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8-Anilinoimidazo[4,5-*g*]quinoline-7-carbonitriles as Src Kinase Inhibitors

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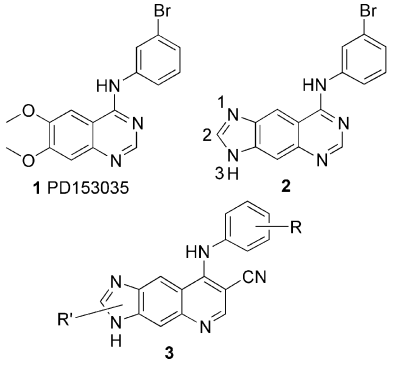
Abstract—A series of 8-anilinoimidazo[4,5-*g*]quinoline-7-carbonitriles was synthesized and evaluated as Src kinase inhibitors. Several aniline substituents were surveyed, as well as water-solubilizing groups at the C-2 and N-3 positions. Potent Src inhibitors were identified, with N-3 providing the best position for an additional water-solubilizing group.

Protein tyrosine kinases (TKs) catalyze the phosphorylation of a tyrosine residue on substrate proteins, a process important for regulation of cell growth and differentiation. The Src family of TKs belong to a class of nonreceptor TKs, which are present in the cytoplasm.[1] Src itself participates in signaling pathways controlling proliferation, migration and angiogenesis. Small molecule inhibitors of Src have thus been considered to be potentially useful agents for therapeutic intervention in cancer[2a,b] and other disease states, such as osteoporosis[3a,b] and stroke.[4]

Several different structural types of compounds have been reported to be inhibitors of Src family TKs, 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]

In recent publications, it has been shown that 6,7-dialkoxy substituted 4-anilino-3-quinolinecarbonitriles are particularly potent EGFr,[11] Src,[12a–c] and MEK[13a,b] kinase inhibitors. These compounds are known to bind at the site normally occupied by ATP and parallel the activity seen with 4-anilinoquinazolines, which also have activity as tyrosine kinase inhibitors.

Thus, for example, structure **1** is a highly potent inhibitor of EGFr.[14] An 8-anilinoimidazo[4,5-*g*]-quinazoline (**2**) had been shown to be even more effective as an EGFr kinase inhibitor.[15] In this work it was shown that water-solubilizing groups attached at the N-1 or N-3 positions decreased enzyme activity (although these compounds were still very potent EGFr inhibitors), but the C-2 position was not similarly explored.



In this communication, we describe the activity of substituted 8-anilinoimidazo[4,5-*g*]quinoline-7-carbonitriles (**3**) as Src kinase inhibitors. We chose to attach water-solubilizing substituents at the C-2 and N-3 positions of the 8-anilinoimidazo[4,5-*g*]quinoline-7-carbonitriles, since it has been shown that attachment of these groups at the C-7 position of the 4-anilinoquinoline-3-carbonitrile series provides optimal activity.[12a–c]

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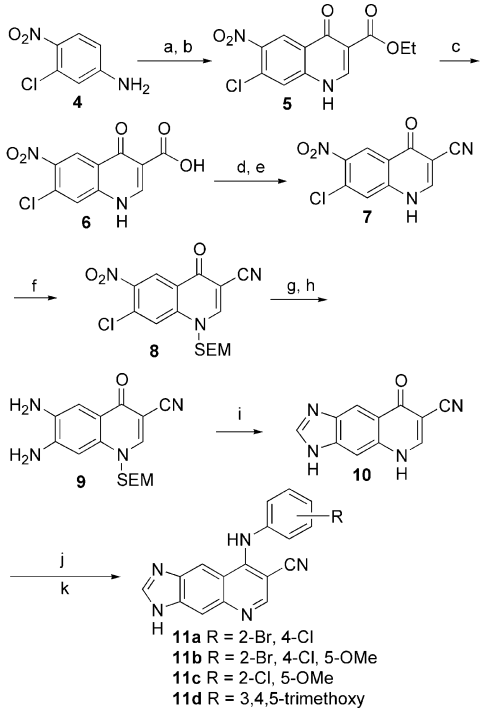
An optimized route to the 8-anilinoimidazo[4,5-*g*]quinoline-7-carbonitriles is outlined in Scheme 1. The reaction of **4** with diethyl ethoxymethylenemalonate, followed by cyclization in refluxing Dowtherm provided intermediate **5** as a single isomer. Hydrolysis to the corresponding carboxylic acid **6** was readily accomplished in refluxing aqueous sodium hydroxide. Conversion of the acid to an amide was accomplished using CDI as an activating agent, followed by reaction with ammonia. Cyanuric chloride was utilized to convert the resulting amide to the 3-cyano substituent, providing **7** (compound **7** could also be prepared directly from the reaction of **4** with ethyl (ethoxymethylene)cyanoacetate, but this reaction provided two regioisomeric cyclization products as an inseparable mixture). Protection of the quinoline nitrogen with SEM chloride provided **8**, which more readily underwent a nucleophilic displacement of the chloro substituent at C-7 and was more soluble in common organic solvents than **7**.

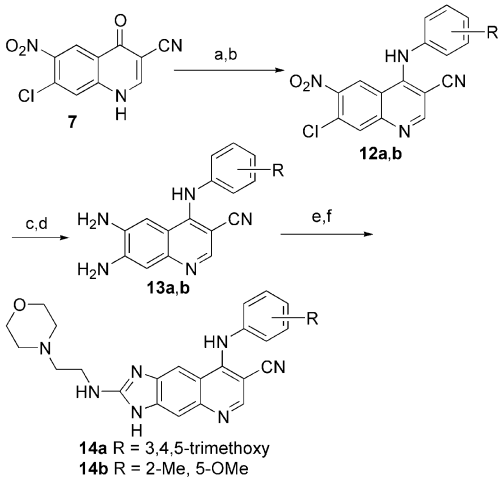
Displacement of the chloro substituent by sodium azide at 60 °C produced a 7-azido intermediate that was readily reduced by catalytic hydrogenation to provide the oxidatively unstable diamine **9** in excellent overall yield. Treatment of **9** with refluxing formic acid simultaneously removed the SEM group and formed the imidazole ring of **10** in one step. It was necessary to add at least 4 equiv of imidazole to this reaction to scavenge the formaldehyde generated by the deprotection. In the absence of a scavenging nucleophile, significant amounts of dimerized **10** were formed, bonded by a methylene linkage. Treatment of **10** with oxalyl chloride provided the corresponding chloride, which was displaced by substituted anilines[12c] in refluxing ethoxyethanol in the presence of pyridine hydrochloride to provide target compounds **11a**–**d**.[16]



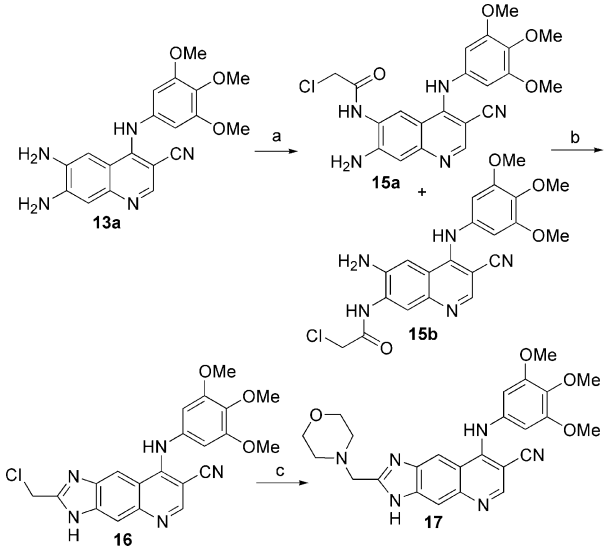
Scheme 1. (a) Diethyl ethoxymethylenemalonate; (b) Dowtherm; (c) NaOH; (d) (1) CDI/DMF, (2) NH_3; (e) cyanuric chloride; (f) (1) NaH (2) SEMCl; (g) NaN_3; (h) H_2, Pd/C; (i) HCO_2H, imidazole; (j) oxalyl chloride; (k) pyridine–HCl, aniline, ethoxyethanol.

Scheme 2 outlines the chemistry utilized to synthesize compounds with water-solubilizing groups attached via a C-2 amino group. Cyanoquinolone **7** was converted to the corresponding 4-chloro intermediate by reaction with oxalyl chloride, followed by displacement with substituted anilines to provide **12a**,**b**. Azide displacement of the chloro group, followed by catalytic hydrogenation provided the diamino intermediates **13a**,**b**. Reaction of the diamine with morpholinoethylisothiocyanate, followed by cyclization with mercuric oxide, afforded products **14a**,**b**.

Scheme 3 outlines the chemistry utilized to synthesize a compound with a water-solubilizing group attached at C-2 via a methylene spacer. The reaction of **13a** with chloroacetyl chloride utilizing diethylaniline as a base



Scheme 2. (a) Oxalyl chloride; (b) aniline, pyridine–HCl, ethoxyethanol; (c) NaN_3; (d) H_2, Pd/C; (e) morpholinoethylisothiocyanate; (f) HgO.

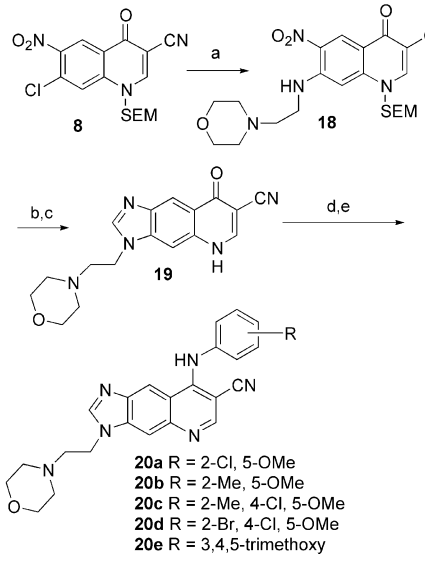


Scheme 3. (a) Chloroacetyl chloride, diethylaniline; (b) acetic acid; (c) morpholine.

provided mixtures of isomers **15a**,**b**. Cyclization to the imidazocyanoquinoline **16** was achieved by refluxing in acetic acid. The reaction of **16** with morpholine provided target compound **17**. Attempts to utilize similar chemistry to attach water-solubilizing groups with longer spacers were unsuccessful.

Compounds with morpholine groups linked via N-3 were synthesized as outlined in Scheme 4. The SEM-protected cyanoquinolone **8** was reacted with substituted diamines to provide **18**. Catalytic hydrogenation over palladium on carbon, followed by reaction in refluxing formic acid (with added imidazole) cleanly provided **19**. Chlorination by oxalyl chloride, followed by reaction with substituted anilines[12c] provided compounds **20a**–**e**.

It has been shown[12c] that the most potent 4-anilino-3-quinolinecarbonitrile Src kinase inhibitors have anilines substituted at the 2, 4, and 5 positions, with only slightly diminished activity for 2,5-substituted anilines. As shown in Table 1, compounds **11a**–**c** show a similar trend, with **11b** being the most potent compound with an IC_{50} of 16 nM, and **11c** being a little less potent (IC_{50}: 30 nM).[17] For 4-anilino-3-quinolinecarbonitriles, 3,4,5-trimethoxyaniline substituents[12b] provide potent Src kinase activity. In this series, the 3,4,5-trimethoxy substituted **11d** was less potent than **11a**. Compounds **11a**–**c** possessed moderate cell activity, the best being **11b**, which inhibited Src transformed rat fibroblasts with an IC_{50} of 2.8 μM.

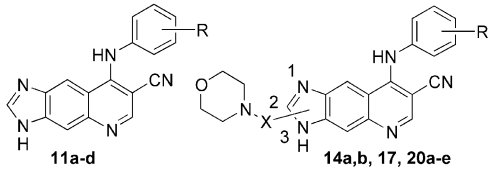


Scheme 4. (a) Diamine; (b) H_2, Pd/C; (c) HCO_2H, imidazole; (d) oxalyl chloride; (e) pyridine–HCl, ethoxyethanol.

At the outset of this work, it was anticipated that the presence of additional amine water-solubilizing substituents would improve cell activity, and possibly enzyme activity as well. Compound **14b** was moderately active in the enzyme assay with an IC_{50} of 67 nM, while the 3,4,5-trimethoxy substituted **14a** had a significantly higher IC_{50}. However, **14a** had comparable activity to **11d**, indicating that the compounds with morpholine groups attached via an ethylamino chain at C-2 retained similar enzyme activity as compared to compounds unsubstituted on the imidazole moiety. Unfortunately, the water-solubilizing group did not provide **14a** or **14b** with cell activity below 10 μM. Compound **17** was the least active of the compounds having a 3,4,5-trimethoxyaniline group at the C-4 position, likely indicating that the short tether to the morpholine ring is not optimal.

Overall, the most potent group of compounds in enzyme and cell assays was the N-3 substituted series: **20a**–**e**. As was seen for **11a**–**d**, compounds with 2,4,5-trisubstituted anilines were the most potent (**20c**,**d**) with IC_{50}s of 29 and 9 nM, respectively, followed by the 2,5-substituted analogues (**20a**,**b**). Compound **20e**, possessing a 3,4,5-trimethoxyaniline, was the least active of this series, with activity comparable to **11d** and **14a**. It appears that the 2-chloro (**20a**) or bromo (**20d**) substituents on the C-4 aniline provide about 3-fold better activity than the 2-methyl substituted analogues (**20c**

Table 1. Inhibition of Src enzymatic and Src cellular activity for compounds **11a–d, 14a,b, 17** and **20a–e**



Compd	R	X	Src (IC_{50}, nM[a])	Cells (IC_{50}, μM[a])
11a	2-Br, 4-Cl	—	75	10.1
11b	2-Br, 4-Cl, 5-OMe	—	16	2.8
11c	2-Cl, 5-OMe	—	30	10.5
11d	3,4,5-trimethoxy	—	330	>10
14a	3,4,5-trimethoxy	2-$NH(CH_2)_2$–	180	>10
14b	2-Me, 5-OMe	2-$NH(CH_2)_2$–	67	>10
17	3,4,5-trimethoxy	2-CH_2–	620	>10
20a	2-Cl, 5-OMe	3-$(CH_2)_2$–	43	1.2
20b	2-Me, 5-OMe	3-$(CH_2)_2$–	120	3.3
20c	2-Me, 4-Cl, 5-OMe	3-$(CH_2)_2$–	29	3.9
20d	2-Br, 4-Cl, 5-OMe	3-$(CH_2)_2$–	9	0.67
20e	3,4,5-trimethoxy	3-$(CH_2)_2$–	200	>10

[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.

and **20b**). This differs somewhat from the SAR observed within the 4-anilino-6,7-dialkoxy-3-quinolinecarbonitriles, where the 2-methyl, chloro or bromo analogues were essentially equivalent in activity.[12c]

Compounds **20d** and **20a** were the most potent in the cellular assay, with IC_{50}s of 0.67 and 1.2 μM, respectively. These compounds do appear to benefit from the presence of the water-solubilizing morpholine group, being more potent in cells than **11b** and **11c** (2.8 and 10.5 μM, respectively). The N-3 substituted **20b** had better cellular activity than the C-2 substituted **14b** (3.3 μM and >10 μM, respectively). It is unclear whether the poor cellular activity of **14b** is due to the C-2 substitution, or whether the length and composition of the tether group to morpholine is the major contributing factor. However, it was not possible to synthesize C-2 ethyl-linked analogues due to their propensity to readily undergo E2 elimination.

In summary, as part of a continuing effort to discover novel and potent Src kinase inhibitors, a series of 8-anilinoimidazo[4,5-*g*]quinoline-7-carbonitriles was synthesized and evaluated. A novel reaction sequence was employed to synthesize the target compounds, the key step utilizing an azide addition at C-7 to place a nitrogen atom at this position under mild reaction conditions. These compounds showed promising activity in enzyme and cell assays, comparable to initial lead compounds within the 4-anilino-6,7-dialkoxy quinoline-3-carbonitrile series. None of the compounds tested showed significant activity against a panel of other kinases, including EGFr, ErbB2, and MEK (IC_{50}s >1 μM). Attachment of water-solubilizing groups at the C-2 position proved to be a significant challenge, due to the difficulty of adding electrophiles to the relatively unreactive 4-anilino-6,7-diamino-3-cyanoquinoline intermediates. The analogues synthesized did not have an increased activity in cells. The N-3 substituted **20d** had the best overall enzyme and cell activity of this series, thereby providing a potential direction for future optimization of these compounds.

Acknowledgements

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16. All final products were characterized by ^{1}H NMR, MS and elemental analysis. A representative example, compound **11d**: ^{1}HNMR (DMSO-d_6 + trifluoroacetic acid) δ 3.73 (s, 3H) 3.81 (s, 6H) 6.90 (s, 2H) 8.20 (s, 1H) 8.85 (s, 1H) 9.13 (s, 1H) 9.15 (s, 1H) 11.40 (broad s, 1H). MS (ES) m/z 376.3 $(M+H)^+$.

Analysis for $C_{20}H_{17}N_5O_3 0.9H_2O$, Calcd: C 61.34; H 4.84; N 17.88, Found: C 61.03; H 4.82; N 17.76.

17. 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.