SKI-606 and beyond

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4-Phenylamino-3-quinolinecarbonitriles have been extensively studied by Wyeth as inhibitors of diverse kinases including EGFR, HER-2, and MEK.[1,2] Screening of a library of 4-phenylamino-3-quinolinecarbonitriles with various substituents on the 4-phenylamino group identified 1 as an inhibitor of Src kinase[3] (Scheme 1).

Optimization of this screening lead led to SKI-606, currently in clinical trials for the treatment of solid tumors.[4] Subsequent to the discovery that SKI-606 was a potent Src inhibitor, it was determined that this compound was also a potent inhibitor of Abl kinase.[5]

Early SAR studies on the 7-alkoxy-3-quinolinecarbonitriles established that the (2,4-dichloro-5-methoxyphenyl)amino group at C-4 of the 3-quinolinecarbonitrile provided the best Src enzyme inhibition. Later, profiling against Abl kinase showed that this group also provided the best Abl enzyme inhibition.

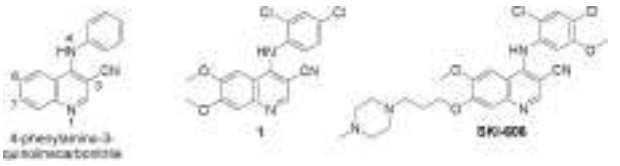
The 1-methylpiperazine group was optimal for cellular and *in vivo* activity. It was also noted that lengthening or shortening the propoxy group at C-7 retained most of the Src inhibitory activity. Therefore we investigated other linkers at C-7 between the 4-[(2,4-dichloro-5-methoxyphenyl)amino]-3-quinolinecarbonitrile and the 1-methylpiperazine group.

We reported earlier that analogs with a 3,5- or 2,5-disubstituted thiophene at C-7 (2 and 3) retained much of the activity of SKI-606 (Table 1)[6] (Scheme 2). These compounds were tested in a LANCE format Src enzymatic assay and in a cell proliferation assay using c-Src transformed rat fibroblasts. We also reported that reduced activity was seen with the 1,4-disubstituted phenyl analog, 4, and that the introduction of a 6-OMe group to these C-7 phenyl analogs reduced the activity even further.[7] Since the thiophene analogs were more potent than the phenyl analog, the 2,5- and 3,5-disubstituted furan analogs were deemed worthy of investigation.

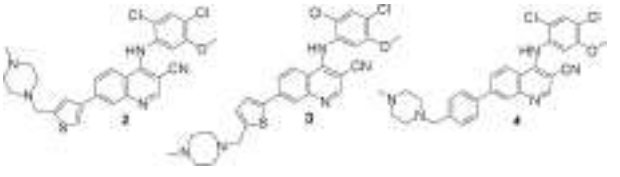
As depicted in Scheme 3, palladium catalyzed coupling of the 7-Br intermediate 56 with a furyl boronic acid or stannane derivative, followed by reductive amination of the key aldehyde intermediates with 1-methylpiperazine, provided 6 and 7. As opposed to what was seen with the corresponding thiophene deriviatives, the 3,5-disubstituted furan analog 6 was about 3-fold more potent than the 2,5- disubstituted furan analog 7. The 6-OMe analogs of 6 and 7 were targeted next. We had prepared the 6-OMe C-7 phenyl derivatives via the C-7 triflate intermediate 8.[7] While this triflate readily reacted with a variety of boronic acids and stannane derivatives, its preparation from methyl vanillate required nine steps. To facilitate the preparation of additional 6-OMe analogs, either an improved route to 8 or a facile route to an alternative intermediate, namely 9 or 10, was required.

Efficient synthesis of both 9 and 10 was achieved based on chemistry first carried out by Wyeth Chemical Development scientists.[8] As shown in Scheme 2, reaction of 2,4-dichloro-5-methoxyaniline with cyanoacetic acid and 1,3-diisopropylcarbodiimide gave the acetamide derivative 11. Treatment of 11 with triethylorthoformate and 3-bromo- or 3-iodo-4-methoxyaniline provided 12a or 12b, with subsequent phosphorus oxychloride mediated ring closure resulted in formation of 9 or 10, respectively. These key intermediates were now readily available in only three steps.

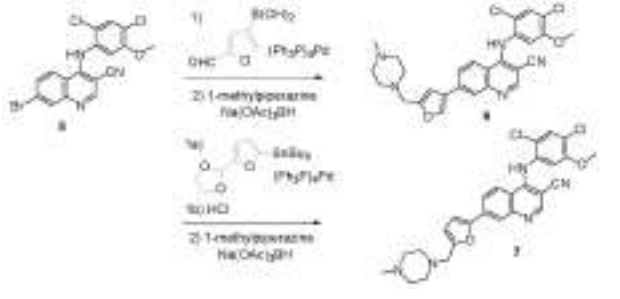
As shown in Scheme 5, following the synthetic route used to prepare 6 and 7, 10 was converted to 13 and 14. While the presence of a 6-OMe group caused a decrease in activity relative to 7, with 14 having an IC_{50} in the Src enzyme assay of 13 nM, addition of a 6-OMe group to 6 resulted in increased activity, with 13 having an IC_{50} of 0.78 nM. Compound 13 also potently inhibited Src cellular activity, having an IC_{50} of 15 nM. Additional analogs of 13 were readily obtained by using various amines in the reductive amination reaction. While the dimethylamine analog, 15,



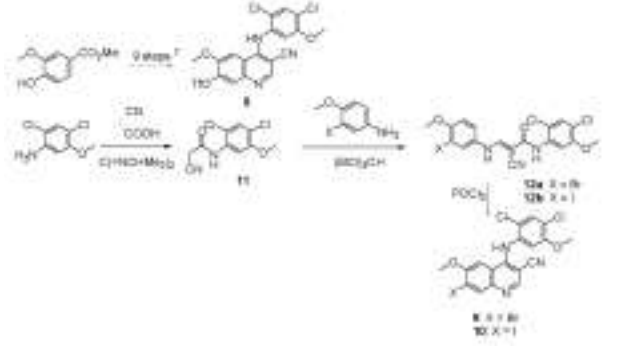
Scheme 1.



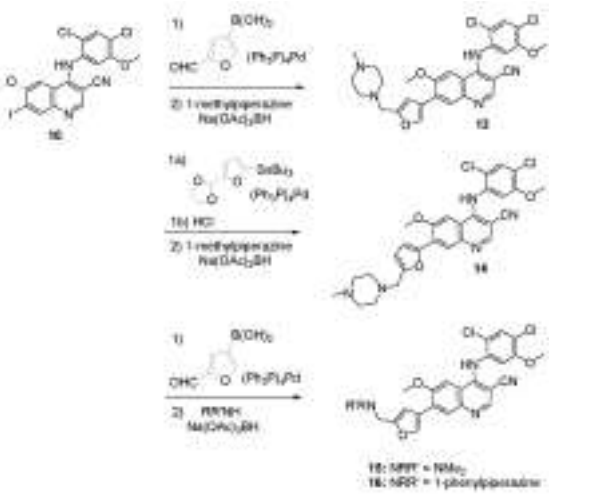
Scheme 2.



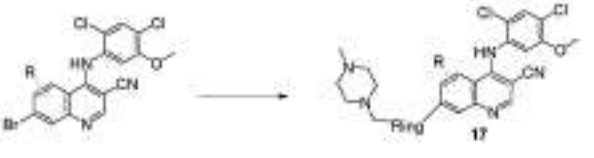
Scheme 3



Scheme 4.



Scheme 5.



Schema 6.

retained most of the activity of 13, reduced activity was observed with the 1-phenylpiperazine analog, 16.

Due to the potent activity observed with 13, additional analogs were prepared, varying the ring at C-7 while retaining the (2,4-dichloro-5-methoxyphenyl) amino group at C-4 and 1-methylpiperazine as the solubilizing group. The general route to these compounds is depicted in Scheme 6. One of these analogs, 17, showed good Src inhibitory activity, having IC_{50}s of 4.0 nM in the enzyme assay and 61 nM in the cell assay.

We previously reported that SKI-606 was a dual inhibitor of Src and Abl kinase activity with the ability to cause regression of K562 tumors in nude mice.[5] These properties positioned this compound as a potential agent for the treatment of CML.[9]

The new analogs, 13 and 17, were compared to SKI-606 in a c-Abl kinase assay as shown in Table 2. Both compounds were more potent Abl inhibitors than SKI-606 and also had improved activity against the T315I Abl variant that correlated with the improved activity against wild type c-Abl. The corresponding antiproliferative activity of 13, 17 and SKI-606 in CML cells is shown in Table 2. The IC_{50}s for 13 in these three-day proliferation assays was significantly improved compared to SKI-606, while 17 was slightly more potent than SKI-606.

As expected, both 13 and 17 showed improved activity in inhibiting signaling from Bcr-Abl in K562 and KU812 cells. Figure 1 shows a comparison of the inhibition of Stat5 phosphorylation by the three compounds. Inhibition of Stat5 phosphorylation correlated with the antiproliferative activity of all three compounds. Both 13 and 17 demonstrated improved efficacy *in vivo* against K562 xenografts compared to SKI-606 (Figure 2). SKI-606 was somewhat more effective in this study than reported earlier, with most animals

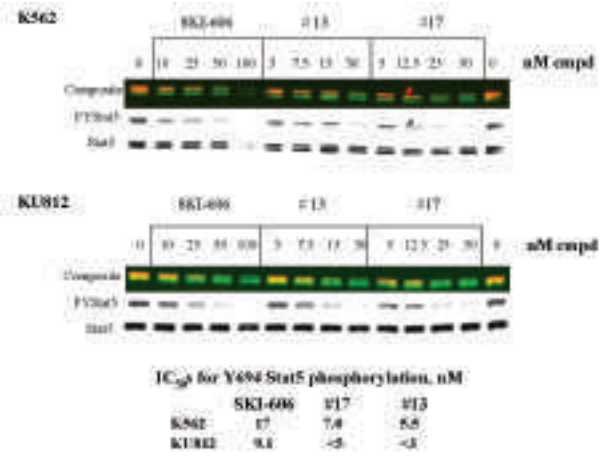


Figure 1. Inhibition of Stat5 phosphorylation on Y694 by SKI-606, 13 and 17. Cells were treated with the indicated compound, or with DMSO alone for four hours, after which extracts were prepared and analyzed by immunoblotting with the indicated antibody. Blots were scanned on an Odyssey Imager. The color panel is a composite of the phosphorylated Stat5 and total Stat5, where the red represents phosphorylated Stat5, green total Stat5 and yellow arises when the red and green overlap. IC50 values were determined by comparing counts in compound treated sample relative to the untreated controls in the phosphoStat5 bands and then related to total Stat5 counts.

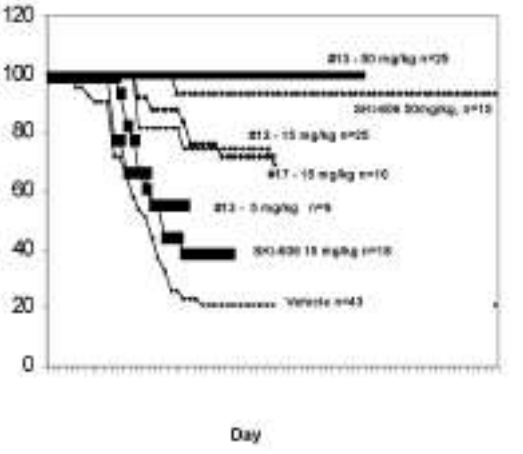


Figure 2. Survival data for animals with K562 tumors staged to 300- 400 mg. Six to seven week-old female nude mice were injected with 5 x 107 K562 cells in Matrigel. When tumors reached the appropriate size, animals were administered 0.2 ml vehicle (0.5% methylcellulose, 0.4% Tween-80) or 0.2 ml compound at the indicated concentration in this vehicle by oral gavage once daily for five days. Animals were sacrificed when tumors reached 2.5 g.

in the 50 mg/kg group surviving for more than two months, compared to 50% in our earlier report.[5] When orally administered for five days at 50 mg/kg, 13 cured all 25 animals in this group. This compound provided some protection even when given at 5 mg/kg, being somewhat more effective than SKI-606 at 15 mg/kg. Compound 17 resulted in complete cures for more than two months when given orally at 25 mg/kg for five days (*not shown*), while at 15 mg/kg, survival was significantly improved compared to SKI-606 at the

Table 1. Inhibition of Src kinase enzymatic and cell activity.

	Ring	R	Src enzyme IC_{50} nM	Src cell IC_{50} nM	
SKI-606			3.8	100	
2		3,5-thiophene	H	3.8	69
3		2,5-thiophene	H	2.3	64
4		1,4-phenyl	H	5.0	640
6		3,5-furan	H	2.7	46
7		2,5-furan	H	7.5	120
13		3,5-furan	OMe	0.78	15
14		2,5-furan	OMe	13	550
15		Dimethylamine	analog of 13	0.75	20
16		N-Ph-piperazine	analog of 13	4.0	33
17		-	-	4.0	61

Table 2. Comparison of enzymatic and CML cell activity of three Src/Abl inhibitors (IC50s in nM).

	Src	WT c-Abl	T315I c-Abl	K562	KU812
SKI-606	3.8	1.4	344	20	4.3
13	0.78	0.35	68	5.7	1.4
17	4.0	0.68	210	12	3.1

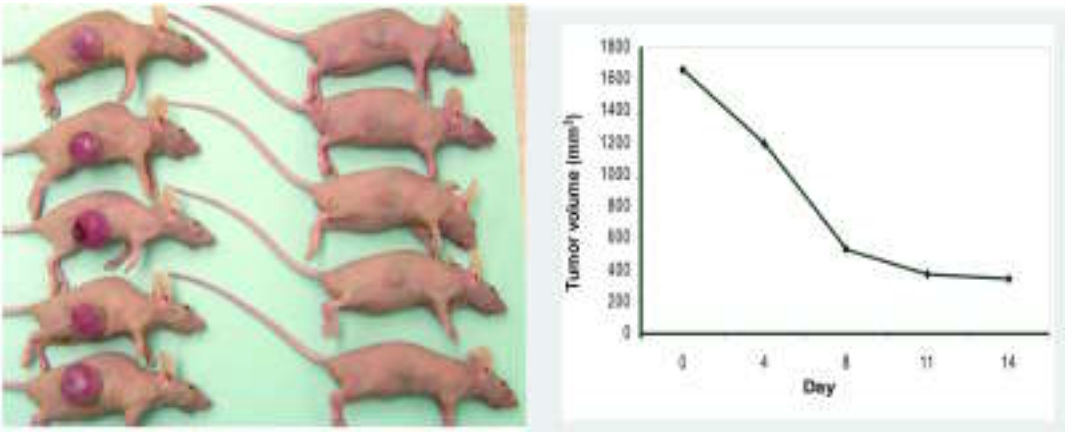


Figure 3. Compound 13 causes regression of large K562 tumors in nude mice. A representative group of mice with tumors as they appeared when dosing was started is shown in the left group of the top panel. The right-hand group received compound 13 for five days at 50 mg/kg. The picture was taken three weeks after compound administration began. The right panel shows the kinetics of tumor regression, with the bulk of the tumor disappearing by 14 days.

same dose. As was observed with SKI-606, compound 13 caused regression in animals with large tumors. Animals with K562 tumors staged to ~1.6 g were administered compound 13 at 50 mg/kg for five days. As shown in Figure 3, three weeks after dosing was stopped complete regression occurred, with no recurrence of tumors more than two months later.

These data show that these new members of the 4-phenylamino-3-quinolinecarbonitrile class of kinase inhibitors share the therapeutic potential of the current clinical lead SKI-606. We are continuing to study these and additional dual Src and Abl inhibitors in the hope of achieving increased therapeutic benefits to those of SKI-606.

Acknowledgements

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