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Investigation of the Effect of Varying the 4-Anilino and 7-Alkoxy Groups of 3-Quinolinecarbonitriles on the Inhibition of Src Kinase Activity

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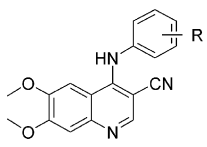
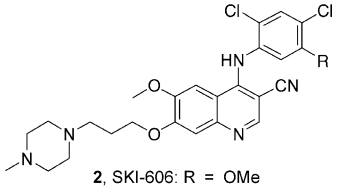
Abstract—Several 7-alkoxy-4-anilino-3-quinolinecarbonitriles were synthesized and evaluated for Src kinase inhibitory activity. Optimal inhibition of both Src enzymatic and cellular activity was seen with analogues having a 2,4-dichloro-5-methoxyaniline group at C-4. Compound **18**, which has a 1-methylpiperidinemethoxy group at C-7, showed in vivo activity in a xenograft model.

Signaling via protein tyrosine kinases (TKs), including Src, is central to many cellular events.[1] Src acts in the regulation of many biological pathways and therefore small molecule Src inhibitors may be useful for the treatment of a variety of disease states, including cancer and osteoporosis.[2] The relevance of Src as a therapeutic target is highlighted by the variety of known classes of Src kinase inhibitors described in the literature including pyrrolo[2,3-*d*]pyrimidines,[3a] pyrido[2,3-*d*]pyrimidines[3b] and oxindoles.[3c]

We previously reported that the 4-anilino-6,7-dimethoxy-3-quinolinecarbonitriles of formula **1a–c** were potent Src inhibitors and that these analogues had greater activity than the corresponding 4-anilino-6,7-dimethoxyquinazolines.[4–6] For compounds **1a–c**, superior Src inhibitory activity was seen with **1c** which has a 2,4-dichloro-5-methoxyaniline group at C-4.[5] Addition of various 3-(dialkylamino)-propoxy groups at C-7 of **1c** increased the inhibition of Src cell activity, with the optimal derivative being **2**, SKI-606.[6,7] While addition of a 3-(4-methyl-1-piperazinyl)-propoxy substituent at C-7 also increased the Src cell activity of **1b**, this analogue **3**, was not as potent as **2**.[5]



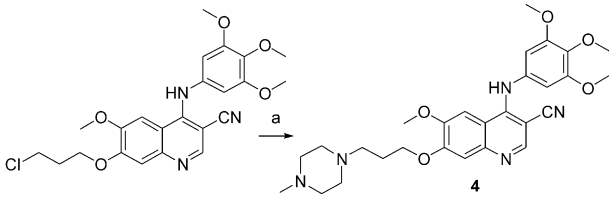
1a: R = 3, 4, 5-tri-OMe
1b: R = 2,4-di-Cl
1c: R = 2,4-di-Cl, 5-OMe



2, SKI-606: R = OMe
3: R = H

The 4-(3,4,5-trimethoxyanilino) derivative **4**[8] was prepared from the known intermediate[4] as shown in Scheme 1. As was observed with **3**, **4** showed decreased Src cell activity compared to **2** (Table 1).

We next looked at the effect on Src inhibitory activity upon retaining these three anilino groups at C-4 and adding solubilizing groups other than 3-(4-methyl-1-piperazinyl)-propoxy at C-7. A survey of various 4-anilinoquinazoline kinase inhibitors currently in clinical trials suggested the use of two rather disparate groups.



Scheme 1. (a) 1-Methylpiperazine, NaI, ethylene glycol dimethyl ether.

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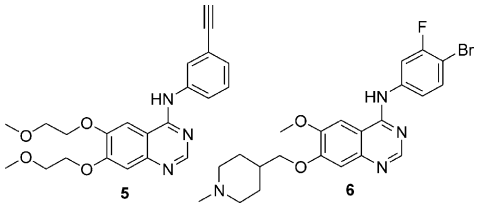
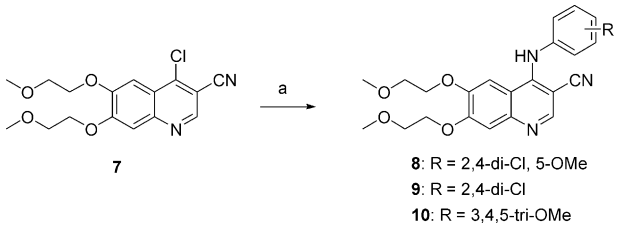
The EGFr inhibitor Tarceva, **5**,[9] has 2-methoxyethoxy groups at both C-6 and C-7, while the KDR inhibitor, ZD6474, **6**,[10] has a methoxy group at C-6 and a 1-methylpiperidinemethoxy group at C-7.

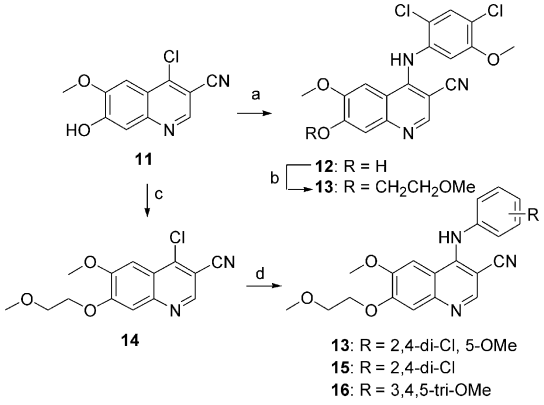


The analogues of **1a–c** with 2-methoxyethoxy groups at both C-6 and C-7 were prepared from the 4-Cl derivative **7**[11] as shown in Scheme 2. Addition of 2,4-dichloro-5-methoxyaniline in the presence of sodium hydride provided **8**. Analogues **9** and **10** were obtained by treatment of **7** with the corresponding aniline in the presence of pyridine hydrochloride. Of these three, the best Src inhibitory activity was seen with the 2,4-dichloro-5-methoxy analogue, **8**. However, this compound was about 2-fold less potent in the enzyme and cell assays than **2**.

Since we had earlier shown that large groups at C-6 could be detrimental to the inhibition of Src activity,[5] the analogues of **8–10** with a methoxy group at C-6 instead of the 2-methoxyethoxy group were prepared (Scheme 3). Starting from **11**,[4] addition of 2,4-dichloro-5-methoxyaniline provided **12**. The phenol group of **12** was alkylated with 2-bromoethyl methyl ether to provide **13**. Since the overall yield by this route was low, an alternative approach was examined. This route utilized a Mitsunobu reaction of **11** with 2-methoxyethanol to provide **14**. Addition of 2,4-dichloro-5-methoxy aniline to **14** resulted in **13**. This route was then also used to prepare analogues **15** and **16** via addition of 2,4-dichloroaniline and 3,4,5-trimethoxyaniline respectively to **14**. As shown in Table 1, the analogues with a 6-OMe group were roughly 2-fold more potent in the Src enzyme assay than those with a 6-(2-methoxyethoxy) group. For unknown reasons **16** showed better Src cell activity than was expected.



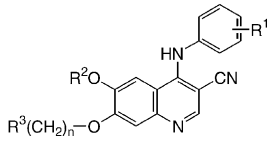
Scheme 2. (a) 2,4-Dichloro-5-methoxyaniline, NaH, THF (for **8**); aniline, pyridine hydrochloride, 2-ethoxyethanol (for **9** and **10**).



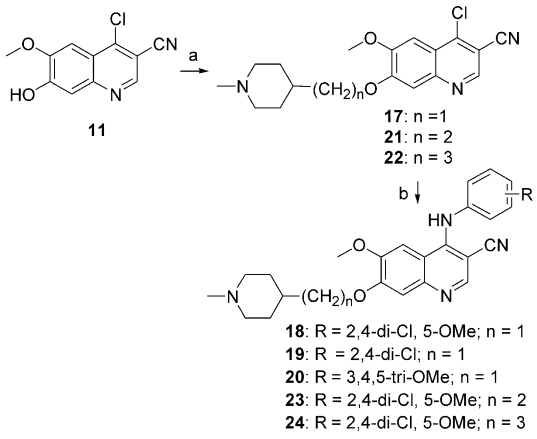
Scheme 3. (a) 2,4-Dichloro-5-methoxyaniline, pyridine hydrochloride, 2-ethoxyethanol; (b) 2-bromoethyl methyl ether, K_2CO_3, DMF; (c) triphenylphosphine, diethylazodicarboxylate, 2-methoxyethanol, THF; (d) aniline, pyridine hydrochloride, 2-ethoxyethanol.

Intermediate **11** was next used to prepare analogues of **1a–c** with the solubilizing tail of ZD6474 at C-7, as shown in Scheme 4. Mitsunobu reaction of **11** with 1-methyl-4-piperidinemethanol provided **17**. The 4-anilino

Table 1.



Compd	R^1	R^2	R^3	*n*	Src enzyme[12] IC_{50} nM (SD)	Src Cells[12] IC_{50} nM (SD)
2	2,4-diCl, 5-OMe	Me	Me-piperazine	3	1.2[6]	100[6]
3	2,4-diCl	Me	Me-piperazine	3	8.7[5]	1000[5]
4	3,4,5-tri-OMe	Me	Me-piperazine	3	5.1 (1.2)	1200 (57)
8	2,4-diCl, 5-OMe	CH_2CH_2OMe	OMe	2	2.8 (0.93)	190 (74)
9	2,4-diCl	CH_2CH_2OMe	OMe	2	12 (0.71)	1200 (250)
10	3,4,5-tri-OMe	CH_2CH_2OMe	OMe	2	25 (8.6)	1400 (400)
13	2,4-diCl, 5-OMe	Me	OMe	2	1.5 (0.52)	370 (211)
15	2,4-diCl	Me	OMe	2	4.7 (2.1)	1400 (390)
16	3,4,5-tri-OMe	Me	OMe	2	14 (1.7)	330 (97)
18	2,4-diCl, 5-OMe	Me	Me-piperidine	1	2.0 (0)	220 (68)
19	2,4-diCl	Me	Me-piperidine	1	6.6 (0.56)	1400 (210)
20	3,4,5-tri-OMe	Me	Me-piperidine	1	8.3 (1.1)	1600 (320)
23	2,4-diCl, 5-OMe	Me	Me-piperidine	2	2.4 (0.74)	230 (56)
24	2,4-diCl, 5-OMe	Me	Me-piperidine	3	1.4 (0.07)	100 (42)



Scheme 4. (a) Triphenylphosphine, diethylazodicarboxylate, 1-methylpiperidine-$(CH_2)_nOH$, THF; (b) 3,4,5-trimethoxyaniline, pyridine hydrochloride, 2-ethoxyethanol (for **20**); aniline, NaH, THF (for **18**, **19**, **23** and **24**).

derivatives **18**–**20** were prepared by the usual reaction conditions. Again, when tested in the Src kinase and cell proliferation assays, the best activity of the three analogues was seen with **18**, which contains the 2,4-dichloro-5-methoxyaniline at C-4. However, **18** was about 2-fold less potent than **2**. To investigate the effect of lengthening the chain, the ethoxy and propoxy analogues of **18** were prepared as shown in Scheme 4. Mitsunobu reaction of **11** with 1-methyl-4-piperidineethanol or 1-methyl-4-piperidinepropanol provided **21** and **22**, respectively. Addition of 2,4-dichloro-5-methoxyaniline to **21** and **22** resulted in **23** and **24**, both of which had Src enzyme and cell activity in the same range as **18** and **2**.

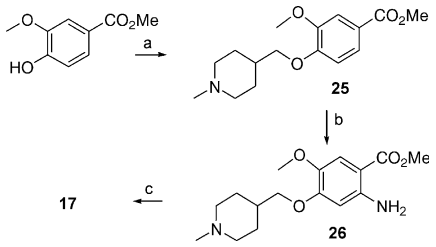
Compounds **8**, **13**, **18**, and **24** had fairly comparable activity in the Src cell assay and were chosen for a PK study. Blood samples were drawn at 0.5, 4, and 8 h after a 50 mg/kg ip dose administered to nude mice in a Tween/Methocel vehicle.[6] As shown in Table 2, the group at C-7 had a significant effect on the observed plasma concentrations. Of these four analogues, **18** had superior plasma levels and was therefore selected for further investigation.

In order to prepare additional amounts of **18**, an alternate route to key intermediate **17** was investigated. As shown in Scheme 5, methyl vanillate was converted to **25** which contains the desired 1-methylpiperidinemethoxy group. Nitration and subsequent reduction of the nitro group provided **26**.[13] Formation of the amidine of **26** followed by addition of the anion of acetonitrile and then chlorination of the resultant quinoline-4-one with phosphorous oxychloride provided **17**. This route, while only one step shorter than that utilized earlier, proved more suitable for larger scale synthesis.

Table 2.

Compd	0.5 h	4.0 h	8.0 h
8	1.7	1.2	0.20
13	0.37	0.23	0.14
18	4.5	2.9	1.6
24	0.40	0.50	0.50

Nude mouse plasma concentrations in μg/mL at 0.5, 4.0 and 8.0 h following a 50 mg/kg ip dose.



Scheme 5. (a) (1) *N-tert*-Butoxycarbonyl-4-(4-toluenesulphonyloxymethyl)piperidine, K_2CO_3, DMF; (2) HCO_2H, 37% aqueous formaldehyde; (b) (1) fuming nitric acid, TFA, CH_2Cl_2; (2) H_2/Pd/C, MeOH; (c) (1) *N,N*-dimethylformamide dimethyl acetal; (2) *n*-BuLi, acetonitrile, THF; (3) phosphorous oxychloride.

Compound **18** was tested in a xenograft assay employing Src transformed rat fibroblasts.[6] In a staged model, where the tumors were implanted in nude mice prior to compound administration, a 50 mg/kg ip dose of **18** for 5 days gave a statistically significant inhibition of tumor growth (T/C = 35% at day 5) as depicted in Figure 1.

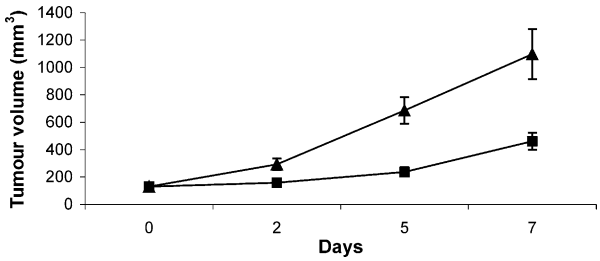


Figure 1. Antitumor activity of **18** versus a Src-transformed fibroblast xenograft. Mice ($n = 10$) were treated with **18** for 5 days at 50 mg/kg ip qd (-■-); Vehicle alone (-▲-).

We have shown here that 3-quinolinecarbonitriles substituted at C-4 with 2,4-dichloroanilino, 3,4,5-trimethoxyanilino or 2,4-dichloro-5-methoxyanilino groups inhibit Src enzymatic and cell activity. The best Src enzymatic and cell inhibitory activity was seen with those analogues with a 4-(2,4-dichloro-5-methoxyanilino) group. In addition, it was demonstrated that **18** can inhibit Src activity in vivo. We are continuing to investigate the biological properties of **18** and other 4-(2,4-dichloro-5-methoxyanilino)-3-quinolinecarbonitriles with additional alkoxy groups at C-7.

Acknowledgements

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