7-Alkoxy-4-phenylamino-3-quinolinecarbonitriles as Dual Inhibitors of Src and Abl Kinases

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Received January 19, 2004

Abstract: We previously reported that several 7-alkoxy-4-phenylamino-3-quinolinecarbonitriles were potent inhibitors of Src kinase activity. We disclose here a new highly efficient and versatile route to these compounds, which are also potent inhibitors of Abl kinase.

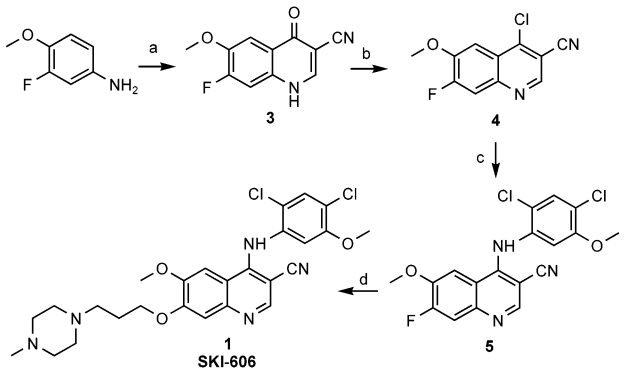
Src is the prototype member of a family of nonreceptor tyrosine kinases (SFKs), consisting of Fyn, Lck, Lyn, Hck, Yes, Blk, and Fgr. The SFKs share a common structural organization and are highly homologous in their ATP-binding regions. In the past 20 years since Src was characterized as a kinase, there has been a plethora of studies on Src's significant involvement in diverse biological pathways. These reports continue to generate considerable efforts to identify low molecular weight inhibitors of Src activity that could be used to treat various diseases, including cancer, osteoporosis, and metastatic bone disease.[1−6]

We previously reported that the potent Src inhibitor **1**, SKI-606, was also an inhibitor of Abl kinase.[7] The molecular hallmark of chronic myelogenous leukemia (CML) is the expression of Bcr-Abl, an activated form of the tyrosine kinase Abl. Gleevec, also known as STI-571 or imatinib, is an Abl kinase inhibitor marketed by Novartis for the treatment of CML patients.[8,9] The remarkable clinical effectiveness of Gleevec validated the premise that molecularly targeted therapy with a kinase inhibitor was possible.

Although the SFKs have a close homology with Abl, Gleevec does not inhibit SFKs. However, several compounds of various structural classes initially identified as Src inhibitors were later reported to also inhibit Abl.[10−14] These findings can be explained by crystallographic studies that showed that while Gleevec binds the inactive form of the Abl catalytic domain, which is structurally distinct from the active or inactive forms of the catalytic domains of the SFKs, PD173955, a dual Src/Abl inhibitor, can bind to both the inactive and active conformations of Abl.[15]

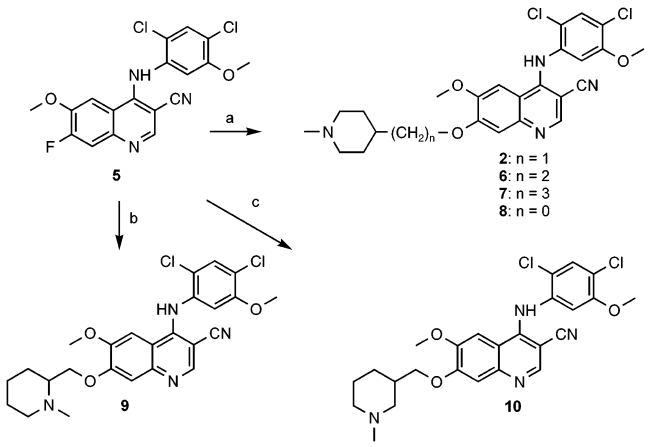
The 4-[(2,4-dichloro-5-methoxyphenyl)amino]-3-quinolinecarbonitrile Src inhibitor, SKI-606, and its 7-[(1-methylpiperidin-4-yl)methoxy] analogue **2**, were initially prepared by multistep reaction sequences. These routes involved either (1) addition of the C-7 alkoxy group at an early stage in the synthesis[16] or (2) a penultimate Mitsunobu reaction utilizing a 7-OH intermediate that, while being more flexible, was not opportune for large scale synthesis.[17] We report here an improved route to **1** and **2** and apply this approach to the preparation of other 7-alkoxy-3-quinolinecarbonitriles. We also disclose additional biological properties of these compounds.

Scheme 1[a]



[a] Reagents: (a) (1) Ethyl (ethoxymethylene)cyanoacetate, toluene; (2) biphenyl, diphenyl ether; (b) $POCl_3$; (c) 2,4-diCl-5-OMe-aniline, pyridine-HCl, 2-ethoxyethanol; (d) 1-(3-hydroxypropyl)-4-methylpiperazine, NaH, DMF.

Scheme 2[a]



[a] Reagents: (a) Various 4-alkoxy-*N*-methylpiperidines, NaH, DMF; (b) 1-methylpiperidine-2-methanol, NaH, DMF; (c) 1-methylpiperidine-3-methanol, NaH, DMF.

On the basis of work initially done in the area of quinolone antibiotics, displacement of a 7-F group of a 3-quinolinecarbonitrile intermediate by an alkoxide should readily provide 7-alkoxy analogues.[18,19] As shown in Scheme 1, treatment of 3-fluoro-*p*-anisidine with ethyl (ethoxymethylene)cyanoacetate, followed by thermal cyclization provided **3**. Subsequent chlorination of **3** with phosphorus oxychloride afforded the key intermediate **4**. Reaction of **4** with 2,4-diCl-5-OMe aniline gave **5**. The 7-F group of **5** was then readily displaced by 1-(3-hydroxypropyl)-4-methylpiperazine in the presence of sodium hydride. This highly efficient route provided **1** in four linear steps from a commercially available starting material.

As shown in Scheme 2, addition of 1-methylpiperidine-4-methanol to **5** provided **2**. The flexibility of this approach was further demonstrated by the conversion of **5** to analogues **6**−**10**, which have additional alkoxy-

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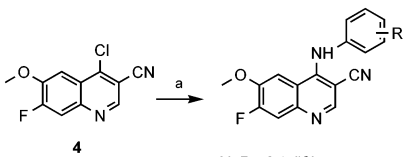
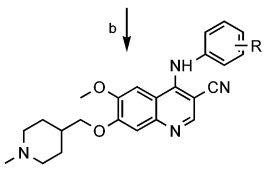
10.1021/jm0499458 CCC: $27.50
Published on Web 02/27/2004

Scheme 3[a]



4

11: R = 2,4-diCl
12: R = 3,4,5-triOMe
13: R = 2-Cl, 5-OMe
14: R = 2-Me, 5-OMe
15: R = 2,4-diMe
16: R = 2,4-diMe, 5-OMe
17: R = 2,4-diCl, 5-OEt



18: R = 2,4-diCl
19: R = 3,4,5-triOMe
20: R = 2-Cl, 5-OMe
21: R = 2-Me, 5-OMe
22: R = 2,4-diMe
23: R = 2,4-diMe, 5-OMe
24: R = 2,4-diCl, 5-OEt

[a] Reagents: (a) Aniline, pyridine-HCl, 2-ethoxyethanol; (b) 1-methylpiperidine-4-methanol, NaH, DMF.

Table 1. Inhibition of Src and Abl Kinase Activity

compound	Src enzyme IC_{50}, nM (SD)	Src cell IC_{50}, nM (SD)	Abl enzyme IC_{50}, nM (SD)
1	3.8 (0.3)	100[16]	1.1 (0.01)
2	7.0 (0.7)	220[17]	2.9 (0.6)
6	7.7 (1.1)	230[17]	2.9 (0.7)
7	2.7 (0.1)	100[17]	0.78 (0.04)
8	230 (9.0)	3800 (120)	89 (1.4)
9	87 (10)	2300 (630)	56 (9.3)
10	9.0 (0.5)	360 (150)	4.6 (0.8)
18	21 (1.4)	1400[17]	16 (1.0)
19	35 (10)	1600[17]	12 (1.9)
20	12 (0.8)	230 (30)	3.5 (0.3)
21	24 (0.8)	390 (100)	8.3 (0.5)
22	63 (1.6)	2500 (790)	38 (7.4)
23	13 (0.1)	510 (170)	8.3 (0.4)
24	1400 (260)	>10000	1500 (70)

N-methylpiperidine groups at C-7. It should be noted that there are reports in the literature of the displacement of a 7-F group of a quinazoline with an alcohol, but in these examples the intermediate is either a 4-one[20] or a 4-anilino derivative with an activating 6-nitro group.[21]

Compound **4** proved to be a very useful intermediate in that an aniline group could be added at C-4 first, followed by variation of the C-7 alkoxy group. As shown in Scheme 3, reaction of **4** with various anilines and subsequent displacement of the 7-F group of **11**−**17** with 1-methylpiperidine-4-methanol provided **18**−**24**.

Compounds were tested in a LANCE format Src enzyme assay and in a Src-dependent cell proliferation assay.[22] We previously reported that varying the chain length of **2** to 2 and 3 methylene groups, analogues **6** and **7** respectively, had a minimal effect on the Src inhibitory activity.[16] However as shown in Table 1, analogue **8**, where the piperidine group is directly attached to the oxygen at C-7, showed a large decrease in activity. The same loss of potency was observed with **9**, the 2-piperidinyl isomer of **2**. This is in contrast to the 3-piperidinyl isomer **10**, which retained the activity of **2**.

Variation of the C-4 aniline substituents of **2** from 2,4-diCl-5-OMe to 2,4-diCl or 3,4,5-triOMe, as in **18** and **19**, decreased the ability of the compound to inhibit Src activity. Compound **20**, the 2-Cl,5-OMe analogue of **2**, showed comparable activity to that of **2**. Compound **21**, the 2-Me,5-OMe aniline analogue, was about 2-fold less potent than **20** in the Src enzyme and cell assays, showing the importance of the 2-Cl group on the aniline. This was further demonstrated by the finding that **22**, the 2,4-diMe aniline analogue, was less potent than **18**, the 2,4-diCl analogue, and that **23**, the 2,4-diMe-5-OMe analogue, was less potent than **2**. Replacing the 5-OMe group on the aniline of **2** with a 5-OEt group led to a large reduction in activity, with **24** having an IC_{50} in the enzyme assay of only 1.4 μM and an IC_{50} of greater than 10 μM in the cell assay.

When the Src inhibitors disclosed here were tested in an Abl kinase assay (Table 1), a very close correlation in the activities was observed, with the most potent Src inhibitors being the most potent Abl inhibitors. Changes at the C-7 position or on the aniline at C-4 that resulted in a decrease in Src inhibition also resulted in a decrease in Abl inhibition. Of those analogues with a 2,4-diCl-5-OMe aniline at C-4, the best Src inhibitor was **7** which was also the most potent Abl inhibitor. Furthermore, the weakest Src inhibitor, **8**, was also the weakest Abl inhibitor. For those analogues where the aniline group was varied, **18**−**24**, a very similar trend was seen and is exemplified by the large loss in Abl inhibition observed with the 2,4-diCl-5-OEt analogue **24**.

We previously reported that **1** inhibited the proliferation of two Bcr-Abl positive leukemia lines, KU812 and K562, with IC_{50}s of 5.0 and 20 nM, respectively.[7] While **2** also blocked the proliferation of these two cell lines, it was less potent than **1**, having IC_{50}s of 13 and 39 nM in the KU812 and K562 assays. Cell activity comparable to that of **1**, was seen with **7**, which had IC_{50}s of 5.8 and 18 nM in the KU812 and K562 assays. As expected, **18** and **19**, analogues of **2** with aniline groups other than 2,4-diCl-5-OMe at C-4, showed reduced activity in both the KU812 (IC_{50}s of 59 and 70 nM) and K562 (IC_{50}s of 260 and 140 nM) cell lines. Therefore, in all cases, the CML cell activity reflected the Abl kinase inhibitory activity.

In summary, a new route was developed for the preparation of a series of 3-quinolinecarbonitriles that exhibit potent dual inhibition of Src and Abl kinases. The biological properties of these compounds are being investigated further. It should be noted that while Gleevec is extremely effective in treating patients with chronic CML, patients in late-stage (blast-phase) CML have a less durable response. In most cases acquired resistance to Gleevec has been attributed to mutations in the Abl kinase domain that prevent the binding of Gleevec.[23−26] Some Src/Abl dual inhibitors retain activity against mutated Bcr-Abl proteins and may therefore be effective in treating Gleevec resistant CML.[27−31] In addition since SFKs are implicated in Bcr-Abl signaling, the compounds reported here, may also represent an alternative therapy to Gleevec.[32−34]

Acknowledgment. We thank the Wyeth Discovery Analytic Chemistry Department for the spectral analysis and combustion analysis. We also thank Drs. Dennis Powell and Tarek Mansour for their support of this work.

Supporting Information Available: Experimental details, ^{1}H NMR, HRMS, and analytical data for all compounds and the enzyme assay protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM0499458