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4-Anilino-3-cyanobenzo[*g*]quinolines as Kinase Inhibitors

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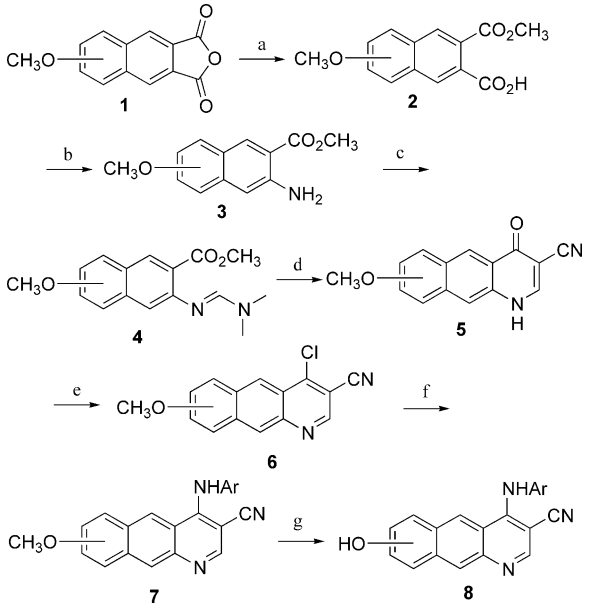
Abstract—A series of 4-anilino-3-cyanobenzo[*g*]quinolines was prepared as potent kinase inhibitors. Compared with their bicyclic 4-anilino-3-cyanoquinoline analogues, the tricyclic 4-anilino-3-cyanobenzo[*g*]quinolines are less active against EGF-R kinase, equally active against MAPK kinase (MEK), and more active against Src kinase. For Src kinase inhibition, the best activity is obtained when both the 7- and 8-positions are substituted with alkoxy groups. Several of these kinase inhibitors show potent growth inhibitory activity in tumor cells.

Protein kinases, which transfer phosphoryl groups from ATP to tyrosine, serine, and threonine residues on proteins, play a key role in signal transduction pathways in the regulation of cell growth and cell cycling in response to external stimuli. However, under certain conditions, as a result of over-expression, mutation, or, in the case of receptor kinases, co-expression of the ligand and the receptor, these enzymes can become hyper-activated, resulting in uncontrolled cell proliferation. Inhibition of kinase activity presents a unique opportunity to block uncontrolled cell growth and, therefore, has potential therapeutic utility in developing cancer treatments. We recently reported 4-anilino-3-cyanoquinolines as potent inhibitors of Epidermal Growth Factor Receptor (EGF-R) kinase,[1] Mitogen-Activated Protein Kinase (MAPK) kinase,[2] and Src kinase.[3] The differential selectivity for these kinases depends on the nature of the anilino group at the 4-position. Dialkoxy substituents at the 6- and 7-positions increase activity, while alkoxy substituents at the 5- and 8-positions lead to decreased activity. These results suggest there is room to expand the bicyclic core from the 6- and 7-positions. We thus introduced a novel tricyclic 3-cyanobenzo[*g*]quinoline core by fusing an additional phenyl ring to the 6- and 7- positions of the 3-cyanoquinoline core. We now report the synthesis and inhibitory activity of this series of 4-anilino-3-cyanobenzo[*g*]quinolines in EGF-R kinase, MAPK kinase, and Src kinase. The activity of the corresponding bicyclic 4-anilino-3-cyanoquinolines is also shown for comparison.

As shown in Scheme 1, the synthesis of these tricyclic 4-anilino-3-cyanobenzo[*g*]quinolines started from the corresponding 6,7-dimethoxynaphthalene-2,3-dicarboxylic anhydride[4] or 6-methoxynaphthalene-2,3-dicarboxylic anhydride[5] **1**. Treatment with sodium methoxide in methanol opened the anhydrides, yielding mono-acid



Scheme 1. (a) $NaOCH_3$, CH_3OH; (b) $(PhO)_2PON_3$, then acetone/H_2O; (c) DMF·DMA, reflux; (d) $LiCH_2CN$, THF, −78 °C, then HOAc, rt; (e) $POCl_3$, reflux; (f) $ArNH_2$, $EtOCH_2CH_2OH$, reflux; (g) pyridinium chloride, 215 °C.

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mono-esters **2** (a 1:1 mixture of regio-isomers in the case of asymmetric mono-methoxy substitution). The carboxylic acid group was then converted to an amino group via Curtius rearrangement. We found that quenching the isocyanate intermediate with a large quantity of wet acetone led to the desired amino naphthoate directly. In the case of mono-methoxy substitution, the regio-isomers were separated at this amino naphthoate stage. The amino group was then converted to the corresponding formamidine **4** in refluxing DMF·DMA. Addition of 2 equiv of lithium anion of acetonitrile followed by acid-catalyzed cyclization with acetic acid gave the 3-cyanobenzo[*g*]quinolones **5**. Chlorination with $POCl_3$ followed by replacement of the chloride with anilines provided the desired 4-anilino-3-cyanobenzo[*g*]quinolines **7**. Demethylation of selected compounds was accomplished in pyridinium chloride at 215 °C, providing the corresponding hydroxy analogues **8**.

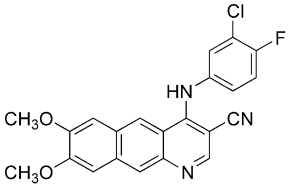
The tricyclic 4-anilino-3-cyanobenzo[*g*]quinolines (**7** and **8**), with appropriate anilino groups at the 4-position, were tested against three different kinases: EGF-R kinase, MAPK kinase (MEK), and Src kinase. They were also tested in tumor cell lines relevant to these kinases. To evaluate the effect of fusing an additional ring to form the tricyclic 3-cyanobenzo[*g*]quinoline core, the corresponding bicyclic analogues, 4-anilino-3-cyanoquinolines, were also tested at the same time for comparison.

We first introduced a 3-chloro-4-fluoroanilino group, one of the best anilino groups for EGF-R kinase inhibition,[1] at the 4-position of the tricyclic core. Compared with its bicyclic analogue **10**, compound **9** is less active as an EGF-R kinase inhibitor (Table 1). The compounds were evaluated for their ability to inhibit the growth of certain cell lines. Two human carcinoma cell lines were used: A431 (epidermoid), which highly over-expresses EGF-R, and SW620 (colon), which serves as a control line expressing low levels of EGF-R. Compound **10** showed a 10-fold increase in activity in A431 cells versus the control SW620 cells, while compound **9** showed a 3-fold increase. These results suggest that both compounds inhibit A431 cell growth, at least in part, by interfering with the function of the EGF-R receptor.

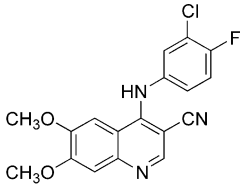
We next studied the effect of the tricyclic core on MAPK kinase (MEK) inhibition. We introduced a 4-phenoxyanilino group, one of the best anilino groups for MAPK kinase inhibition,[2] at the 4-position. As shown in Table 2, the tricyclic compound **11** showed activity comparable to that of the the bicyclic compound **12** in both the enzymatic assay and the inhibition of LOVO human colon tumor cell growth. This result indicates that ring expansion of the bicyclic 3-cyanoquinoline does not decrease potent MAPK kinase inhibitory activity.

As shown in Table 3, in contrast to the effects on EGF-R kinase and MAPK kinase (MEK), the tricyclic 3-cyanobenzo[*g*]quinoline core provides better potency against Src kinase activity compared to the bicyclic analogue. Compound **13**, with a 4-chloro-5-methoxy-2-methylanilino group, one of the most active anilino groups for Src kinase inhibitory activity, at the 4-position,[3b] was prepared. Compared with its bicyclic 3-cyanoquinoline analogue **14**, the tricyclic 3-cyanobenzo[*g*]quinoline **13** was found to be more potent in the enzymatic assay (3×) as well as in the Src-transformed fibroblast cell proliferation assay (10×).

Table 1. EGF-R kinase and cell growth inhibitory activity of **9** and **10**



9

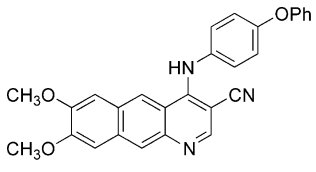


10

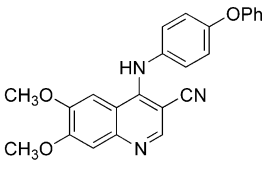
Compd	IC_{50} (μM) EGF-R kinase	IC_{50} (μM) A431 cell	IC_{50} (μM) SW620 cell
9	18.0	0.68	1.99
10	5.17	1.03	9.89

IC_{50} values for EGF-R inhibition represent means of three separate determinations with variations less than 30% of the means.

Table 2. MAPK kinase (MEK) and cell growth inhibitory activity of **11** and **12**



11

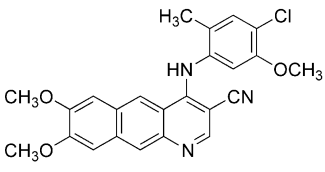


12

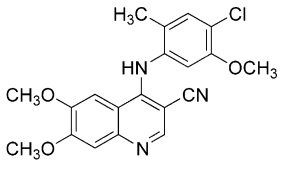
Compd	IC_{50} (μM) coupled MEK[4]	IC_{50} (μM) LOVO cell
11	0.010	0.67
12	0.009	0.63

IC_{50} values for MEK inhibition represent means of three separate determinations with variations less than 10% of the means.

Table 3. Src kinase and cell growth inhibitory activity of **13** and **14**



13

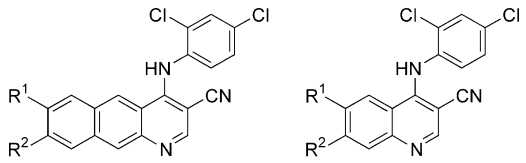


14

Compd	IC_{50} (μM) Src kinase	IC_{50} (μM) Src-transformed fibroblast cell
13	0.0014	0.21
14	0.0040	2.4

IC_{50} values for Src inhibition represent means of three separate determinations with variations less than 20% of the means.

Table 4. Src kinase inhibitory activity of **15–19**



15-18 **19**

Compd	R^1	R^2	IC_{50} (μM)
15	OCH_3	OCH_3	0.018
16	H	OCH_3	30% inh. @ 0.1
17	OCH_3	H	30% inh. @ 0.1
18	OH	OH	10% inh. @ 0.01
19	OCH_3	OCH_3	0.038

IC_{50} values for Src inhibition represent means of at least two separate determinations with variations less than 20% of the means.

The structure–activity relationship of 7- and 8-substitution patterns of this series of tricyclic 3-cyanobenzo[*g*]quinolines against Src kinase activity was explored by comparing compound **15** with its monomethoxy analogues **16** and **17**, and its demethylated analogue **18**. Instead of a 4-chloro-5-methoxy-2-methylanilino, a 2,4-dichloroanilino group was introduced at the 4-position to avoid complications in the last demethylation step in the synthesis of **18**. The activity of these compounds against Src enzyme is shown in Table 4.

Several conclusions can be reached from the Src kinase inhibition results. First, the tricyclic 3-cyanobenzo[*g*]quinoline **15** was again found to be more potent in the enzymatic assay (2×) than its bicyclic 3-cyanoquinoline analogue **19**. As for the anilino group at the 4-position on the tricyclic core, we found the same trend as in the case of the bicyclic 3-cyanoquinoline core,[3b] that is, the 4-chloro-5-methoxy-2-methylanilino substituted compound is more potent than the 2,4-dichloroanilino substituted compound (**13** vs **15**). Compound **15**, with 7,8-dimethoxy substitutions, is more potent than the corresponding mono-methoxy analogues (**16** and **17**) or the 7,8-dihydroxy analogue (**18**). Similar conclusions were reached with the bicyclic 3-cyanoquinoline series,[3a] where 6,7-dimethoxy substitutions were found to be much more active than the corresponding monomethoxy analogues or 6,7-dihydroxy analogue. These similarities in structure–activity relationships strongly suggest that the tricyclic 3-cyanobenzo[*g*]quinolines bind to the Src enzyme in a fashion similar to the bicyclic 3-cyanoquinolines.

In summary, as part of a continuing effort to develop potent and selective kinase inhibitors, we have extended our work on the bicyclic 4-anilino-3-cyanoquinolines by fusing an additional ring onto the 6- and 7-positions of the quinoline core to form the tricyclic 4-anilino-3-cyanobenzo[*g*]quinolines. Presumably, these tricyclic 4-anilino-3-cyanobenzo[*g*]quinolines are competitive inhibitors with ATP as are the bicyclic analogues.[1a,6] The tricyclic core is inferior to the corresponding bicyclic 3-cyanoquinoline core with respect to EGF-R kinase inhibition. For MAPK kinase inhibition, the tricyclic 3-cyanobenzo[*g*]quinoline with a 4-phenoxyanilino group at the 4-position of the quinoline provides activity equivalent to that of the corresponding bicyclic 3-cyanoquinoline. However, the tricyclic 3-cyanobenzo[*g*]quinoline with one of the Src optimal anilino groups at the 4-position is a more potent inhibitor of Src kinase than the corresponding bicyclic 3-cyanoquinoline. SAR studies of the substitutions at the 7- and 8-positions of the tricyclic core on Src kinase inhibition showed patterns similar to the SAR of 6- and 7-substituted bicyclic cyanoquinoline analogues, thus suggesting a similar mode of binding to the enzyme for both the bicyclic and tricyclic cores.

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6. Unpublished results.