase inhibitors.[15]

The corresponding C-7 alkene analogs of **2c** where X is N were prepared as shown in Scheme 1. Palladium catalyzed coupling of 2-bromo-4-(hydroxymethyl)pyridine with tributyl(vinyl)tin provided intermediate **3a**. The 2,5- and 2,6-isomers, **3b–c**, were prepared in a similar fashion from the isomeric starting materials. A second palladium catalyzed coupling reaction, now between **3a–c** and the 7-triflate-3-quinolinecarbonitrile **4**,[16] provided **5a–c**. Conversion of the primary alcohol group of **5a–c** to a mesylate group and subsequent displacement of the mesylate with dimethylamine led to the desired 7-alkene analogs **6a–c**. As seen in Table 1, the 2,5- and 2,6-isomers **6b** and **6c** were more potent Src inhibitors than the 2,4-isomer **6a**.



1: Bosutinib



2a: X = N or CH

2b: X = N or CH; R = H
2c: X = N; R = CH_2NMe_2

The C-7 alkene analogs of **2c** where X is CH were prepared as shown in Scheme 2. Palladium catalyzed coupling of 4-vinylbenzaldehyde[17] and 3-vinylbenzaldehyde with triflate **4**, provided **7a** and **7b**, respectively. Subsequent reductive amination with dimethylamine led to the desired 7-alkene analogs **8a** and **8b**.

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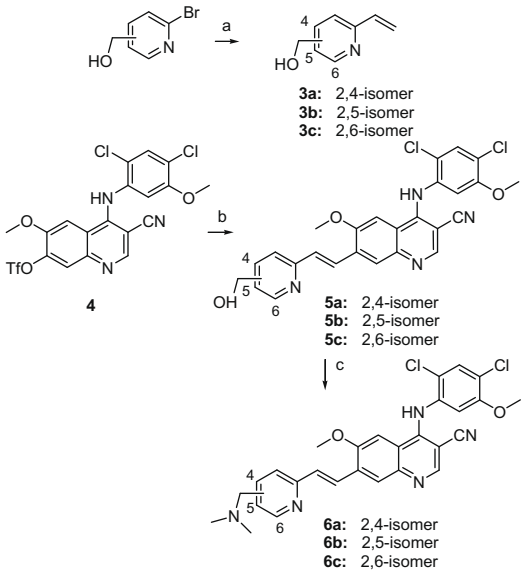
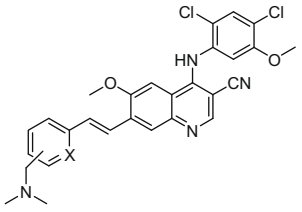
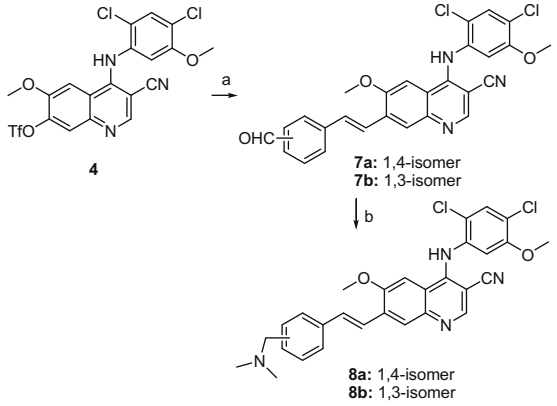


Scheme 1. Reagents: (a) Bu_3Sn-CH=CH_2, $Pd(PPh_3)_2Cl_2$, CuI, toluene; (b) **3a–c**, $Pd(OAc)_2$, P(*o*-tol)$_3$, DMF, Et_3N; (c) (1) MsCl, Et_3N, THF, DMF; (2) Me_2NH, THF.

Table 1
Src enzyme and cell inhibitory activity and nude mouse 24 h plasma levels of 7-alkenylaryl-3-quinolinecarbonitriles



Compound number	X	Isomer	Src enzyme IC_{50} (nM)[22]	Src cell IC_{50} (nM)[24]	24 h Plasma level (ng/mL)
Bosutinib			3.8	100	40
6a	N	2,4	6.8	280	
6b	N	2,5	2.2	58	36
6c	N	2,6	2.2	28	28
8a	CH	1,4	2.4	40	10
8b	CH	1,3	3.0	100	

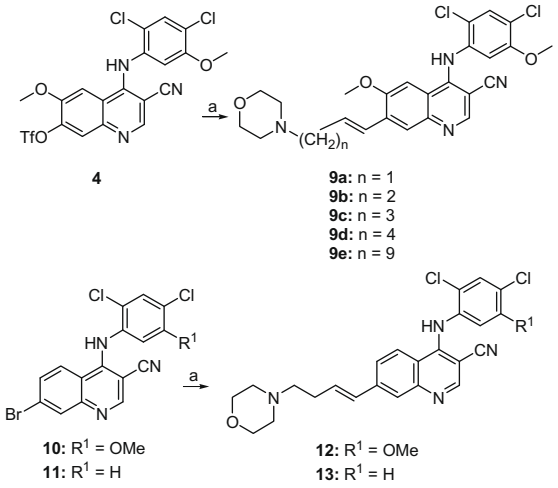


Scheme 2. Reagents: (a) 4 and 3-vinylbenzaldehyde, $Pd(OAc)_2$, P(*o*-tol)$_3$, DMF, Et_3N; (b) Me_2NH, THF, $Na(OAc)_3BH$, CH_2Cl_2, DMF, AcOH.

As shown in Table 1, while both **8a** and **8b** were potent Src inhibitors in the enzyme assay, **8a** was 2.5-fold more potent than **8b** in the Src cell assay. The three most potent compounds in this assay were evaluated for their 24 h plasma levels in nude mice after a single oral 50 mg/kg dose. As shown in Table 1, none of these three compounds had greater plasma levels than those of bosutinib. When the two compounds with the highest plasma levels, **6b** and **6c**, were tested in an HT-29 xenograft model with oral dosing at 150 mg/kg, qd, for 14 days, neither compound was efficacious in blocking tumor growth.

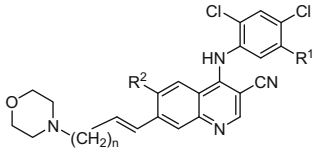
We next replaced the C-7 alkenylaryl group with an alkenylalkyl group. As shown in Scheme 3, the initial analogs were prepared from known vinyltributylstannanes, with the reaction of triflate **4** with 4-[(*E*)-3-(tributylstannyl)prop-2-enyl]morpholine[18] and 4-[(*E*)-4-(tributylstannyl)but-3-enyl]morpholine[19] providing **9a** and **9b**, respectively. The additional reagents with extended alkyl chains were prepared from the respective alkynes via a route analogous to that in the literature. Subsequent Stille coupling of these vinyltributylstannanes with triflate **4** provided **9c–e**.

As shown in Table 2, the best inhibitors of Src enzymatic activity were **9b** and **9c**, having IC_{50} values of 4.8 and 5.4 nM respectively. Extending the alkyl chain caused a substantial decrease in activity. The best inhibition of Src cell activity was seen with **9b**, which had an IC_{50} value of 180 nM. As shown in Scheme 3, the des C-6 methoxy analog of **9b**, namely **12**, was prepared by reaction of 4-[(*E*)-4-(tributylstannyl)but-3-enyl]morpholine with the 7-bromo-3-quinolinecarbonitrile **10**.[20] Reduced activity in both the Src enzyme and cell assays was observed with **12**. Further loss of activity was observed with the analog of **12** lacking the



Scheme 3. Reagents: (a) Bu_3Sn-CH=$CH(CH_2)_n$-morpholine, $Pd(PPh_3)_4$, NMP.

Table 2
Src enzyme and cell inhibitory activity of 7-alkenylalkyl-3-quinolinecarbonitriles



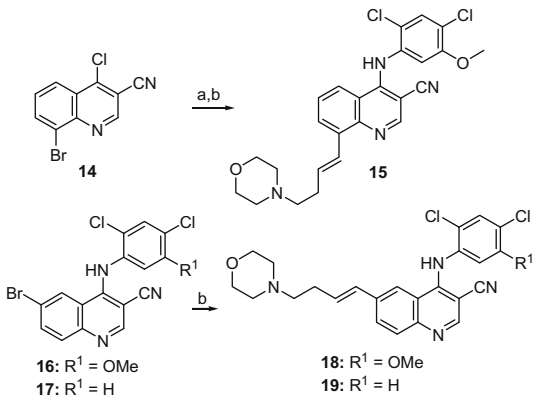
Compound number	R^1	R^2	*n*	Src enzyme IC_{50} (nM)[22]	Src cell IC_{50} (nM)[24]
9a	OMe	OMe	1	8.7	260
9b	OMe	OMe	2	4.8	180
9c	OMe	OMe	3	5.4	350
9d	OMe	OMe	4	18	290
9e	OMe	OMe	9	130	3200
12	OMe	H	2	15	1100
13	H	H	2	41	2800

5-methoxy group on the aniline ring. Reaction of the 7-bromo-3-quinolinecarbonitrile **11**[14] with 4-[(*E*)-4-(tributylstannyl)but-3-enyl]morpholine provided **13** which had IC_{50} values for the inhibition of Src enzyme and Src kinase activity of only 41 nM and 2.8 μM, respectively.

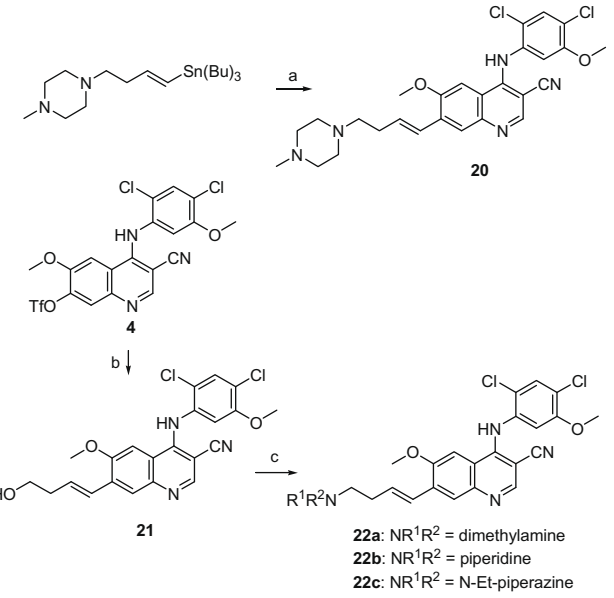
The C-8 and C-6 isomers of **12**, namely **15** and **18**, were prepared as shown in Scheme 4. Addition of 2,4-dichloro-5-methoxyaniline to the 7-bromo-4-chloro-3-quinolinecarbonitrile **14** followed by reaction with 4-[(*E*)-4-(tributylstannyl)but-3-enyl]morpholine provided the 8-alkenyl-3-quinolinecarbonitrile **15**. The corresponding C-6 isomer **18** was prepared from the 6-bromo-3-quinolinecarbonitrile **16**.[20] As shown in Table 3, greatly reduced activity in the Src enzyme assay was observed with both these isomers. Once again further loss of activity was observed with **19**, the analog of **18** lacking the 5-methoxy group on the aniline ring.

The SAR to this point demonstrated the importance of the 5-methoxy group on the aniline, the 6-methoxy on the 3-quinolinecarbonitrile ring, and of the but-3-enyl group at C-7 of the core, in agreement with what was observed in our earlier SAR studies on related 3-quinolinecarbonitrile Src inhibitors. Since **9b** had the best Src enzyme and cell inhibitory activity, additional analogs were prepared varying the water solubilizing amine group, starting with the *N*-methylpiperazine **20**.

As shown in Scheme 5, reaction of triflate **4** with 1-methyl-4-[(3*E*)-4-(tributylstannyl)but-3-enyl]piperazine[21] provided the desired compound. Replacement of the morpholine group of **9b** with an *N*-methylpiperazine increased the Src enzyme inhibitory activity twofold (IC_{50} = 2.1 nM) and the cell inhibitory activity threefold (IC_{50} = 58 nM). In a nude mouse PK study, a 50 mg/kg single oral dose of **20** provided a 24 h plasma level of 38 ng/mL. Bosutinib and **20** were tested side-by-side in an HT-29 xenograft study via oral administration at 150 mg/kg, qd, for 14 days. When the tumors were measured on day 21, both compounds were efficacious with bosutinib having a T/C of 0.43 and **20** having a T/C of 0.36.



Scheme 4. Reagents: (a) 2,4-dichloro-5-methoxyaniline, 2-ethoxyethanol; (b) Bu_3Sn-CH=CH$(CH_2)_2$-morpholine, $Pd(PPh_3)_4$, NMP.

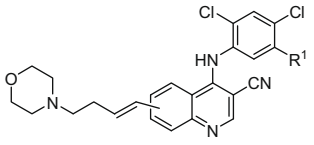


Scheme 5. Reagents: (a) **4**, $Pd(PPh_3)_4$, NMP; (b) $Bu_3SnCH{=}CH(CH_2)_2$-OH, $Pd(PPh_3)_4$, NMP; (c) (1) MsCl, Et_3N, THF, DMF; (2) R^1R^2NH.

Additional profiling of **20** revealed that like bosutinib[22] this 7-alkene analog was also an inhibitor of Abl kinase activity, with **20** having an IC_{50} value of 0.4 nM. Weak activity was observed against EGFR (IC_{50} = 3.3 μM) and **20** was inactive against several other kinases including IKK, AKT, TPL2, PDK1, IGFR and mTOR (IC_{50}s >10 μM). In a rat liver microsome stability assay, **20** had a half-life of only 10 min. A short half-life was also observed in human microsomes (11 min), while in nude mouse microsomes **20** had a half-life of 33 min. Metabolite ID studies showed no metabolism occurred on the alkene group and that the major metabolites included oxidation and dealkylation of the *N*-methylpiperazine.

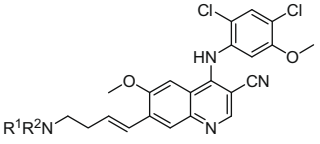
To determine if replacement of the *N*-methylpiperazine group of **20** with other amines would increase the microsomal stability, additional analogs were targeted. As shown in Scheme 5, coupling of triflate **4** with (*E*)-4-(tributylstannyl)-3-buten-1-ol[23] provided **21**, which contains a terminal alcohol group. Reaction of **21** with mesyl chloride followed by treatment of the intermediate mesylate with various amines gave the desired analogs **22a–c**. As shown in Table 4, all three compounds had similar Src inhibitory activity to that of **20**. In addition, all three had half-lives of greater than 30 min in a rat liver microsome stability assay.

Table 3
Src enzyme inhibitory activity of C-7, C-8 and C-6 substituted 4-(morpholin-4-yl)but-1-enyl-3-quinolinecarbonitriles



Compound number	Isomer	R^1	Src enzyme IC_{50} (nM)[22]
12	7	OMe	15
15	8	OMe	52% at 10 μM
18	6	OMe	340
19	6	H	1200

Table 4
Src enzyme and cell inhibitory activity of 7-(but-3-enyl)-3-quinolinecarbonitriles



Compound number	R^1R^2N	Src enzyme IC_{50} (nM)[22]	Src cell IC_{50} (nM)[24]
9b	Morpholine	4.8	180
20	*N*-Me-Piperazine	2.1	58
22a	Me_2N	2.5	62
22b	Piperidine	2.6	78
22c	*N*-Et-Piperazine	1.7	67

While the Stille reactions of **4** with the vinyltributylstannanes provided the desired products as mixtures of the *E* and *Z* isomers in a ratio of approximately 98:2, Suzuki reaction of **4** with an (*E*)-vinylboronic ester, namely 1-methyl-4-[(3*E*)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-buten-1-yl]piperazine, provided **20** as >99% of the trans isomer.[21] The identification of this highly stereoselective route to the *E* alkenes, coupled with the in vivo efficacy of **20** and the increased microsomal stability of **22a–c** provides rationale for further study of these 7-alkene-3-quinolinecarbonitrile Src inhibitors.

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