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Substituted 4-Anilino-7-phenyl-3-quinolinecarbonitriles as Src Kinase Inhibitors

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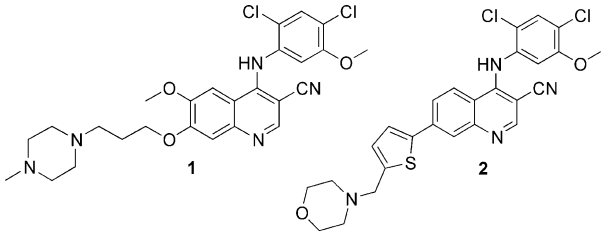
Abstract—A series of substituted 4-anilino-7-phenyl-3-quinolinecarbonitriles has been prepared as Src kinase inhibitors. Optimal activity is observed with compounds that have basic amines attached via the *para* position of the 7-phenyl ring, and a hydrogen atom at the C-6 position. The best compounds are low nanomolar inhibitors of Src kinase, and have potent activity against Src-transformed fibroblast cells.

Protein tyrosine kinases (TKs) play an important role in cell growth and differentiation. These enzymes catalyze the transfer of a phosphate group from ATP to a tyrosine residue on an appropriate substrate, thereby bringing about cell signaling events. Several nonreceptor TKs have been identified, among them the Src family of cytoplasmic protein TKs.[1] The TK Src has been implicated in several disease states, including cancer,[2a,b] osteoporosis,[3a,b] and stroke.[4] Therefore, inhibition of Src kinase could prove useful in the treatment of these and other diseases. A number of Src family kinase inhibitors have been reported in the literature, including 4-anilinoquinazolines,[5] pyrazolo[3,4-*d*]pyrimidines,[6] pyrrolo [2,3-*d*]pyrimidines,[7a–e] pyrido[2,3-*d*]pyrimidin-7(8*H*)-ones,[8] 1,6-naphthyridin-2(1*H*)-ones,[9] and aminopyrido-[2,3-*d*] pyrimidin-7-yl ureas.[10]

A series of 4-anilino-3-quinolinecarbonitrile compounds has been reported to be potent inhibitors of EGFr,[11] Src[12a–c] and MEK[13a,b] kinases. While earlier synthetic efforts were directed at 4-anilino-6,7-dialkoxy-3-quinolinecarbonitrile Src kinase inhibitors, such as compound **1**,[12c] recent efforts have focused on 4-anilino-7-thienyl-3-quinolinecarbonitriles (e.g., **2**) as potent Src kinase inhibitors.[14]

As part of our research effort to explore the optimal aryl substitution at the C-7 position, we synthesized a series of phenyl substituted compounds possessing different water-solubilizing groups. With the phenyl ring spacer, the attached water-solubilizing groups are oriented differently as compared to those on the C-7 thiophene substituted quinolinecarbonitriles. Compounds were synthesized with these substituents at the *ortho*, *meta*, and *para* positions. Finally, we also looked at the effect of these 7-phenyl compounds with an additional methoxy group attached at the C-6 position. This substitution enhances the activity of compounds with 7-alkoxy substituents,[12a] and thus it was of interest to determine the effects on the aryl-linked compounds.



The synthesis of the substituted phenyl intermediates **5a–c** is shown in Scheme 1. Reductive amination of cyclic amines **4a**,**b** with 3-bromobenzaldehyde or 4-bromobenzaldehyde provided the substituted benzylamines.

A two-step reaction sequence was utilized to synthesize intermediates **7a–c**, which have the amine substituents

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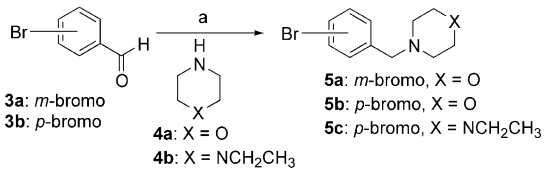


Scheme 1. (a) $NaCNBH_3$, HOAc, EtOH.

attached to the phenyl ring via a two carbon chain. The phenylacetic acid derivatives **6a** and **6b** were first reacted with amines **4a** or **4b** to provide the corresponding amides, followed by reduction to form the amines **7a–c** (Scheme 2).

The intermediate 3-quinolinecarbonitrile **13** was prepared as shown in Scheme 3. Methyl vanillate **8** was benzylated and nitrated to provide **9**. Treatment of **9** with boron trichloride selectively removed only the benzyl group. The resulting phenol was reacted with triflic anhydride, then with iron/ammonium chloride to provide aniline **10**. Compound **10** was reacted with DMF-dimethylacetal to produce an intermediate amidine, which was cyclized to 3-quinolinecarbonitrile **11** with the lithium anion of acetonitrile.

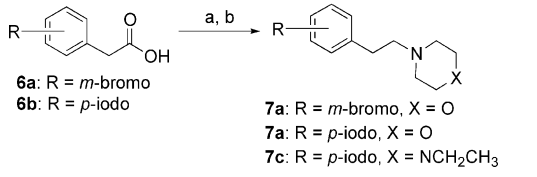
Compound **11** was converted to **12** using refluxing phosphorus oxychloride. The reaction of **12** with 2,4-dichloro-5-methoxyaniline[12c] in the presence of pyridine hydrochloride provided intermediate **13**.

Compounds **15a–k** were synthesized by the chemistry outlined in Scheme 4. Intermediates **5a–c** and **7a–c** were converted to the corresponding arylboronic acids by reaction with dipinacoldiboron using catalytic [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane.[15] Reaction of the resulting boronic acids with triflate **13**, or bromide **14**[14] in the presence of catalytic tetrakis triphenylphosphine palladium(0) provided the final products.[16]

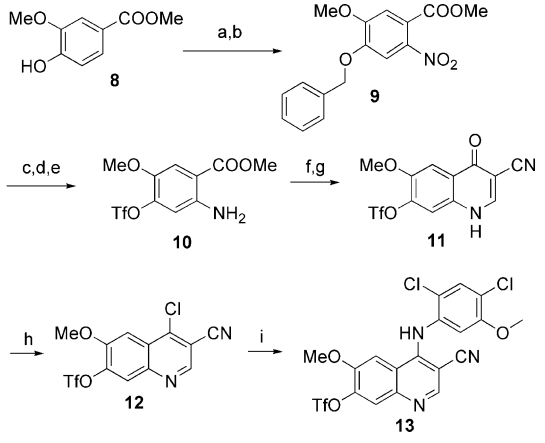
Several analogues were synthesized via an alternative reaction sequence, as outlined in Scheme 5. Intermediate **14** was first reacted with the *o*-, *m*-, and *p*-substituted arylboronic acids **16a–c** to provide **17a–c**. Reductive amination of intermediates **17a–c** with the appropriately substituted cyclic amines provided the target compounds **15l–o**.[17]

The biological activity of **15a–o** is summarized in Table 1.[18] Several trends are evident. Compounds possessing a C-6 methoxy group (**15a–e**) are generally less active (up to 5-fold) than the corresponding compounds with hydrogen at C-6 (**15f–15j**). Since a C-6 methoxy group is beneficial for activity in the 6,7-dialkoxy-3-quinolinecarbonitrile series,[12a] we speculate that the decrease in activity observed for this series may be due to the steric effect of the C-6 methoxy group on the rotation of the C-7 phenyl ring. Rotating the phenyl ring further from the plane of the quinolinecarbonitrile core might cause less favorable steric interactions in the active site of Src.

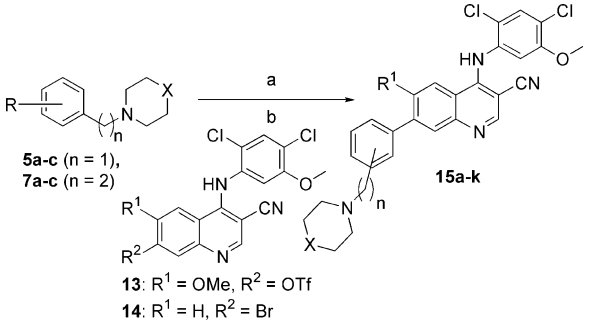
Another clear trend can be observed with regard to the position of attachment of the water-solubilizing group.



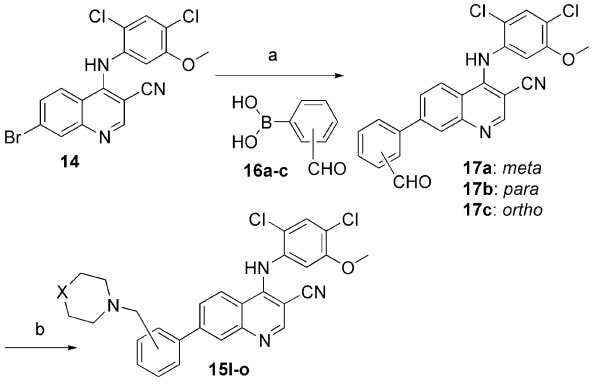
Scheme 2. (a) CH_2Cl_2, EDCI, DMA, **4a** or **4b**; (b) $BH_3.SMe_2$, THF.



Scheme 3. (a) K_2CO_3, $PhCH_2Br$, DMF; (b) HNO_3, HOAc; (c) BCl_3, CH_2Cl_2; (d) $(TfO)_2O$, pyridine, CH_2Cl_2; (e) Fe, NH_4Cl, H_2O/MeOH; (f) DMF-dimethylacetal; (g) *n*-BuLi /CH_3CN, THF; (h) $POCl_3$; (i) 2,4-dichloro-5-methoxyaniline,[12c] pyridine hydrochloride, ethoxyethanol.

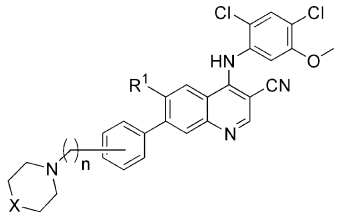


Scheme 4. (a) $PdCl_2dppf.CH_2Cl_2$, DMSO, KOAc, dipinacoldiboron; (b) $Pd(PPh_3)_4$, DME, $NaHCO_3$.



Scheme 5. (a) $Pd(PPh_3)_4$, DME, $NaHCO_3$; (b) $NaB(O_2CCH_3)_3H$, amine, HOAc, DMF.

Table 1. Inhibition of Src enzymatic and Src cellular activity for **15a–o**



Compd	Phenyl subst.	R^1	*n*	X	Src IC_{50}, nM[a]	Cells IC_{50}, nM[a]
15a	*m*	OMe	1	O	150	8100
15b	*p*	OMe	1	O	15	1500
15c	*p*	OMe	1	NCH_2CH_3	7.0	2000
15d	*m*	OMe	2	O	74	12,000
15e	*p*	OMe	2	O	11	1300
15f	*m*	H	1	O	28	3900
15g	*p*	H	1	O	3.3	520
15h	*p*	H	1	NCH_2CH_3	3.0	960
15i	*m*	H	2	O	29	3200
15j	*p*	H	2	O	14	1000
15k	*p*	H	2	NCH_2CH_3	3.7	1900
15l	*m*	H	1	NCH_3	14	2800
15m	*p*	H	1	NCH_3	3.8	390
15n	*p*	H	1	$N(CH_2)_2OH$	4.7	71
15o	*o*	H	1	O	4000	>10,000
1					1.3	100

[a]The IC_{50} values reported represent the means of at least two separate determinations with typical variations of less than 40% between replicate values.

The most active compounds are the *p*-substituted phenyl compounds, with less activity for the *m*-substituted analogues, and essentially no activity for *o*-substituted **15o**. This result correlates with the SAR of the 4-anilino-7-thienyl-3-quinolinecarbonitrile series, in which it was observed that the 'linear' analogues were more potent than the corresponding 'angular'compounds.[14]

Compounds with water-solubilizing groups attached via different chain lengths ($n=1$ or 2) had comparable activity against Src enzyme. However, none of the compounds with $n=2$ had submicromolar activity in the Src dependent cell proliferation assay.[12a,b] Generally, the different water-solubilizing groups provided similar enzyme activity for compounds in which all other structural features were identical. Overall, the most potent group of compounds were the *p*-substituted phenyl analogues, with C-6 = H, and $n=1$: **15g**,**h** and **15m**,**n**, which only differ by the nature of the water-solubilizing substituent. Of these, the most active compound in cells was **15n**, which was comparable to compound **1** with an IC_{50} of 71 nM. These compounds showed good selectivity for Src versus a panel non-Src family kinases. For example, **15g** was significantly less active against EGFr (3000 nM), cyclin-dependent kinase 4 (10% inhibition at 10 μg/mL), and MEK (280 nM). However, **15g** and **15m** did show some activity in an assay measuring Fyn dependent cell proliferation[12c] (1.17 and 0.83 μM, respectively). Therefore, these compounds might be only marginally selective for Src over the other Src family members. Work is in progress to further enhance the potency and selectivity of these Src kinase inhibitors.

In conclusion, a series of 7-phenyl substituted 3-quinolinecarbonitriles was synthesized and evaluated as Src kinase inhibitors. Several of these were very potent enzyme inhibitors, with the overall most active compound in cells being **15n**, which possesses a hydroxyethylpiperazine water-solubilizing substituent.

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16. A typical preparation of target compounds **15a–k** is illustrated by the following procedure for **15f**: To a dry flask under a nitrogen atmosphere was added 200 mg (0.78 mmol) of **5a**, 0.218 g (0.86 mmol) of bis(pinacolato)diboron, 230 mg (2.34 mmol) of potassium acetate, 5 mL of dimethylsulfoxide and 32 mg (0.04 mmol) of [1,1′-bis(diphenylphosphino)ferrocene] dichloropalladium(II), complex with dichloromethane. The reaction mixture was heated at 80 °C for 2 h. After cooling, the mixture was partitioned between 20 mL of toluene, 40 mL of ethyl acetate and 40 mL of water. The layers were separated and the aqueous layer was further extracted with 30 mL of ethyl acetate. The organic layers were combined and washed with 4×40 mL water. After drying over magnesium sulfate, removal of the solvents gave crude 4-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl]morpholine as a dark oil.

A mixture of 110 mg (0.26 mmol) of **14**, crude 4-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl]morpholine and 45 mg (0.04 mmol) of tetrakis(triphenylphosphine) palladium(0) was heated at reflux in 4 mL of ethylene glycol dimethyl ether and 2.5 mL of saturated aqueous sodium bicarbonate for 2 h. After cooling, the mixture was partitioned between 50 mL of ethyl acetate and 40 mL of water. The layers were separated and the ethyl acetate layer was dried over magnesium sulfate. Removal of the solvent in vacuo gave a dark residue which was purified by flash silica gel chromatography eluting with a gradient of ethyl acetate to 95:5 ethyl acetate/methanol, to provide 70 mg of **15f** as a yellow solid, mp 88–91 °C; ^{1}H NMR (DMSO-d_6) δ 10.05 (s, 1H), 8.61 (broad s, 2H), 8.19 (s, 1H), 8.08–7.97 (m, 1H), 7.85–7.72 (m, 3H), 7.51 (t, J = 8 Hz, 1H), 7.43 (s, 1H), 7.41 (s, 1H), 3.87 (s, 3H), 3.61 (s, 2H), 3.59 (s, 4H), 2.42 (s, 4H). MS (ES) m/z 519.1, 521.0 (M + 1). Analysis for $C_{28}H_{24}Cl_2N_4O_2$0.5 EtOAc: Calcd: C, 63.95; H, 5.01; N, 9.94. Found: C, 63.64; H 4.93; N, 9.97.

17. A typical preparation of target compounds **15l–o** is illustrated by the following procedure for **15m**: By the procedure of the final step utilized to synthesize **15f**, **14** (3.00 g, 7.1 mmol) was reacted with 4-formylphenyl boronic acid (**16b**) (1.27 g, 8.5 mmol) and tetrakis(triphenylphosphene) palladium (0) (0.30 g, 0.25 mmol) in a mixture of ethylene glycol dimethyl ether (20 mL) and a saturated aqueous solution of sodium bicarbonate (20 mL) to provide 3.00 g (94%) of **17b** as a yellow solid, mp 248–251 °C; ^{1}H NMR (DMSO-d_6) δ 10.11 (s, 1H), 10.10 (s, 1H), 8.67 (t, J = 9 Hz, 2H), 8.32 (s, 1H), 8.16 (m, 3H), 8.09 (s, 1H), 7.99 (s, 1H), 7.78 (s, 1H), 7.43 (s, 1H), 3.87 (s, 3H); MS (ES) m/z 450.0 (M + 1). Analysis for $C_{24}H_{15}Cl_2N_3O_2$- 0.5 CH_2Cl_2: calcd: C, 56.91; H, 5.20; N, 10.30. Found: C, 57.20; H, 5.10; N, 9.91.

4-Methylpiperidine (63 mg, 0.63 mmol) was added to a suspension of **17b** (200 mg, 0.44 mmol) in 4 mL of methylene chloride and 1 mL of *N*,*N*-dimethylformamide. The mixture was cooled to 0 °C and sodium triacetoxyborohydride (500 mg, 2.36 mmol) was added followed by a drop of acetic acid. The reaction mixture was allowed to warm to room temperature and stirred at room temperature for 2 h to give a yellow solution. The reaction was quenched by the addition of water and partitioned between saturated sodium bicarbonate and methylene chloride. The organic layer was dried over sodium sulfate, filtered and concentrated in vacuo. The residue was purified by flash chromatography, eluting with 10% methanol in methylene chloride to provide 183 mg (82%) of **15m** as an off-white solid, mp 120–123 °C; ^{1}H NMR (DMSO-d_6) δ 10.05 (broad s, 1H), 8.58 (broad s, 2H), 8.20–8.11 (m, 1H), 8.06–7.97 (m, 1H), 7.90–7.78 (m, 2H), 7.77–7.63 (m, 1H), 7.47 (dd, J = 3.3, 8.1 Hz, 2H), 7.47–7.37 (m, 1H), 3.86 (s, 3H), 3.55 (s, 2H), 2.53–2.36 (m, 8H), 2.24 (s, 3H); MS (ES) m/z 531.2, 532.0 (M + 1). Analysis for $C_{29}H_{27}Cl_2N_5O$1.5 CH_2Cl_2: Calcd: C, 55.52; H, 4.58; N, 10.61. Found: C, 55.17; H, 4.50; N, 10.78.

18. Compounds were tested in a modified format of the enzymatic assay previously reported.[12a–c] Peptide was bound to the streptavidin plate prior to the kinase reaction, and peptide phosphorylation reaction was monitored by europium fluorescence as recommended by the manufacturer (Perkin–Elmer). For cell assays, Costar ultra-low binding plates were coated with Sigma-Cote to block residual cell attachment.