Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 2477–2480

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Inhibitors of Src Tyrosine Kinase: The Preparation and Structure–Activity Relationship of 4-Anilino-3-cyanoquinolines and 4-Anilinoquinazolines

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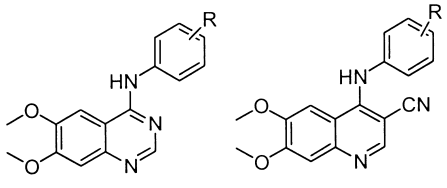
Received 20 July 2000; accepted 22 August 2000

Abstract—Src is a nonreceptor tyrosine kinase involved in signaling pathways that control proliferation, migration, and angiogenesis. Increased Src expression and activity are associated with an increase in tumor malignancy and poor prognosis. Several quinolines and quinazolines were identified as potent and selective inhibitors of Src kinase activity.

The nontransmembrane or nonreceptor tyrosine kinases (TKs) have intrinsic kinase activity, are present in the cytoplasm and nucleus, and participate in diverse signaling pathways. A large number of nonreceptor TKs have been identified, including Src, Abl, Jak, Fak, Syk, and ZAP 70. The Src family consists of Src, Fyn, Lyn, Yes, Lck, Fgr, Hck, and Blk.[1] Src is involved in signaling pathways controlling proliferation, migration, and angiogenesis. Experimental evidence suggests that increased Src expression and activity are associated with an increase in tumor malignancy and poor prognosis.[2] Therefore, inhibitors of Src kinase activity may prove useful for therapeutic intervention in cancer as well as other proliferative diseases. Several structural types of compounds have been reported to be inhibitors of Src family kinases,[3] including the quinazoline **1**,[4] reported by Rhone-Poulenc Rorer to be a 500 nM inhibitor of Lck. A similar quinazoline **2**, was reported by Parke-Davis to be an inhibitor of epidermal growth factor receptor (EGFr),[5] a receptor tyrosine kinase. Recently Wyeth-Ayerst disclosed that **3**, the 3-cyanoquinoline analogue of **2**, was also an effective inhibitor of EGFr.[6] We found that while **1** had an IC_{50} of 280 nM for the inhibition of Src enzymatic activity,[7] the corresponding 3-cyanoquinoline **4** had an IC_{50} of 35 nM. In this paper, we present the synthesis and structure–activity relationships toward Src for additional analogues of **1** and **4**.



1 R = 3,4,5-trimethoxy
RPR-108518A
2 R = 3-bromo
PD 153035

3 R = 3-bromo
4 R = 3,4,5-trimethoxy

The 4-anilinoquinazoline **6** or 4-anilinoquinolines **7**–**9** were obtained by the reaction of anilines with 4-chloroquinazoline **5a**[8a] or 4-chloroquinolines **5b**–**5d**[8b–d] in 2-ethoxyethanol using pyridine–HCl as the catalyst as shown in Scheme 1.[6,9] The 3-CH_2OH quinoline **10** was prepared by the reduction of **9** with DIBAL-H. The phenoxy derivative **11** and benzylamine derivative **12** were prepared by the reaction of the corresponding phenol or benzylamine with 4-chloro-3-cyanoquinoline **5b**. In order to further explore the SAR of the aniline portion of **1** and **4**, several 4-alkoxy-3,5-dimethoxy anilines **15a**–**15e** were prepared. While the desired anilines could be obtained by regioselective demethylation of compound **7** followed by alkylation and reduction as documented in the literature,[10] the yield of the demethylation step was only 10%. To circumvent this low yield, the *para*-methoxy group may be directly replaced by different nucleophiles,

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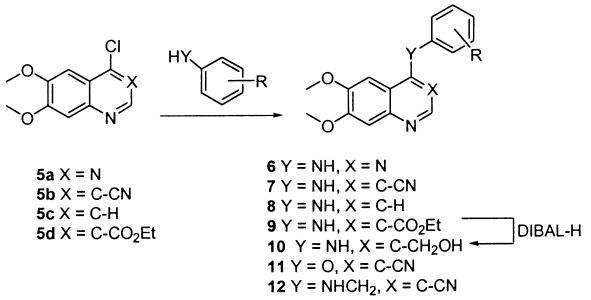
0960-894X/00/$ - see front matter
PII: S0960-894X(00)00493-5

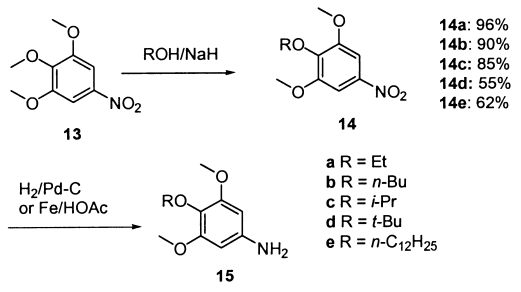


Scheme 1.

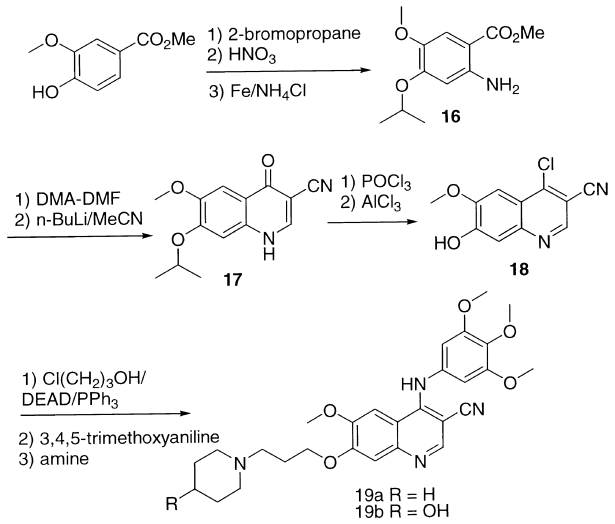
such as alcohols and amines.[11] However, the literature procedure[11] used vigorous reaction conditions and often resulted in unsatisfactory yields of the desired product. We found a mild and facile reaction condition[12] (NaH/ROH/DMF/THF, room temperature) that effected complete conversion of **13** to the desired products regioselectively. Furthermore, this reaction condition tolerates a wide range of alcohol nucleophiles including primary, secondary and tertiary alcohols. As shown in Scheme 2, compounds **14a**–**14e** were all generated in good to excellent yields and were then converted to compounds **15a**–**15e** by either catalytic hydrogenation or Fe/HOAc reduction in good yields.

3-Cyanoquinolines **19a** and **19b**, which contain water solubilizing groups, were prepared as shown in Scheme 3.[9] Protection of the hydroxy group of commercially available methyl vanillate followed by nitration and reduction gave compound **16**, which was converted to the amidine derivative and cyclized with the anion generated from acetonitrile to provide compound **17**. Chlorination of **11** with $POCl_3$ and deprotection of the isopropoxy group with $AlCl_3$ both went smoothly to give **18**. Mitsunobu reaction of the phenol with 3-chloropropanol proceeded readily to give the dichloride. Subsequent replacement of the 4-chloro by 3, 4, 5-trimethoxyaniline and of the alkyl chloride by 4-hydroxypiperidine or piperidine provided **19a** and **19b**.

The 4-anilinoquinazolines and 4-anilino-3-cyanoquinolines were evaluated for their inhibitory activity against Src kinase using an ELISA assay, and the activities are reported in Table 1.[7] In the quinazoline series, **1** showed moderate activity with an IC_{50} of 280 nM. However, only small alkoxy groups were tolerated at the C-4 position of the aniline. When the 4-methoxy group in the aniline was replaced with a bulkier alkoxy group, the activity dropped consistently. Replacement of 4-



Scheme 2.



Scheme 3.

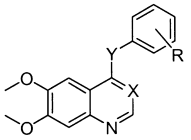
methoxy with isopropoxy gave a 3-fold reduction in activity. We speculate that this is due to a size-limited hydrophobic interaction of the aniline with the kinase.

We found that the 3-cyanoquinolines were far more potent Src inhibitors than the corresponding quinazolines. Compound **4** inhibited Src activity with an IC_{50} of 35 nM. A similar trend was observed to that seen in the quinazoline series when a bulkier alkoxy group replaced the 4-methoxy group in the aniline. Changing 4-methoxy to isopropoxy again caused a 3-fold loss of activity. It is interesting that when either one of the *meta* or the *para* methoxy groups of **4** was removed, the activity dropped dramatically (from an IC_{50} of 35 nM for **4** to IC_{50}s of 980 nM and 950 nM for **7d** and **7e**, respectively). This is analogous to what was observed by Rhone-Poulenc Rorer[4] for the analogues of **1**. Therefore, all three methoxy groups in the aniline appear to be required for an energy favored molecular conformation that can have an effective hydrophobic interaction with the enzyme. Introduction of a bromo or methyl group into the 2-postion of the aniline (**7f** and **7g**) may substantially change the optimal conformation, resulting in the observed reduced activity.

To further identify the other structural features of **4** required for the inhibition of Src activity, several analogues of **4** were prepared where the 3,4,5-trimethoxyphenyl portion was maintained. Substitution of the NH linker at C-4 with O (**11**) resulted in an 8-fold loss of activity, while inserting one methylene group between NH and trimethoxybenzene (**12**) led to an even greater loss of activity. Replacement of the C-3-cyano group with CO_2Et (**9**) or CH_2OH (**10**) decreased Src inhibitory activity while the C-3 unsubstituted quinoline **8** was a moderate Src inhibitor. These results imply that the NH linker at C-4 and the cyano group at C-3 are both required for good Src inhibitory activity.

The 3-cyanoquinolines with water solubilizing groups at C-7, **19a** and **19b**, were better Src inhibitors than **4**, having IC_{50}s of 5.5 nM and 5.3 nM, respectively. This

Table 1.



Compounds	X	Y	R	IC_{50} (nM)
1	N	NH	3,4,5-Trimethoxy	280
6a	N	NH	3,5-Dimethoxy-4-ethoxy	340
6b	N	NH	3,5-Dimethoxy-4-*n*-butoxy	490
6c	N	NH	3,5-Dimethoxy-4-isopropoxy	830
6d	N	NH	3,5-Dimethoxy-4-*t*-butoxy	2700
6e	N	NH	3,5-Dimethoxy-4-*n*-dodecanoxy	5200
4	C-CN	NH	3,4,5-Trimethoxy	35
7a	C-CN	NH	3,5-Dimethoxy-4-ethoxy	63
7b	C-CN	NH	3,5-Dimethoxy-4-isopropoxy	110
7c	C-CN	NH	3,5-Dimethoxy-4-*n*-dodecanoxy	180
7d	C-CN	NH	3,5-Dimethoxy	980
7e	C-CN	NH	3,4-Dimethoxy	950
7f	C-CN	NH	2-Methyl-3,4,5-trimethoxy	470
7g	C-CN	NH	2-Bromo-3,4,5-trimethoxy	170
8	C-H	NH	3,4,5-Trimethoxy	200
9	C-CO_2Et	NH	3,4,5-Trimethoxy	>10,000
10	C-CH_2OH	NH	3,4,5-Trimethoxy	>10,000
11	C-CN	O	3,4,5-Trimethoxy	290
12	C-CN	$NHCH_2$	3,4,5-Trimethoxy	>10,000

increased activity may be a result of an additional interaction of the heteroatom-rich side chain with the kinase. In an anchorage independent cellular assay[13] measuring the inhibition of Src dependent cell proliferation, **19a** was more potent than **19b**, with IC_{50}s of 1.3 μM and >10 μM, respectively.

In an ELISA study examining the effect of increasing the concentration of ATP (100, 500, 1000, and 5000 μM), the IC_{50} values for the inhibition of Src activity by **4** steadily increased (34, 108, 190, and 650 nM, respectively), suggesting that **4** is an ATP competitive inhibitor. In addition, **4** was much less active in inhibiting a panel of other kinases including EGFr[6b] (IC_{50} = 1.5 μM). However, activity was seen with **19a** in an assay measuring Fyn dependent cell proliferation (IC_{50} = 6.7 μM).[14] Therefore, while these compounds may be selective for the Src family kinases over other kinase families, they appeared to be marginally selective for Src over other Src family members. Further work towards identifying more potent and selective Src inhibitors is in progress.

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7. Src kinase activity was measured in an ELISA format (Roche Diagnostics Tyrosine Kinase Assay Kit). Src (human c-Src protein, 3 units/reaction; Upstate Biotechnologies), reaction buffer (50 mM Tris–HCl pH 7.5, 10 mM $MgCl_2$, 0.1 mM EGTA, 0.5 mM Na_3VO_4) and cdc2 substrate peptide were added to compound and incubated at 30 °C for 10 min. The reaction was started by the addition of ATP to a final concentration of 100 μM, incubated at 30 °C for 1 h and stopped by addition of EDTA. Instructions from the manufacturer were followed for subsequent steps. Compounds were tested in duplicate and the value given is an average of two determinations with the exception of **4**, which was tested seven times resulting in values of 15, 90, 21, 36, 20, 27, and 40 nM (average = 35 nM).
8. (a) Preparation of **5a**: Bridges, A. J.; Zhou, H.; Cody, D. R.; Rewcastle, G. W; McMichael, A.; Showalter, H. D. H; Fry, D. W.; Kraker, A. J.; Denny, W. A. *J. Med. Chem.* **1996**, *39*, 267. (b) Preparation of **5b**: see ref 6a. (c) Preparation of **5c**: Kubo, K.; Shimizu, T.; Ohyama, S.; Murooka, H.; Nishitoba, T.; Kato, S.; Kobayashi, Y.; Yagi, M.; Isoe, T.; Nakamura, K. Osawa, T.; Izawa, T. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2935.

(d) Preparation of **5d**: Marquez, V. E.; Li, Z.; Bolen, J. B.; Stefanova, I.; Horake, I. *J. Med. Chem.* **1993**, *36*, 425.
9. The final compounds were analytically pure as determined by elementary analysis, ^{1}H NMR and MS.
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12. The general procedure of regioselective substitution of **13**: NaH (276 mg, 6.9 mmol) was suspended in THF:DMF (1:1, 5 mL) and isopropanol (625 mg, 10.4 mmol) in THF:DMF (1:1, 5 mL) was added dropwise. The resulting mixture was stirred at room temperature for 40 min. Compound **13** (500 mg, 2.3 mmol) in DMF (15 mL) was added via syringe. The reaction mixture was stirred at room temperature for 15 min and quenched with saturated aqueous NH_4Cl. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organics were dried over Na_2SO_4, concentrated and column chromatographed to give 473 mg (85% yield) of compound **14c**.
13. Anchorage independent Src cellular assay: Rat2 fibroblasts were stably transformed with a pCDNA plasmid (Invitrogen) containing a v-Src:human c-Src fusion under control of the CMV promoter. The v-Src/Hu c-Src fusion gene has the catalytic domain of human c-Src fused with the remainder of v-Src (the amino terminal half of v-Src and the v-Src C-terminal tail). Ultra-low cluster plates (Costar No. 3474), which do not support cell attachment, were seeded with 10,000 cells per well on day one. Compound was added on day two and MTS reagent (Promega) was added on day five to measure relative cell number according to manufacturer specifications.
14. This assay is similar to the anchorage independent Src cellular assay, except that a v-Src:human fynB fusion gene was employed. An IC_{50} >10 μM was obtained for **19b** in this assay.