# 4-Anilino-6,7-dialkoxyquinoline-3-carbonitrile Inhibitors of Epidermal Growth Factor Receptor Kinase and Their Bioisosteric Relationship to the 4-Anilino-6,7-dialkoxyquinazoline Inhibitors

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The synthesis and SAR of a series of 4-anilino-6,7-dialkoxyquinoline-3-carbonitrile inhibitors of epidermal growth factor receptor (EGF-R) kinase are described. Condensation of 3,4dialkoxyanilines with ethyl (ethoxymethylene)cyanoacetate followed by thermal cyclization gave, regiospecifically, 6,7-dialkoxy-4-oxo-1,4-dihydroquinoline-3-carbonitriles. Chlorination (POCl<sub>3</sub>) followed by the reaction with substituted anilines furnished the 4-anilino-6,7-dialkoxyquinoline-3-carbonitrile inhibitors of EGF-R kinase. An alternate synthesis of these compounds starts with a methyl 3,4-dialkoxybenzoate. Nitration followed by reduction (Fe,  $NH_4Cl$ ,  $MeOH-H_2O$ ) gave a methyl 2-amino-4.5-dialkoxybenzoate. Amidine formation using DMF-acetal followed by cyclization using LiCH<sub>2</sub>CN furnished a 6,7-dialkoxy-4-oxo-1,4-dihydroquinoline-3-carbonitrile, which was transformed as before. Compounds containing acid, ester, amide, carbinol, and aldehyde groups at the 3-position of the quinoline ring were also prepared for comparison, as were several 1-anilino-6,7-dimethoxyisoquinoline-4-carbonitriles. The compounds were evaluated for their ability to inhibit the autophosphorylation of the catalytic domain of EGF-R. The SAR of these inhibitors with respect to the nature of the 6,7-alkoxy groups, the aniline substituents, and the substituent at the 3-position was studied. The compounds were further evaluated for their ability to inhibit the growth of cell lines that overexpress EGF-R or HER-2. It was found that 4-anilinoquinoline-3-carbonitriles are effective inhibitors of EGF-R kinase with activity comparable to the 4-anilinoquinazoline-based inhibitors. A new homology model of EGF-R kinase was constructed based on the X-ray structures of Hck and FGF receptor-1 kinase. The model suggests that with the quinazoline-based inhibitors, the N3 atom is hydrogen-bonded to a water molecule which, in turn, interacts with Thr 830. It is proposed that the quinoline-3-carbonitriles bind in a similar manner where the water molecule is displaced by the cyano group which interacts with the same Thr residue.

## Introduction

Protein tyrosine kinases play a role in normal cell growth. Many of the growth factor receptor proteins have intracellular domains that function as tyrosine kinases, and it is with this that they effect signaling. The interaction of growth factors with these receptors is a necessary event in normal regulation of cell growth. However, under certain conditions, as a result of overexpression, mutation, or coexpression of the ligand and the receptor, these receptors can become hyperactivated; the result of this is uncontrolled cell proliferation.<sup>1</sup> Among the growth factor receptor kinases that have been identified as being important in cancer is epidermal growth factor receptor (EGF-R) kinase (also known as erb-B1 or HER-1) and the related human epidermal growth factor receptor HER-2 (also known as erbB-2 or neu). EGF-R is overexpressed in numerous tumors<sup>2</sup> including those derived from the brain, lung, bladder, head, and neck. Such overexpression has been correlated with poor prognosis in some of these diseases. The gene product is frequently mutated in the extracytoplasmic domain of the receptor in gliomas,<sup>3</sup> prostate cancer,<sup>4</sup> breast cancer,<sup>5</sup> and non-small-cell lung cancer;<sup>6</sup> this mutation makes the receptor constitutively active.<sup>7</sup> In addition, the receptor need not be overexpressed or mutated to be activated, since the ligands for the receptor (EGF or transforming **g**rowth **f**actor- $\alpha$  (TGF $\alpha$ )) can be produced within the same cancerous tissue or cell that expresses EGF-R. This suggests that paracrine or autocrine loops stimulate hyperproliferation as in prostate cancer,<sup>8</sup> head and neck cancer,<sup>9</sup> ovarian cancer,<sup>10</sup> non-small-cell lung cancer,<sup>11</sup> and bladder cancer.<sup>12</sup> EGF-R hyperactivation has also been implicated in other diseases including polycystic kidney disease,<sup>13</sup> psoriasis,<sup>14</sup> and asthma.<sup>15</sup>

Since in many types of proliferative diseases, the phosphorylation event mediated by EGF-R or HER-2 kinases is a necessary signal for cell division to occur and since hyperactivation of these kinases has been associated with these diseases, an inhibitor of this event, an EGF-R or HER-2 kinase inhibitor, may have potential therapeutic value. Since these two receptor kinases have a high sequence homology in their catalytic

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domains,<sup>16</sup> one might expect that an inhibitor of one would have similar effects on the other.

The middle of the past decade was marked by the discovery of the 4-anilinoquinazolines, a series of potent and selective ATP-competitive inhibitors of EGF-R kinase.<sup>17</sup> Several pharmaceutical firms,<sup>18</sup> including ourselves,<sup>19</sup> established programs based on these inhibitors, and now at least three such compounds have entered or will be entering clinical trials.<sup>20</sup> In extending our work with this series of inhibitors, we realized that the nitrogen atom located at the 3-position of these quinazoline inhibitors is an important feature needed for good activity. Replacing this atom with a carbon leads to a significant loss in the ability of the compound to inhibit the enzyme.<sup>21</sup> We reasoned that given the importance of this nitrogen atom for activity, it is likely that it has a specific interaction with the enzyme. Presumably, it functions as a hydrogen bond acceptor. In designing our inhibitors, we made use of a homology model of the enzyme that is similar to the model previously constructed.<sup>22</sup> More recently we have developed a refined model as discussed below. With either model, docking experiments using our inhibitors did not reveal any residues close by to which the N3 atom could directly interact. We therefore made the hypothesis that this nitrogen atom could be interacting with a water molecule and that this water molecule could then serve as a bridge between the drug and enzyme. Such water bridging is a well-known phenomenon.<sup>23</sup> Since the N3 atom of the quinazoline inhibitor, in solution, surely forms a hydrogen bond with a water molecule, retaining this interaction after binding to the enzyme should have a minimal effect on the entropy of binding. In addition, it occurred to us that if this nitrogen atom were removed and replaced with a carbon atom that had an attached electron-withdrawing group (such as a cyano group), such a molecule should retain a similar overall shape and charge distribution compared to the quinazoline hydrogen-bonded to a water molecule. In this communication we would like to report that applying the above concept has resulted in a new series of 4-anilinoquinoline-3-carbonitriles that are potent inhibitors of EGF-R kinase.

## Chemistry

Since earlier work by the Parke-Davis group with the quinazoline-based inhibitors of EGF-R established that 6,7-dialkoxy substitution is compatible with good activity,<sup>24</sup> we decided to retain this feature in our initial compounds. One of the methods we used to prepare the intermediate quinolones 3a-c follows the method of Bredereck<sup>25</sup> as shown in Scheme 1. Condensation of anilines 1a,b with diethyl ethoxymethylenemalonate or ethyl (ethoxymethylene)cyanoacetate followed by thermal cyclization in refluxing Dowtherm gave, regiospecifically, **3a-c**. These quinolones were converted, in good yield, to the corresponding chloroquinolines by refluxing in an excess of POCl<sub>3</sub>. The preparations of  $3c^{25b}$  and  $4a^{26}$  have been described previously. In the final step of the sequence, refluxing a solution of a chloroquinoline and a substituted aniline derivative in ethoxyethanol gave the 4-anilino-6,7-dialkoxyquinoline-3-carbonitriles 5-38 (see Table 1). In most cases, the

Scheme 1<sup>a</sup>



<sup>a</sup> (a) For **2a**:  $C_2H_5OCH=C(CO_2C_2H_5)_2$ , neat, 100 °C; for **2b**,c:  $C_2H_5OCH=C(CO_2C_2H_5)CN$ , toluene, reflux; (b) Dowtherm, 258 °C; (c) POCl<sub>3</sub>, reflux; (d)  $C_2H_5O(CH_2)_2OH$ , reflux; for **35**: NaH, DMF, reflux; (e) (i) NaNO<sub>2</sub>, HOAc, H<sub>2</sub>O, 0 °C, (ii) NaN<sub>3</sub>; (f) (CH<sub>3</sub>CO)<sub>2</sub>O, CH<sub>3</sub>CO<sub>2</sub>H.

products can be isolated as the hydrochloride salt by filtering directly from the reaction mixture, or alternatively, the free base can be isolated after neutralization of the salt with base. Due to the hindered nature of 2-bromoaniline, it was necessary to prepare compound **35** using sodium hydride in refluxing DMF. Compound **40** was prepared by acetylation of **16** with acetic anhydride in acetic acid. Compound **39** was prepared by a two-step process involving formation the diazonium salt of **16** using sodium nitrite and acetic acid followed by the reaction with sodium azide.

An alternate synthesis of some of these compounds is shown in Scheme 2. Compounds containing unsymmetrical 6,7-dialkoxy substitution were prepared from the isomeric methylbenzoates **41a**,**b** by alkylation with ethyl iodide and potassium carbonate in refluxing DMF. Compound **42c** was prepared as already described.<sup>27</sup> Nitration of **42a**-**c** in acetic acid at 50 °C gave the nitro derivatives **43a**-**c**. Reduction using iron and NH<sub>4</sub>Cl in a water-methanol mixture at reflux followed by amidine formation using *N*,*N*-dimethylformamide dimethylacetal in DMF at reflux gave **45a**-**c** which were used without additional purification. The lithium anion of acetonitrile was prepared in THF by the addition of *n*-butyllithium at -78 °C. Addition of the amidines to



<sup>*a*</sup> (a)  $C_2H_5I$ ,  $K_2CO_3$ , DMF, 100 °C; (b) HNO<sub>3</sub>, HOAc, 50 °C; (c) Fe, NH<sub>4</sub>Cl, CH<sub>3</sub>OH, H<sub>2</sub>O, reflux; (d) DMA–DMF, DMF, reflux; (e) (i) *n*-BuLi, CH<sub>3</sub>CN, THF, -78 °C, (ii) HOAc, -78 to 25 °C; (f) for **47a,c**: POCl<sub>3</sub>, reflux; for **47b**: (COCl)<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>, reflux; (g) C<sub>2</sub>H<sub>5</sub>O(CH<sub>2</sub>)<sub>2</sub>OH or C<sub>2</sub>H<sub>5</sub>OH, reflux.

this solution containing 2 equiv of the anion followed by the addition of acetic acid and warming to 25 °C furnished the quinolones 46a-c. These were then converted to the 4-anilino-6,7-dialkoxyquinoline-3-carbonitriles 48-50 as described above.

Compounds containing varying substitution at the 3-position were prepared by simple functional group manipulations as shown in Scheme 3. Reduction of the ester **5** with DIBAL gave the carbinol derivative **51** which, in turn, was oxidized to the aldehyde **52** using pyridinium chlorochromate buffered with sodium acetate in methylene chloride. Base hydrolysis of **5** furnished the carboxylic acid **53** which was then converted to the amide **54** using a two-step procedure consisting of first activating the carboxylate group as the acyl imidazole derivative followed by the addition of ammonia.

Additional variation of the 6,7-alkoxy groups was accomplished as shown in Scheme 3 by first demethylating **8** by heating in neat pyridine hydrochloride at 210 °C to give the 6,7-dihydroxy derivative **55**. Bisalkylation of **55** with ethyl iodide, chloromethyl methyl ether, or bromoethyl methyl ether using  $K_2CO_3$  in refluxing DMF gave compounds **6**, **56**, and **57**, respectively. Compounds **58** and **59** which have, respectively, a fused five- or seven-membered diether ring were prepared by alkylation using bromochloromethane and Scheme 3<sup>a</sup>



 $^a$  (a) DIBAL, toluene; (b) PCC, NaOAc, CH<sub>2</sub>Cl<sub>2</sub>; (c) NaOH, H<sub>2</sub>O, C<sub>2</sub>H<sub>5</sub>OH, reflux; (d) (i) CDl, DMF, (ii) NH<sub>3</sub>; (e) pyridine·HCl, 210 °C; (f) C<sub>2</sub>H<sub>5</sub>I, K<sub>2</sub>CO<sub>3</sub>, DMF; (g) CH<sub>3</sub>OCH<sub>2</sub>Cl, K<sub>2</sub>CO<sub>3</sub>, DMF; (h) CH<sub>3</sub>O(CH<sub>2</sub>)<sub>2</sub>Br, K<sub>2</sub>CO<sub>3</sub>, DMF; (i) BrCH<sub>2</sub>Cl, Cs<sub>2</sub>CO<sub>3</sub>, DMF; (j) Br(CH<sub>2</sub>)<sub>3</sub>Br, K<sub>2</sub>CO<sub>3</sub>, DMF; (k) substituted aniline, isopropanol, reflux.

 $Cs_2CO_3$  in DMF or 1,3-dibromopropane and potassium carbonate in DMF, respectively.

Finally, we were interested in seeing if replacement of the N1 atom of a quinazoline inhibitor with a carbon bearing a cyano group would also lead to an active EGF-R inhibitor. Accordingly, we prepared the 1-anilinoisoquinoline-4-carbonitrile derivatives **61** and **62** from the corresponding known<sup>28</sup> chloro derivative **60** by treating it with the substituted aniline in refluxing 2-propanol.

### **Molecular Modeling**

In a prior publication,<sup>19</sup> we discussed modeling work on EGF-R kinase using a homology model similar to that described by Knighton et al.<sup>22</sup> This model was based on the first crystal structure of a protein kinase, viz. cAMPdependent protein kinase, a serine/threonine kinase. While the sequence homology between cAMP-dependent protein kinase and EGF-R kinase is 41%, the sequence identity is only 27%. Since then, a number of additional kinase structures have been solved, including several tyrosine kinases. We therefore decided to build a homology model of EGF-R kinase using more closely related crystal structures as templates. We used the BLAST server at NCBI to search the Protein Data Bank. The top-ranked structure was fibroblast growth factor receptor-1 kinase,<sup>29</sup> FGF receptor-1 (PDB code: 1fgk), with a sequence identity of 33%. However, several residues in the activation loop are missing from this



Figure 1. Proposed binding models for (a) quinazoline 63 and (b) 3-cyanoquinoline 8 at the ATP site in EGF-R based on the homology model.

structure. Another high-ranked hit in the BLAST search, hematopoietic cell kinase,<sup>30</sup> Hck (PDB code: 1qcf) was also explored as a possible template. However, its alignment with EGF-R involved a major insertion in the glycine loop which is close to the ATP binding site. The resulting model had major distortions in the ATP binding site. We therefore decided to use a combination of these two templates: FGF receptor-1 for the N-terminal lobe and Hck for the C-terminal lobe. We used the homology modeling facility within the Molecular Operating Enviroment (MOE) software to do this. The resulting model was minimized using Quanta/CHARMm and used for subsequent studies.

A recent paper from a group at Glaxo Wellcome<sup>31</sup> revealed the crystal structures of the 4-anilinoquinazoline class of compounds complexed with two kinases: CDK2 and p38-MAP kinase. The binding mode of the quinazoline ring in both structures is similar, the N1 atom of the quinazoline being hydrogen-bonded to the backbone nitrogen of a residue on the domain connector strand (Leu 83 in CDK2 and Met 109 in p38). In the p38 structure, the N3 atom forms a hydrogen bond with a water molecule, which, in turn, makes an interaction with the hydroxyl group of a Thr residue. These two crystal structures strongly suggest that there is a single binding orientation of the quinazoline ring in kinases. We therefore revised our earlier binding model to reflect these new data from the crystal structures. The water molecules found in the Hck structure were added to the homology model. We also used a pentapeptide model substrate AEYLR by positioning it so that the tyrosine hydroxyl is close to the catalytic aspartate (Asp 813) and close to the  $\gamma$ -phosphate of ATP. The enzyme-ATPsubstrate model was built and optimized separately. We then removed the ATP molecule and replaced it with quinazoline inhibitor 63 (PD 153035),<sup>24</sup> using the figures and descriptions of the published X-ray structures as a guide to orient the molecule. The entire complex was

optimized by several cycles of molecular dynamics and minimization using Quanta/CHARMm.

In the final model with the quinazoline **63** (see Figure 1a), the N1 atom of the quinazoline forms a hydrogen bond with the backbone NH of Met 769 and the N3 atom forms a hydrogen bond to a water molecule. This water molecule, in turn, forms a hydrogen bond with the hydroxyl group of Thr 830. The C2 atom of the quinazoline is 3.28 Å from the backbone carbonyl oxygen of Gln 767 and the C8 atom is 4.20 Å from the backbone carbonyl oxygen of Met 769.

The corresponding model with the quinoline-3-carbonitrile **8** (see Figure 1b) was generated in a similar manner. The orientation of the molecule and its interactions with the protein are similar to that observed in the quinazoline model. The hydrogen bond of the N1 atom to the backbone NH of Met 769 is retained. In this model the 3-cyano group displaces the water molecule that was previously hydrogen-bonded to the N3 atom of the quinazoline and instead makes its own interaction with the hydroxyl group of Thr 830.

As indicated by these models, the similar manner in which **63** and **8** bind suggests that the carbon with its attached cyano group (C-CN) of **8** is bioisosteric with the N3 atom of **63** hydrogen-bonded to a water molecule. To lend more credence to this idea, we performed single-point AM1 calculations on **63** and **8** each in their binding conformations as extracted from the models. The results of these calculations are shown in Figure 2. It is evident from these calculations that both systems are similar in terms of their overall shape and the location of charge.

After this work was completed, a report appeared that described a series of quinazoline and benztriazine inhibitors of Scytalone dehydratase along with the 3-cyanoquinoline and 3-cyanocinnoline inhibitors derived from them, respectively.<sup>32</sup> This report compared the X-ray structures of a benztriazine and its corre-



**Figure 2.** (a) Electrostatic potential surfaces (0.005 contour level) calculated for **63** hydrogen-bonded to the water molecule and (b) the corresponding surface for **8**. Single-point calculations of the structures extracted from the binding models were accomplished using the semiempirical AM1 method as implemented in MacSpartan.

sponding 3-cyanocinnoline bound at the active site of this enzyme. Although this protein is not a kinase, the manner in which the molecules bind has relevance to the discussion at hand. In this structure, the N3 atom of the benztriazine forms a hydrogen bond with a crystallographic water molecule. The water molecule, in turn, forms a bridge to the hydroxyl groups of two Tyr residues. In addition, the authors prepared a corresponding 3-cyanocinnoline, and in the resulting X-ray structure, the cyano group occupies a position corresponding to the position of the water molecule in the benztriazine structure where the nitrogen atom of the cyano groups bridges the same two Tyr residues. This is analogous to the situation we are now proposing for the binding of the quinazoline and quinoline-3carbonitrile inhibitors at the ATP binding pocket of EGF-R.

Our homology model of EGF-R differs somewhat from the model proposed by the Parke-Davis group<sup>33</sup> in that while the orientation of the bound quinazoline is similar and has the same interaction between N1 and Met 769, the latter model has a direct hydrogen bond between N3 and the hydroxyl group of Thr 766; it would seem that the EGF-R inhibition activity exhibited by the quinoline-3-carbonitriles, as discussed below, is not easily explained by this latter model.

#### **Results and Discussion**

The compounds shown in Table 1 were evaluated for their ability to inhibit EGF-R kinase. To compare these data with similar data already reported for the quinazoline-based inhibitors, the inhibitory activity of the standard compound, **63**, under our assay conditions, is incorporated into the data set. First, it is immediately evident that the IC<sub>50</sub> value we obtain with **63** for inhibition of EGF-R is significantly higher than was found by previous workers.<sup>24</sup> Additionally, for our compounds, we do not see the extremely large range in potency found by these workers for the quinazoline inhibitors. We attribute these observations to differences in the nature of the enzyme and substrate as well as to the overall assay conditions. We use a solid-phase ELISA-based assay. Our enzyme consists of the purified cytoplasmic domain of EGF-R, and we measure the inhibition of autophosphorylation of this protein. Other workers have measured inhibition using, in a soluble format, the entire enzyme, purified from A431 cells, along with an exogenous peptide substrate. To further address this issue, two compounds, **63** and its corresponding quinoline-3-carbonitrile analogue **8**, were additionally evaluated in EGF-R kinase assays using a soluble format, purified cytoplasmic domain, and exogenous substrate as described previously.<sup>19</sup>

In the ELISA-based EGF-R assay **63** is only about 3 times more potent than **8**. The difference in potency between the two compounds is similar when using the alternate protocol with the same enzyme preparation but in a soluble format and with an exogenous peptide substrate where the IC<sub>50</sub> values for **63** and **8** are 0.0022 and 0.0075  $\mu$ M, respectively. As expected, the IC<sub>50</sub> values are lower in the latter assay. These results establish that the quinoline-3-carbonitriles are potentially comparable to the quinazolines in their ability to inhibit EGF-R kinase.

We were interested in determining the effect on activity of replacing the 3-cyano group with other functionalities. With respect to EGF-R inhibition, replacement with an ester (5), carboxylic acid (53), carbinol (51), or amide (54) group leads to significant (79-fold to greater than 530-fold) loss in potency. While the aldehyde derivative 52 was closest in activity to 8, it is still about 18-fold less potent. A possible rationalization of these data is that one might expect for 5, 53, 54, and 52 that the low-energy conformations would be characterized by an intramolecular hydrogen bond between the carbonyl oxygen of the 3-substituent and the NH group of the aniline moiety. Our binding model would suggest that this hydrogen bond would have to be sacrificed for these compounds to adopt an appropriate binding conformation; this will result in a commensurate loss in binding energy. In addition, the sphybridization of the cyano substituent could result in a more favorably directed interaction with Thr 830 than when the substituents are sp<sup>2</sup>-hybridized.

Since replacement of the N3 atom of the quinazoline with a carbon atom bearing a cyano group is compatible with good activity, it was obviously of interest to see what would happen when the same replacement was made with the N1 atom. Accordingly, we found that the isoquinoline-4-carbonitruiles **61** and **62** are, respectively, 368- and 496-fold less potent than their corresponding quinoline-3-carbonitrile isomers **8** and **9**. This is an expected result in view of the binding model, which indicates a direct interaction of N1 of the quinoline, and quinazoline-based inhibitors with the backbone NH of Met 769.

We surveyed a variety of substituents at different positions on the aniline ring of these quinoline-3carbonitriles. Our molecular model suggests that the aniline ring resides in a large, mostly lipophilic, pocket buried deep within the active site. Good activity is seen when the aniline ring is monosubstituted with a bromine (8) or chlorine (9) atom at the *meta*-position. A similar observation was reported for the quinazoline

#### Table 1. Inhibition of EGF-R Kinase and Inhibition of Cell Proliferation



						IC <sub>50</sub> (μM)			
compd <sup>a</sup>	Х	Y	$\mathbb{R}^1$	$\mathbb{R}^2$	R″	EGF-R <sup>b</sup>	A431 <sup>c</sup>	SKBR3 <sup>c</sup>	SW620 <sup>c</sup>
5	C-CO <sub>2</sub> Et	Ν	CH <sub>3</sub>	CH <sub>3</sub>	3-Br	>81.3	3.01	6.21	6.93
6	C-CN	Ν	$C_2H_5$	$C_2H_5$	3-Br	0.42	0.11	0.09	0.16
7	C-CN	N	$C_2H_5$	$C_2H_5$	3-Cl, 4-F	0.98	0.27	0.49	0.49
8	C-CN	N	$CH_3$	$CH_3$	3-Br	$0.19^{e}$	0.78	0.48	3.72
9	C-CN	Ν	$CH_3$	$CH_3$	3-Cl	0.18	0.59	1.76	7.06
10	C-CN	Ν	$CH_3$	$CH_3$	$3-CF_3$	1.45	2.53	13.42	35.63
11	C-CN	N	$CH_3$	$CH_3$	$3,4$ -di-OCH $_3$	50.49	5.28	13.16	8.76
12	C-CN	N	$CH_3$	$CH_3$	3-F	0.74	3.07	8.29	13.52
13	C-CN	N	$CH_3$	$CH_3$	3-CN	14.18	3.51	25.97	43.29
14	C-CN	N	$CH_3$	CH <sub>3</sub>	4-F	47.94	1.43	0.74	2.87
15	C-CN	N	$CH_3$	$CH_3$	3-COCH <sub>3</sub>	7.38	7.37	5.12	6.30
16	C-CN	N	CH <sub>3</sub>	CH <sub>3</sub>	$3-NH_2$	0.82	6.62	2.45	14.33
17 <sup>a</sup>	C-CN	N	$CH_3$	CH <sub>3</sub>	$3-N(CH_3)_2$	0.85	0.65	0.36	0.99
18	C-CN	N	$CH_3$	CH <sub>3</sub>	$3-NO_2$	0.87	2.14	2.00	17.27
19	C-CN	N	CH <sub>3</sub>	CH <sub>3</sub>	3-Cl, 4-F	0.54	0.70	0.47	2.3
20	C-CN	N	CH <sub>3</sub>	CH <sub>3</sub>	4-CI, 2-F	13.85	0.818	0.54	5.34
21	C-CN	IN N	CH <sub>3</sub>	CH <sub>3</sub>	3-0H	6.36	1.52	0.95	7.8
22	C-CN	IN N	CH <sub>3</sub>	CH <sub>3</sub>	$4-CH_3$	2.73	0.96	0.64	60.43
23	C-CN	IN N	CH <sub>3</sub>	CH <sub>3</sub>	$3-\text{CUNH}_2$	7.69	5.91	8.44	0.29
24 95	C-CN	IN N		CH <sub>3</sub>	$3-D\Gamma, 4-CH_3$	1.79	0.75	0.00	2.89
20 90	C-CN	IN N		CH <sub>3</sub>	3-UI, 4-UH	9.04	3.01	1.29	2.70
<b>20</b> 97d	C-CN C-CN	IN N			5,5-01-01, 4-0H	3.13	5.130	2.017	3.43
<b>2</b> 1 <sup>a</sup> 99d	C CN	IN N			2-CI, 2-UI	17.02	0.94	1.19	3.01
20ª 20d	C-CN C-CN	IN N			3-3CH3 3 CL 4 CU.	0.70	1.431	1.34	4.90
29 20d	C-CN	N	CH <sub>3</sub>	CH <sub>3</sub>	3-01, 4-0113 2 - F - 4 - Br	>01.40	0.93	1 60	24.90
30	C-CN	N	CH <sub>3</sub>	CH <sub>3</sub>	$\Lambda$ -CH(CH <sub>a</sub> ) <sub>a</sub>	0.23	3.02	2 91	3 51
39	C-CN	N	CH <sub>0</sub>	CH <sub>3</sub>	$2 - CH(CH_{0})_{0}$	0.25	13.02	16 12	1 98
33	C-CN	Ň	CH	CH	3-CH(CH <sub>a</sub> ) <sub>a</sub>	62 32	4 35	6 74	3.80
34	C-CN	Ň	CH <sub>2</sub>	CH <sub>2</sub>	4-Br	6.65	0.76	1.47	3.76
35	C-CN	N	CH <sub>3</sub>	CH <sub>3</sub>	2-Br	4.18	21.03	0.80	17.02
<b>36</b> <sup>d</sup>	C-CN	N	CH <sub>3</sub>	CH <sub>3</sub>	3-CF <sub>3</sub> , 4-F	0.16	7.72	0.52	29.53
37	C-CN	N	CH <sub>3</sub>	CH <sub>3</sub>	2-CH <sub>3</sub> , 3-Br	0.84	3.64	0.39	11.83
38	C-CN	Ν	CH <sub>3</sub>	CH <sub>3</sub>	3-CH <sub>3</sub> , 4-Br	0.62	0.73	1.38	3.09
39	C-CN	Ν	$CH_3$	$CH_3$	3-N <sub>3</sub>	2.21	1.48	1.16	4.74
40	C-CN	Ν	$CH_3$	CH <sub>3</sub>	3-NHCOCH <sub>3</sub>	69.40	2.62	1.55	2.74
<b>48</b>	C-CN	Ν	$C_2H_5$	$CH_3$	3-Br	21.19	0.57	0.64	4.24
<b>49</b>	C-CN	Ν	$CH_3$	$C_2H_5$	3-Br	33.02	0.14	0.06	0.06
50	C-CN	Ν	$-CH_2$	$CH_2-$	3-Br	>104.7	0.42	0.27	0.43
51	$C-CH_2OH$	N	$CH_3$	$CH_3$	3-Br	>102.2	7.91	15.36	23.15
52	C-CHO	N	$CH_3$	$CH_3$	3-Br	3.37	1.95	2.97	5.24
53	$C-CO_2H$	Ν	$CH_3$	$CH_3$	3-Br	34.22	18.23	35.71	53.82
54	$C-CONH_2$	Ν	$CH_3$	$CH_3$	3-Br	15.17	4.65	7.78	9.87
55	C-CN	N	Н	H	3-Br	0.35	2.23	2.74	4.52
56	C-CN	N	CH <sub>3</sub> OCH <sub>2</sub>	CH <sub>3</sub> OCH <sub>2</sub>	3-Br	6.75	14.50	14.20	9.79
57	C-CN	N	$CH_3O(CH_2)_2$	$CH_3O(CH_2)_2$	3-Br	1.10	0.71	0.56	4.32
58	C-CN	N	$-CH_2-$		3-Br	6.79	2.62	1.94	4.18
59	C-CN	N	-(CH	$(1_2)_3 - $	3-Br	5.15	2.08	5.07	2.90
61 <sup>a</sup>	N	C-CN	CH <sub>3</sub>	CH <sub>3</sub>	3-Br	70.84	18.76	24.96	48.49
62	N	C-CN	CH <sub>3</sub>	CH <sub>3</sub>	3-CI	88.29	26.13	27.67	64.45
63	N	N	$CH_3$	$CH_3$	3-Br	$0.07^{t}$	1.52	0.82	13.94

<sup>*a*</sup> Unless indicated compounds were tested as the free base. <sup>*b*</sup> Concentration needed to inhibit the autophosphorylation of the cytoplasmic domain of EGF-R by 50% as determined from the dose–response curve. Determinations were done in duplicate and repeat values agreed, on average, within 40%. <sup>*c*</sup> Dose–response curves were determined at five concentrations. The IC<sub>50</sub> values are the concentrations needed to inhibit cell growth by 50% as determined from these curves. <sup>*d*</sup> Compound tested as the HCl salt. <sup>*e*</sup> Using the assay protocol described in ref 19 and purified cytoplasmic domain, the IC<sub>50</sub> for inhibition is 0.0022  $\mu$ M.

series.<sup>24</sup> Moving the bromine atom to the *ortho*- or *para*position as in **35** and **34** results in a 22- and 35-fold decrease, respectively, in the ability of the compound to inhibit EGF-R. Surprisingly, with the weakly electrondonating isopropyl group, this trend is reversed; the *meta*-substituted derivative **33** is by far less potent than either the *para*- or *ortho*-isomers **31** and **32**, respectively. Modeling offers no immediate explanation for this trend. For the *meta*-trifluoromethyl-substituted compounds **10** and **36**, adding an additional *para*-fluoro substituent leads to a 9-fold increase in potency, while for the *meta*-chloro derivatives **9** and **19** this same modification leads to a modest (3-fold) decrease. Compounds **21** and **25–27**, having a hydroxyl group in various positions on the aniline ring, each showed poor activity, while compounds **16** and **17**, which bear amino groups at the *meta*-position, had only a moderate loss in potency.

We made a number of modifications to the 6,7dialkoxy substituents. For both compounds **8** and **19**, replacing 6,7-dimethoxy groups with ethoxy groups (**6** and **7**, respectively) leads, in each case, to a small (2-fold) decrease in EGF-R inhibitory activity. This is in contrast to what was reported in the quinazoline series, where replacing the 6,7-dimethoxy groups with 6,7-diethoxy groups led to a gain in potency.<sup>24</sup> Surprisingly, the 7-ethoxy-6-methoxy and 6-ethoxy-7-methoxy derivatives **48** and **49**, respectively, are both very much less potent than **8**; we can offer no explanation for this result. The 6,7-dihydroxy derivative **55** has comparable activity to **8**. Incorporation of the 6,7-alkoxy substituents into a fused ring as in **50**, **58**, and **59** results in significant loss of activity. Likewise, placing polyether chains at the 6,7-positions as in **56** and **57** decreases potency compared to **8**.

The compounds were also evaluated for their ability to inhibit the growth of certain cell lines. Three human carcinoma cell lines were used: A431 (epidermoid) which highly overexpresses EGF-R, SKBR3 (breast) which highly overexpresses HER-2 and, to a lesser extent, EGF-R, and SW620 (colon) which serves as a control line expressing low levels of EGF-R and HER-2. It is immediately obvious from the data shown in Table 1 that inhibition of cell proliferation exhibited by these compounds is likely the result of multiple mechanisms; cell lines such as SW620 that have little dependence on EGF-R or HER-2 are inhibited. It is possible that this result reflects a lack of selectivity and that these compounds inhibit other kinases. In fact, we know that the selectivity of these compounds for a particular kinase can be modulated by changing the substitution pattern on the aniline ring; this will be the subject of future communications. While multiple kinase activities would make a straightforward interpretation of these results difficult, some trends are evident. There is an approximate correlation between IC<sub>50</sub> values observed for growth inhibition of the A431 and SKBR3 lines. In addition, for the majority of the compounds, we find that they are better inhibitors of proliferation of the A431 and SKBR3 lines than the control SW620 line suggesting that, at least in part, inhibition of proliferation of the these lines is the result of EGF-R or HER-2 kinase inhibition. In comparing the quinoline-3-carbonitrile 8 with its guinazoline counterpart 63, we find that while 8 is about twice as potent as 63 in inhibiting the growth of both A431 and SKBR3 lines, it is also a more potent inhibitor of the SW620 control line. Some of the most potent inhibitors of cell growth are compounds that contain an ethoxy group at the 7-position such as 6, 7, and 49, but these are also some of the least selective inhibitors since they inhibit the control line at comparable levels suggesting that with these compounds, a major component of the inhibition is unrelated to the EGF-R or HER-2 kinase activity.

#### Conclusions

The above results demonstrate that 4-anilino-6,7dialkoxyquinoline-3-carbonitriles are effective inhibitors of EGF-R kinase. The SAR profile exhibited by these compounds is, in a number of respects, similar to that shown by the quinazoline series of EGF-R inhibitors. In addition, we constructed a homology model of EGF-R with which we proposed a binding model for the quinazoline- and quinoline-3-carbonitrile-based inhibitors that is consistent with much of the observed activities. In future reports, we will attempt to show the versatility of this new series of tyrosine kinase inhibitors.

Of additional significance is our proposal that a carbon atom bearing a cyano group can sometimes be bioisosteric with an azomethine group that is hydrogenbonded to a water molecule. Potentially, this concept may have utility in other areas of drug design.

## **Experimental Section**

**Biology. Preparation and purification of EGF-R DNA** constructs: A 1.6-kb cDNA for the EGF-R cytoplasmic domain (amino acids 45-1186) was cloned in baculoviral expression vectors pBlueBacHis2B (Invitrogen, Carlsbad, CA) and pFASTBacHTc (GIBCO, Rockville, MD). A sequence that encodes (His)<sub>6</sub> is located 5' upstream to the EGF-R sequence. Sf-9 cells were infected at moi = 10 for 3 days for protein expression. Sf-9 cell pellets were solubilized at 0 °C in a buffer at pH 7.4 containing 50 mM HEPES, 10 mM NaCl, 1% Triton, 10  $\mu$ M ammonium molybdate, 100  $\mu$ M sodium vanadate, 10  $\mu$ g/mL aprotinin, 10  $\mu$ g/mL leupeptin, 10  $\mu$ g/mL pepstatin, and 16 µg/mL benzamidine HCl for 20 min followed by 20-min centrifugation. Crude extract supernatant was passed through an equilibrated Ni-NTA superflow packed column (Qiagen, Valencia, CA) and washed with 10 and 100 mM imidazole to remove nonspecifically bound material. Histidine-tagged proteins were eluted with 250 and 500 mM imidazole and dialyzed against 50 mM NaCl, 20 mM HEPES, 10% glycerol and 1  $\mu$ g/ mL each of aprotinin, leupeptin and pepstatin for 2 h. The entire purification procedure was performed at 4 °C or on ice. Purified materials were subjected to electrophoresis followed by Coomassie blue stain to determine the purity of the preparation and Western blot for the identity of protein. Enzyme preparations were stored at -80 °C.

EGF-R kinase autophosphorylation assay by DELFIA/ time-resolved fluorometry: The EGF-R kinase assay was set up to assess the level of autophosphorylation based on DELFIA/time-resolved fluorometry. Compounds were dissolved in 100% DMSO and diluted to the appropriate concentrations with 25 mM HEPES at pH 7.4. In each well, 10  $\mu$ L of compound was incubated with  $10 \ \mu L$  of recombinant enzyme (1:80 dilution in 100 mM HEPES) for 10 min at room temperature (>90% EGF-R binds to maxisorp plate within 1 h of incubation). Then, 10  $\mu$ L of 5x buffer (containing 20 mM HEPES, 2 mM MnCl<sub>2</sub>, 100 µM Na<sub>3</sub>VO<sub>4</sub> and 1mM DTT) and 20 µL of 0.1 mM ATP-50 mM MgCl<sub>2</sub> was added and the mixture was incubated for 1 h. Positive and negative controls were included in each plate by incubation of enzyme with or without ATP-MgCl<sub>2</sub>. At the end of incubation, liquid was aspirated and plates were washed three times with wash buffer. A 75- $\mu$ L (400 ng) sample of europium-labeled antiphosphotyrosine antibody was added to each well for another 1 h of incubation. After washing, enhancement solution was added and the signal was detected by Victor (Wallac Inc.) with excitation at 340 nm and emission at 615 nm. All reagents were supplied by Wallac Inc. (Wallac/Perkin-Elimer, Gaithersburg, MD). The percentage of autophosphorylation inhibition by the compound was calculated using the equation: 100 - [(test - negative control)/(positive control - negative control)]. The  $IC_{50}$  was obtained from curves of percentage inhibition with 8 concentrations of compound. As the purity of EGF-R enzyme preparation is greater than 80%, the majority of the signal detected by the anti-phosphotyrosine antibody is from EGF-R. The IC<sub>50</sub> values reported in Table 1 are averages of duplicate determinations.

**Cell proliferation assay:** The cell proliferation assays were done with three human carcinoma cell lines: A431 (epidermoid carcinoma), SKBR3 (breast carcinoma), and SW620 (colon carcinoma), as previously described.<sup>19</sup> All cell lines were obtained from the American Type Culture Collection. Cells were plated in 96-well plates at densities of  $5.0 \times 10^4$ /mL in RPMI-1640 medium supplemented with 5% fetal bovine

serum. On the next day, compounds were dosed at 0.5, 5, 50, 500, and 5000 ng/mL concentrations and the cells were cultured for 2 days. At the end of incubation, cell survival was determined by the sulforhodamine B assay as previously described.<sup>34</sup> The IC<sub>50</sub> values were obtained from the growth curves.

**Molecular Modeling.** Semiempirical calculations (AM1 basis) were carried out on the quinazoline and quinoline-3-carbonitrile compounds. In the quinazoline case, the bound molecule along with the water molecule hydrogen-bonded to the N3 atom were extracted from the modeled complex, and a single-point calculation was carried out. In the quinoline-3-carbonitrile case, only the bound compound was extracted and used for the single-point calculation. The electrostatic potential was mapped onto the surface (electron density contoured at the 0.005 level) and color-coded with red representing negative potential and blue representing positive potential. These calculations were done using MacSpartan Plus (Wavefunction Inc., 18401 Von Karman, Suite 370, Irvine, CA 92612).

Homology modeling was done using the MOE (Molecular Operating Environment) software (Chemical Computing Group Inc., 1255 University St., Suite 1600, Montreal, Quebec, Canada H3B 3X). Initial crude minimization was done within the homology modeling function of MOE. However, the resulting model needed further energy minimization. This was done using Quanta/CHARMm (Molecular Simulations Inc., 9685 Scranton Rd., San Diego, CA 92121). The crude model from MOE was minimized with a few thousand cycles of minimization using the ABNR (adopted-basis Newton–Raphson) method.

Ligands were modeled by positioning them in the active site in accordance with the published crystal structures of quinazoline derivatives bound to CDK2 and MAP kinase (p38).<sup>31</sup> The entire complex was then subjected to alternate cycles of minimization and dynamics. Each dynamics run was short, about 3 ps. The intent was to get a satisfactory structure for the complex that was consistent with the published crystal structure.

**Chemistry.** <sup>1</sup>H NMR spectra were determined with a NT-300 WB spectrometer at 300 MHz. Chemical shifts ( $\delta$ ) are expressed in parts per million relative to the internal standard tetramethylsilane. Electrospray mass spectra were recorded in positive mode on a Micromass Platform spectrometer. Electron impact and high-resolution mass spectra were obtained on a Finnigan MAT-90 spectrometer. Chromatographic purifications were by flash chromatography using Baker 40- $\mu$ m silica gel. Melting points were determined in open capillary tubes on a Meltemp melting point apparatus and are uncorrected.

**2-Cyano-3-(3,4-dimethoxyphenylamino)acrylic** Acid Ethyl Ester (2c). A mixture of 3,4-dimethoxyaniline (30.6 g, 200 mmol), ethyl (ethoxymethylene)cyanoacetate (33.8 g, 200 mmol), and 80 mL of toluene was stirred at 100 °C for 1 h and at 125 °C for 15 min. After evaporation of the toluene, the residue was recrystallized from EtOAc to give 40.0 g (72%) of a tan solid: mp 166–170 °C; MS (ES+) *m/z* 277.2 (M + H)<sup>+1</sup>. Anal. ( $C_{14}H_{16}N_2O_4$ ) C, H, N.

**6,7-Dimethoxy-4-oxo-1,4-dihydroquinoline-3-carbonitrile (3c).** A stirred mixture of **2c** (40 g, 145 mmol) and 1.2 L of Dowtherm A was heated at reflux under N<sub>2</sub> for 10 h. After cooling to 50 °C the mixture was diluted with hexane. The product was filtered off, washed with hexane followed by methylene chloride, and dried to give 21.1 g (63%) of a brown solid: mp 330–350 °C dec; <sup>1</sup>H NMR (DMSO-*d*<sub>*\nethylelow*})  $\delta$  12.57 (s, 1H), 8.59 (s, 1H), 7.44 (s, 1H), 7.03 (s, 1H), 3.89 (s, 3H), 3.87 (s, 3H); MS (ES+) *m*/*z* 231.0 (M + H)<sup>+1</sup>. Anal. (C<sub>12</sub>H<sub>10</sub>N<sub>2</sub>O<sub>3</sub>) C, H, N.</sub>

**4-Chloro-6,7-dimethoxyquinoline-3-carbonitrile (4c).** A stirred mixture of **3c** (20 g, 87 mmol) and 87 mL of POCl<sub>3</sub> was heated at reflux for 2 h. Volatile materials were removed under vacuum at about 70 °C. The residue was stirred at 0 °C with methylene chloride and H<sub>2</sub>O as solid K<sub>2</sub>CO<sub>3</sub> was carefully added until the pH was 8–9. After stirring for 30 min at 25 °C the organic layer was separated, washed with H<sub>2</sub>O, dried, filtered through Celite, and concentrated to give 19.8 g (92%)

of an off-white solid. A sample recrystallized from  $CH_2Cl_2$  gave an off-white solid: mp 220–223 °C; <sup>1</sup>H NMR (DMSO- $d_{\dot{e}}$ )  $\delta$  8.98 (s, 1H), 7.54 (s, 1H), 7.42 (s, 1H), 4.02 (s, 3H), 4.01 (s, 3H).

**4-(3-Bromophenylamino)-6,7-dimethoxyquinoline-3carboxylic Acid Ethyl Ester (5).** A stirred mixture of **4a**<sup>26</sup> (14.8 g, 50 mmol), 3-bromoaniline (9.46 g, 55 mmol), pyridine (4.05 mL, 50 mmol), and 150 mL of EtOH was heated at reflux for 30 min. The EtOH was evaporated, and the residue was partitioned between  $CH_2Cl_2$  and aqueous NaHCO<sub>3</sub>. The organic layer was washed with water, dried over MgSO<sub>4</sub>, and concentrated. The product was recrystallized from EtOH to give 15.4 g (71%) of a white solid: mp 155–158 °C; <sup>1</sup>H NMR (DMSO- $d_{cl}$ )  $\delta$  9.44 (s, 1H), 8.82 (s, 1H), 7.37 (s, 1H), 7.30 (s, 1H), 7.25–6.90 (m, 5H), 4.03 (q, J = 7.0 Hz, 2H), 3.95 (s, 3H), 3.71 (s, 3H), 1.16 (t, J = 7.0 Hz, 2H); MS (ES+) m/z 431.2, 433.2 (M + H)<sup>+1</sup>. Anal. ( $C_{20}H_{19}BrN_2O_4$ ) C, H, N.

4-(3-Bromophenylamino)-6,7-diethoxyquinoline-3-carbonitrile (6). To a stirred mixture of 55 (5.34 g, 15 mmol), K<sub>2</sub>CO<sub>3</sub> (8.29 g, 60 mmol), and 60 mL of DMF at 0 °C was added 4.8 mL (60 mmol) of iodoethane. The resulting mixture was warmed to 25 °C and stirred for 4.5 h. The reaction mixture was partitioned between EtOAc and H<sub>2</sub>O as 4N HCl was added slowly with cooling until the pH reached about 8. The organic layer was separated, washed well with H<sub>2</sub>O, dried and concentrated. The residue was subjected to flash chromatography on silica gel with 60:30:1 CH<sub>2</sub>Cl<sub>2</sub>-EtOAc-TEA to give the product (3.27 g, 53%); homogeneous on TLC with the above solvent system. Recrystallization from EtOAc gave a white solid: mp 173-175 °C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 9.50 (s, 1H), 8.54 (s, 1H), 7.67 (s, 1H), 7.35 (s, 1H), 7.55-7.15 (m, 4H), 4.35-4.10 (m, 4H), 1.45-1.35 (m, 6H); MS (ES+) m/z 412.3, 414.2  $(M + H)^{+1}$ . Anal.  $(C_{20}H_{18}BrN_3O_2)$  C, H, N.

**4-(3-Bromophenylamino)-6,7-dimethoxyquinoline-3carbonitrile (8).** A stirred mixture of **4c** (0.40 g, 1.61 mmol), 3-bromoaniline (0.28 g, 1.61 mmol), and 15 mL of 2-ethoxyethanol was heated at reflux for 3 h and cooled to 25 °C. The crude HCl salt was filtered off and washed with 2-propanol and Et<sub>2</sub>O: yield 0.62 g (92%). The free base was obtained by partition between EtOAc and aqueous Na<sub>2</sub>CO<sub>3</sub>. The organic layer was dried (MgSO<sub>4</sub>) and solvent was removed to give 0.58 g of crude product. Recrystallization (EtOAc–hexane) gave a white solid: mp 224–228 °C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  9.55 (s, 1H), 8.55 (s, 1H), 7.69 (s, 1H), 7.41 (s, 1H), 7.45–7.15 (m, 4H), 3.96 (s, 3H), 3.92 (s, 3H); MS (ES+) *m*/*z* 384.1, 386.1 (M + H)<sup>+1</sup>. Anal. (C<sub>18</sub>H<sub>14</sub>BrN<sub>3</sub>O<sub>2</sub>) C, H, N, Br.

**4-(3-Chloro-4-fluorophenylamino)-6,7-diethoxyquinoline-3-carbonitrile (7).** The compound was prepared in 88% yield according to the procedure for **8** from 4-chloro-6,7diethoxyquinoline-3-carbonitrile (**4b**) and 3-chloro-4-fluoroaniline: mp 194–198 °C (EtOAc); <sup>1</sup>H NMR (DMSO- $d_6$ )  $\delta$  9.50 (s, 1H), 8.47 (s, 1H), 7.70 (s, 1H), 7.54–7.25 (m, 3H), 7.32 (s, 1H), 4.26–4.15 (m, 4H), 1.42 (t, 6H, J = 6.0 Hz); MS (ES+) m/z 386.2 (M + H)<sup>+1</sup>. Anal. (C<sub>20</sub>H<sub>17</sub>ClFN<sub>3</sub>O<sub>2</sub>) C, H, N.

**4-(3-Chlorophenylamino)-6,7-dimethoxyquinoline-3-carbonitrile (9).** The compound was prepared in 55% yield according to the procedure for **8** using 3-chloroaniline: mp 214–217 °C (EtOAc-hexane); <sup>1</sup>H NMR (DMSO-*d<sub>d</sub>*)  $\delta$  9.66 (s, 1H), 8.58 (s, 1H), 7.58 (s, 1H), 7.39 (s, 1H), 7.52–7.20 (m, 4H), 3.97 (s, 3H), 3.92 (s, 3H); MS (ES+) *m*/*z* 340.1, 342.2 (M + H)<sup>+1</sup>. Anal. (C<sub>18</sub>H<sub>14</sub>ClN<sub>3</sub>O<sub>2</sub>) C, H, N.

**6,7-Dimethoxy-4-(3-trifluoromethylphenylamino)quinoline-3-carbonitrile (10).** The compound was prepared in 72% yield according to the procedure for **8** using 3-trifluoromethylaniline: mp 190–193 °C (EtOAc–hexane); <sup>1</sup>H NMR (DMSO- $d_{\theta}$ )  $\delta$  9.67 (s, 1H), 8.58 (s, 1H), 7.71 (s, 1H), 7.65– 7.45 (m, 4H), 7.39 (s, 1H), 3.97 (s, 3H), 3.92 (s, 3H); MS (ES+) m/z 374.2 (M + H)<sup>+1</sup>. Anal. (C<sub>19</sub>H<sub>14</sub>F<sub>3</sub>N<sub>3</sub>O<sub>2</sub>) C, H, N.

**4-(3,4-Dimethoxyphenylamino)-6,7-dimethoxyquinoline-3-carbonitrile (11).** The compound was prepared in 66% yield according to the procedure for **8** using 3,4-dimethoxyaniline: mp 230–240 °C (EtOAc–hexane); <sup>1</sup>H NMR (DMSO $d_{cl}$ )  $\delta$  9.45 (s, 1H), 8.36 (s, 1H), 7.98 (s, 1H), 7.30 (s, 1H), 7.00– 6.83 (m, 3H), 3.94 (s, 3H), 3.92 (s, 3H) 3.78 (s, 3H), 3.76 (s, H); MS (ES+) *m*/*z* 366.3 (M + H)<sup>+1</sup>. Anal. (C<sub>20</sub>H<sub>19</sub>N<sub>3</sub>O<sub>4</sub>) C, H, N. **4-(3-Fluorophenylamino)-6,7-dimethoxyquinoline-3-carbonitrile (12).** The compound was prepared in 74% yield according to the procedure for **8** using 3-fluoroaniline: mp 226–230 °C (EtOAc); <sup>1</sup>H NMR (DMSO- $d_{\theta}$ )  $\delta$  9.57 (s, 1H), 8.54 (s, 1H), 7.70 (s, 1H), 7.37 (s, 1H), 7.45–6.95 (m, 4H), 3.96 (s, 3H), 3.92 (s, 3H); MS (ES+) *m*/*z* 324.3 (M + H)<sup>+1</sup>. Anal. (C<sub>18</sub>H<sub>14</sub>-FN<sub>3</sub>O<sub>2</sub>) C, H, N.

**4-(3-Cyanophenylamino)-6,7-dimethoxyquinoline-3carbonitrile (13).** The compound was prepared in 89% yield according to the procedure for **8** using 3-cyanoaniline: mp 285–288 °C; <sup>1</sup>H NMR (DMSO- $d_6$ )  $\delta$  9.66 (s, 1H), 8.58 (s, 1H), 7.58 (s, 1H), 7.75–7.50 (m, 4H), 7.39 (s, 1H), 3.97 (s, 3H), 3.92 (s, 3H); MS (ES+) *m*/*z* 331.3 (M + H)<sup>+1</sup>. Anal. (C<sub>19</sub>H<sub>14</sub>N<sub>4</sub>O<sub>2</sub>· 0.5H<sub>2</sub>O) C, H, N.

**4-(4-Fluorophenylamino)-6,7-dimethoxyquinoline-3carbonitrile (14).** The compound was prepared in 91% yield according to the procedure for **8** using 4-fluoroaniline: mp 282–285 °C (EtOAc); <sup>1</sup>H NMR (DMSO- $d_{\theta}$ )  $\delta$  9.49 (s, 1H), 8.42 (s, 1H), 7.76 (s, 1H), 7.40–7.23 (m, 4H), 7.32 (s, 1H), 3.95 (s, 3H), 3.92 (s, 3H); MS (ES+) *m*/*z* 324.3 (M + H)<sup>+1</sup>. Anal. (C<sub>18</sub>H<sub>14</sub>-FN<sub>3</sub>O<sub>2</sub>) C, H, N.

**4-(3-Acetylphenylamino)-6,7-dimethoxyquinoline-3carbonitrile (15).** The compound was prepared in 69% yield according to the procedure for **8** using 3-acetylaniline: mp 204–206 °C (acetone–hexane); <sup>1</sup>H NMR (DMSO- $d_6$ )  $\delta$  9.62 (s, 1H), 8.53 (s, 1H), 7.77 (d, J = 1.7 Hz, 1H), 7.74 (s, 1H), 7.65– 7.45 (m, 4H), 3.96 (s, 3H), 3.92 (s, 3H), 2.61 (s, 3H); MS (ES+) m/z 347.9 (M + H)<sup>+1</sup>. Anal. (C<sub>20</sub>H<sub>17</sub>N<sub>3</sub>O<sub>3</sub>) C, H, N.

**4-(3-Aminophenylamino)-6,7-dimethoxyquinoline-3carbonitrile (16).** A stirred mixture of **4c** (3.73 g, 15.0 mmol), 1,3-phenylenediamine (4.86 g, 45 mmol), pyridine (1.21 mL, 15 mmol), and 45 mL of 2-ethoxyethanol was heated at reflux for 30 min, cooled to 25 °C, and stirred with aqueous NaHCO<sub>3</sub>. The resulting solid was filtered, washed with H<sub>2</sub>O, and dried. Recrystallization from EtOH gave 4.32 g (90%) of a brown solid: mp 222–228 °C; <sup>1</sup>H NMR (DMSO-*d<sub>0</sub>)*  $\delta$  9.28 (s, 1H), 8.43 (s, 1H), 7.74 (s, 1H), 7.31 (s, 1H), 6.99 (m, 1 H) 6.43 (m, 3H), 5.21 (bs, 2H) 3.94 (s, 3H), 3.90 (s, 3H); MS (ES+) *m/z* 321.0 (M + H)<sup>+1</sup>. Anal. (C<sub>18</sub>H<sub>16</sub>N<sub>4</sub>O<sub>2</sub>·0.5H<sub>2</sub>O) C, H, N.

**4-(3-Dimethylaminophenylamino)-6,7-dimethoxyquinoline-3-carbonitrile (17).** The compound was prepared according to the procedure for **8** using *N*,*N*-dimethyl-1,3-phenylenediamine dihydrochloride. The product was recrystallized from water to give 0.4 g (18%) of a yellow solid: mp 246–249 °C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  8.88 (s, 1H), 8.12 (s, 1H), 7.48 (s, 1H), 7.28 (m, 1H), 6.78 (m, 2H), 6.70 (d, 1H, *J* = 11.49), 3.99 (s, 3H), 3.98 (s, 3H), 2.94 (s, 6H). MS (ES+) *m*/*z* 349.2 (M + H)<sup>+1</sup>, 174.9 (M + 2H)<sup>+2</sup>. Anal. (C<sub>20</sub>H<sub>20</sub>N<sub>4</sub>O<sub>2</sub>·HCl·0.75H<sub>2</sub>O) C, H, N.

**4-(3-Nitrophenylamino)-6,7-dimethoxyquinoline-3-carbonitrile (18).** The compound was prepared according to the procedure for **8** using 3-nitroaniline. Recrystallization from EtOH gave a 69% yield of **18** as yellow crystals: mp 221–222 °C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  3.92 (s, 3H), 3.98 (s, 3H), 7.42 (s, 1H), 7.65 (m, 3H), 7.96 (m, 2H), 8.64 (s, 1H), 9.79 (s, 1H); MS (ES+) *m/z* 351.0 (M + H)<sup>+1</sup>. Anal. (C<sub>18</sub>H<sub>14</sub>N<sub>4</sub>O<sub>4</sub>) C, H, N.

**4-(3-Chloro-4-fluorophenylamino)-6,7-dimethoxyquinoline-3-carbonitrile (19).** The compound was prepared in 87% yield according to the procedure for **8** using 3-chloro-4fluoroaniline: mp 223–225 °C; <sup>1</sup>H NMR (DMSO- $d_6$ )  $\delta$  9.65 (s, 1H), 8.52 (s, 1H), 7.75 (s, 1H), 7.62–7.28 (m, 3H), 7.35 (s, 1H), 3.96 (s, 3H), 3.93 (s, 3H); MS (ES+) *m*/*z* 358.2 (M + H)<sup>+1</sup>. Anal. (C<sub>18</sub>H<sub>13</sub>ClFN<sub>3</sub>O<sub>2</sub>) C, H, N.

**4-(4-Chloro-2-fluorophenylamino)-6,7-dimethoxyquinoline-3-carbonitrile (20).** Prepared in 52% yield according to the procedure for **8** using 4-chloro-2-fluoroaniline: mp 184– 186 °C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  9.49 (br s, 1H), 8.47 (s, 1H), 7.79 (s, 1H), 7.60 (dd, *J* = 10.7, 2.1 Hz, 1H), 7.48 (t, *J* = 8.7 Hz, 1H), 7.35 (m, 2H), 3.96 (s, 3H), 3.94 (s, 3H); MS (ES+) *m*/*z* 358 (M + H)<sup>+1</sup>. Anal. (C<sub>18</sub>H<sub>13</sub>ClFN<sub>3</sub>O<sub>2</sub>) C, H, N.

**4-(3-Hydroxyphenylamino)-6,7-dimethoxyquinoline-3carbonitrile (21).** Prepared (91% yield) according to the procedure for **8** using 3-hydroxyaniline: mp > 250 °C; <sup>1</sup>H NMR (DMSO- $d_6$ )  $\delta$  3.90 (s, 3H), 3.95 (s, 3H), 6.58–6.66 (m, 3H), 7.17 (t, J = 8.25 Hz, 1H) 7.33 (s, 1H), 7.72 (s, 1H), 8.46 (s, 1H), 9.37 (s, 1H), 9.52 (s, 1H). Anal. (C<sub>18</sub>H<sub>15</sub>N<sub>3</sub>O<sub>3</sub>·0.2H<sub>2</sub>O) C, H, N.

**4-(4-Methylphenylamino)-6,7-dimethoxyquinoline-3carbonitrile (22)**. Prepared in 88% yield according to the procedure for **8** using 4-methylaniline: mp 128–130 °C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  2.33 (s, 3H), 3.91 (s, 3H), 3.94 (s, 3H), 7.15 (d, J = 6.1, 2H Hz), 7.22 (d,, J = 6.1 Hz, 2H), 7.32 (s, 1H), 7.76 (s, 1H), 8.41 (s, 1H), 9.40 (s, 1H). Anal. (C<sub>19</sub>H<sub>17</sub>N<sub>3</sub>O<sub>2</sub>· 0.5H<sub>2</sub>O) C, H, N.

**3-(3-Cyano-6,7-dimethoxyquinolin-4-ylamino)benzamide (23).** Prepared in 89% yield according to the procedure for **8** using 3-aminobenzamide: mp 253–255 °C; <sup>1</sup>H NMR (DMSO- $d_6$ )  $\delta$  9.56 (br s, 1H), 8.51 (s, 1H), 8.02 (s, 1H), 7.76 (s, 2H), 7.71 (d, J = 7.8 Hz, 1H), 7.47 (t, J = 7.8 Hz, 1H), 7.41 (s, 1H), 7.38 (d, J = 7.8 Hz, 1H), 7.36 (s, 1H), 3.96 (s, 3H), 3.92 (s, 3H); MS (ES+) m/z 349 (M + H)<sup>+1</sup>. Anal. (C<sub>19</sub>H<sub>16</sub>N<sub>4</sub>O<sub>3</sub>· 0.3HCl) C, H, N.

**4-(3-Bromo-4-methylphenylamino)-6,7-dimethoxyquinoline-3-carbonitrile (24).** Prepared in 72% yield according to the procedure for **8** using 3-bromo-4-methylaniline: mp 292–294 °C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  11.05 (br s, 1H), 8.10 (s, 1H), 7.71 (d, J = 2.1 Hz, 1H), 7.48 (d, J = 8.3 Hz, 1H), 7.44 (s, 1H), 7.38 (dd, J = 8.3, 2.1 Hz, 1H), 4.00 (s, 3H), 3.98 (s, 3H), 2.41 (s, 3H); MS (ES+) m/z 398 (M + H)<sup>+1</sup>. Anal. (C<sub>19</sub>H<sub>16</sub>-BrN<sub>3</sub>O<sub>2</sub>·0.3H<sub>2</sub>O) C, H, N.

**4-(3-Chloro-4-hydroxyphenylamino)-6,7-dimethoxyquinoline-3-carbonitrile (25).** Prepared in 65% yield according to the procedure for **8** using 4-amino-2-chlorophenol: mp 230–232 °C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  10.29 (br s, 1H), 9.39 (br s, 1H), 8.37 (s, 1H), 7.76 (s, 1H), 7.31 (d, J = 2.4 Hz, 1H), 7.30 (s, 1H), 7.12 (dd, J = 8.4, 2.8 Hz, 1H), 6.99 (d, J = 8.4Hz, 1H), 3.94 (s, 3H), 3.92 (s, 3H); MS (ES+) *m/z* 356 (M + H)<sup>+1</sup>. Anal. (C<sub>19</sub>H<sub>16</sub>BrN<sub>3</sub>O<sub>2</sub>•0.4EtOAc) C, H, N.

**4-(3,5-Dichloro-4-hydroxyphenylamino)-6,7-dimethoxyquinoline-3-carbonitrile (26).** By the procedure as described above for **8** using 3,5-dichloro-4-hydroxyaniline, the title compound was obtained in 89% yield: mp > 250 °C; <sup>1</sup>H NMR (DMSO-*d<sub>6</sub>*)  $\delta$  3.96 (s, 3H), 3.98 (s, 3H), 7.37 (s, 1H), 7.49 (s, 2H), 7.94 (s, 1H), 8.75 (s, 1H), 10.34 (bs,1H), 10.40 (bs, 1H); MS (ES+) *m*/*z* 389.8, 391.8 (M + H)<sup>+1</sup>. Anal. (C<sub>18</sub>H<sub>13</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>3</sub>· 1.0H<sub>2</sub>O) C, H, N.

**4-(5-Chloro-2-hydroxyphenylamino)-6,7-dimethoxyquinoline-3-carbonitrile Hydrochloride (27)**. By the procedure as described above for **8**, using 5-chloro-2-hydroxyaniline, the title compound was obtained in 83% yield as the hydrochloride salt directly from the reaction mixture: mp >  $250 \,^{\circ}$ C; <sup>1</sup>H NMR (DMSO- $d_6$ )  $\delta$  3.98 (s, 3H), 3.99 (s, 3H), 7.02 (d,  $J = 9 \,\text{Hz}$ , 1H), 7.33 (dd,  $J = 2.7, 8.7 \,\text{Hz}$ , 1H), 7.41 (s, 1H), 7.44 (d,  $J = 2.7 \,\text{Hz}$ , 1H), 8.07 (s, 1H), 8.90 (s, 1H), 10.51 (bs, 1H), 10.63 (bs, 1H); MS (ES+) m/z 355.8, 357.8 (M + H)<sup>+1</sup>. Anal. (C<sub>18</sub>H<sub>14</sub>ClN<sub>3</sub>O<sub>3</sub>·0.9HCl) C, H, N.

**6,7-Dimethoxy-4-(3-methylthiophenylamino)quinoline-3-carbonitrile Hydrochloride (28).** By the procedure as described above for **8**, using 3-methylthioaniline, the title compound was obtained in 35% yield as the hydrochloride salt directly from the reaction mixture: mp > 250 °C; <sup>1</sup>H NMR (DMSO-*d<sub>d</sub>*)  $\delta$  2.51 (s, 3H), 3.98 (s, 3H), 4.00 (s, 3H), 7.21 (d, *J* = 8.3 Hz, 1H), 7.29 (d, *J* = 8.1 Hz, 1H), 7.34 (s, 1H), 7.41 (dd, *J* = 7.0, 7.8 Hz, 1H), 7.45 (s, 1H), 8.10 (s, 1H), 8.96 (s, 1H), 11.10 (bs, 1H); MS (ES+) *m*/*z* 351.9 (M + H)<sup>+1</sup>. Anal. (C<sub>19</sub>H<sub>17</sub>N<sub>3</sub>O<sub>2</sub>S-1HCl) C, H, N.

**4-(3-Chloro-4-methylphenylamino)-6,7-dimethoxyquinoline-3-carbonitrile Hydrochloride (29).** By the procedure as described above for **8**, using 3-chloro-4-methylaniline, the title compound was obtained in 90% yield as the hydrochloride salt directly from the reaction mixture: mp > 250 °C; <sup>1</sup>H NMR (DMSO- $d_{\dot{e}}$ )  $\delta$  2.39 (s, 3H), 4.00 (s, 3H), 4.02 (s, 3H), 7.34 (dd, J = 2.13, 8.1 Hz, 1H), 7.43 (s, 1H), 7.48 (d, J = 8.3 Hz, 1H), 7.56 (d, J = 2.0 Hz, 1H), 8.09 (s, 1H), 8.96 (s, 1H), 10.98 (bs, 1H); MS (ES+) m/z 353.9, 355.8 (M + H)<sup>+1</sup>. Anal. (C<sub>19</sub>H<sub>16</sub>-ClN<sub>3</sub>O<sub>2</sub>·1HCl) C, H, N.

4-(4-Bromo-2-fluorophenylamino)-6,7-dimethoxyquinoline-3-carbonitrile Hydrochloride (30). By the procedure as described above for 8, using 4-bromo-2-fluoroaniline, the title compound was obtained in 52% yield as the hydrochloride salt directly from the reaction mixture: mp > 250 °C;  $^{1}\rm H$  NMR (DMSO- $d_{cl}$   $\delta$  4.00 (s, 6H), 7.46 (s, 1H), 7.58 (m, 2H), 7.83 (d, J=9.21 Hz, 1H), 8.16(s, 1H), 9.00 (s, 1H), 11.00 (bs, 1H); MS (ES+) m/z 410.8, 403.8 (M + H)+1. Anal. (C18H13BrFN3O2• 1HCl•0.2H2O) C, H, N.

**4-(4-Isopropylphenylamino)-6,7-dimethoxyquinoline-3-carbonitrile (31).** Prepared in 79% yield according to the procedure for **8** using 4-isopropylaniline giving a pale yellow solid: mp 198–200 °C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  9.41 (s, 1H), 8.42 (s, 1H), 7.72 (s, 1H), 7.32 (s, 1H), 7.27 (d, 2H, *J* = 8.4 Hz, 2H), 7.17 (d, 2H, *J* = 8.4 Hz, 2H), 3.94 (s, 3H), 3.89 (s, 3H), 2.50 (m, 1H), 1.21 (d, *J* = 6.9 Hz, 6H); HRMS *m*/*z* 348.1705 (M + H)<sup>+1</sup>. Anal. (C<sub>21</sub>H<sub>21</sub>N<sub>3</sub>O<sub>2</sub>•0.2H<sub>2</sub>O) C, H, N.

**4-(2-Isopropylphenylamino)-6,7-dimethoxyquinoline-3-carbonitrile (32).** Prepared in 60% yield according to the procedure for **8** using 2-isopropylaniline giving a gray solid: mp 90–91 °C; <sup>1</sup>H NMR (DMSO- $d_{cl}$ )  $\delta$  9.35 (s, 1H), 8.30 (s, 1H), 7.87 (s, 1H), 7.42 (m, 2H), 7.30 (s, 1H), 7.26 (m, 2H), 3.95 (s, 3H), 3.91 (s, 3H), 2.50 (m, 1H), 1.21 (d, J = 6.9 Hz, 6H); HRMS m/z 348.1705 (M + H) <sup>+1</sup>. Anal. (C<sub>21</sub>H<sub>21</sub>N<sub>3</sub>O<sub>2</sub>·0.6H<sub>2</sub>O) C, H, N.

**4-(3-Isopropylphenylamino)-6,7-dimethoxyquinoline-3-carbonitrile (33).** Prepared in 70% yield according to the procedure for **8** using 3-isopropylaniline giving a yellow solid: mp 160–162 °C; <sup>1</sup>H NMR (DMSO-*d<sub>d</sub>*)  $\delta$  9.47 (s, 1H), 8.45 (s, 1H), 7.75 (s, 1H), 7.33 (s, 1H), 7.32 (t, *J* = 7.9 Hz, 1H), 7.11 (s, 1H), 7.08 (m, 2H), 3.93 (d, *J* = 13.3 Hz, 6H), 2.50 (m, 1H); HRMS *m*/*z* 348.1705 (M + H) <sup>+1</sup>. Anal. (C<sub>21</sub>H<sub>21</sub>N<sub>3</sub>O<sub>2</sub>·0.2H<sub>2</sub>O) C, H, N.

**4-(4-Bromophenylamino)-6,7-dimethoxyquinoline-3carbonitrile (34).** The compound was prepared according to the procedure for **8** using 4-bromoaniline in 47% yield, light yellow solid: mp 199–200 °C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  9.49 (s, 1H), 8.51 (s, 1H), 7.71 (s, 1H), 7.56 (d, *J* = 9 Hz, 2H), 7.36 (s, 1H), 7.19 (d, *J* = 9 Hz, 2H), 3.95 (s, 3H), 3.91 (s, 3H); MS (ES+) *m*/*z* 383.8, 385.8 (M + H)<sup>+1</sup>. Anal. (C<sub>18</sub>H<sub>14</sub>BrN<sub>3</sub>O<sub>2</sub>·0.5H<sub>2</sub>O) C, H, N.

**4-(2-Bromophenylamino)-6,7-dimethoxyquinoline-3carbonitrile (35).** To a suspension of NaH (60% in mineral oil, 90 mg, 2.25 mmol) in 5 mL of DMF was added 2-bromoaniline (387 mg, 2.25 mmol). The mixture was heated to reflux then cooled and **4c** (250 mg, 1.05 mmol) was added. The mixture was heated at reflux for 40 min then cooled to room temperature and partitioned between water and ethyl acetate. The organic layer was washed with dilute aqueous ammonium hydroxide, dried over MgSO<sub>4</sub>, filtered and concentrated in vacuo. EtOAc and diethyl ether were added to the residue and the solid was collected to provide 168 mg (43%) of **35** as a tan solid: mp 217–219 °C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  9.58 (s, 1H), 8.38 (s, 1H), 7.85 (s, 1H), 7.76 (d, *J* = 6 Hz, 1H), 7.58–7.42 (m, 2H), 7.36–7.32 (m, 2H), 3.95 (s, 3H), 3.93 (s, 3H); MS (ES+) *m/z* 383.8, 385.8 (M + H)<sup>+1</sup>. Anal. (C<sub>18</sub>H<sub>14</sub>BrN<sub>3</sub>O<sub>2</sub>) C, H, N.

**4-(4-Fluoro-3-trifluoromethylphenylamino)-6,7dimethoxyquinoline-3-carbonitrile Hydrochloride (36).** The compound was prepared as a hydrochloride salt in 72% yield according to the procedure for **8** using 4-fluoro-3trifluoromethylaniline: mp 240–243 °C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  10.21 (broad s, 1H), 8.69 (s, 1H), 7.88 (s, 1H), 7.79–7.69 (m, 2H), 7.64–7.57 (m, 1H), 7.39 (s, 1H), 3.98 (s, 3H), 3.96 (s, 3H); MS (ES+) *m*/*z* 391.9 (M + H)<sup>+1</sup>. Anal. (C<sub>19</sub>H<sub>13</sub>F<sub>4</sub>N<sub>3</sub>O<sub>2</sub>•0.6HCl) C, H. N.

**4-(3-Bromo-2-methylphenylamino)-6,7-dimethoxyquinoline-3-carbonitrile (37).** By the procedure as described above for **8** using 3-bromo-2-methylaniline, the title compound was obtained in 42% yield: mp 183–185 °C; <sup>1</sup>H NMR (DMSO- $d_6$ )  $\delta$  2.28 (s, 3H), 3.93 (s, 3H), 3.94 (s, 3H), 7.21 (dd, J = 7.92, 7.89 Hz, 1H), 7.31 (d, J = 7.70, 1H), 7.33 (s, 1H), 7.63 (d, J = 7.62 Hz, 1H), 7.82 (s, 1H), 8.37 (s, 1H), 9.49 (bs, 1H); MS (ES+) m/z 397.8, 399.8 (M + H)<sup>+1</sup>. Anal. (C<sub>19</sub>H<sub>16</sub>BrN<sub>3</sub>O<sub>2</sub>·0.21C<sub>6</sub>H<sub>14</sub>) C, H. N.

**4-(4-Bromo-3-methylphenylamino)-6,7-dimethoxyquinoline-3-carbonitrile (38).** By the procedure as described above for **8** using 4-bromo-3-methylaniline, the title compound was obtained in 61% yield: mp 168–169 °C; <sup>1</sup>H NMR (DMSO-  $d_{6}$   $\delta$  2.35 (s, 3H), 3.91 (s, 3H), 3.95 (s, 3H), 7.01 (dd, J = 2.67, 8.52 Hz, 1H), 7.24 (d, J = 2.55, 1H), 7.36 (s, 1H), 7.55 (d, J = 8.49 Hz, 1H), 7.72 (s, 1H), 8.51 (s, 1H), 9.46 (bs, 1H); MS (ES+) m/z 397.8, 399.8 (M + H)^+1. Anal. (C19H16BrN3O2  $\cdot$  0.26C6H14  $\cdot$  0.1H2O) C, H, N.

4-(3-Azidophenylamino)-6,7-dimethoxyquinoline-3-carbonitrile (39). Compound 16 (0.643 g, 2.00 mmol) was dissolved in 25 mL of 80% aqueous acetic acid and chilled to 0 °C. A solution of 0.152 g (2.21 mmol) of NaNO2 in 2.2 mL of H<sub>2</sub>O was added, followed 10 min later by a solution of 0.144 g (2.21 mmol) of NaN<sub>3</sub> in 2.2 mL of H<sub>2</sub>O. Volatile material was removed after the reaction had stirred at 25  $^\circ C$  for 1.5 h. The crude product was dissolved in EtOAc and filtered and the filtrate was treated with saturated NaHCO3. The organic layer was separated, washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated. The residue was filtered through silica gel (40% CH<sub>2</sub>-Cl<sub>2</sub> in EtOAc). The eluent was evaporated and the product was dried in vacuo (50 °C) to give 0.526 g (76%) of product as brown crystals: <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 3.91 (s, 3H), 3.96 (s, 3H), 6.98 (m, 3H), 7.43 (m, 2H), 7.69 (s, 1H), 8.54 (s, 1H), 9.55 (s, 1H); HRMS (CI) m/z (M + H)<sup>+1</sup> calcd for C<sub>18</sub>H<sub>15</sub>N<sub>6</sub>O<sub>2</sub> 347.1256, found 347.1255. Anal. (C18H14N6O2) C, H, N.

**N-[3-(3-Cyano-6,7-dimethoxyquinolin-4-ylamino)phenyl]acetamide (40).** A solution of **16** (0.96 g, 3.0 mmol) in 9 mL of HOAc was treated with 0.85 mL of Ac<sub>2</sub>O at 25 °C. After 2 h the solution was evaporated to dryness at 40 °C, stirred in MeOH, and evaporated to dryness. The residue was recrystallized from EtOH to give 0.50 g (46%) of a brown solid: mp 147–150 °C; <sup>1</sup>H NMR (DMSO-*d<sub>d</sub>*)  $\delta$  10.00 (s, 1H), 9.48 (s, 1H), 8.48 (s, 1H), 7.75 (s, 1H), 7.66 (s, 1H), 7.34 (s, 1H), 7.29 (m, 2H), 6.89 (m, 1H), 3.96 (s, 3H), 3.91 (s, 3H), 2.05 (s, 3H); MS (ES+) *m/z* 363.2 (M + H)<sup>+1</sup>. Anal. (C<sub>20</sub>H<sub>18</sub>N<sub>4</sub>O<sub>3</sub>· 1.5H<sub>2</sub>O) C, H, N.

**3-Ethoxy-4-methoxybenzoic Acid Methyl Ester (42a).** A mixture of 24.3 g (134 mmol) of 3-hydroxy-4-methoxybenzoic acid methyl ester, 36.8 g (267 mmol) of K<sub>2</sub>CO<sub>3</sub> and 16 mL (31.4 g, 201 mmol) of C<sub>2</sub>H<sub>5</sub>I in 500 mL of DMF was stirred at 100 °C for 6 h. An additional 16 mL (31.4 g, 201 mmol) of C<sub>2</sub>H<sub>5</sub>I and 18.4 g (133 mmol) of K<sub>2</sub>CO<sub>3</sub> was added and the reaction was heated for 3 h more. The reaction was then filtered, the insoluble material was washed with DMF and the combined filtrate and wash were evaporated. The residue was mixed with H<sub>2</sub>O and dried. Recrystallization from heptane gave 22.5 g (80%) of product as white crystals: mp 81–83 °C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  1.34 (t, *J* = 6.9 Hz, 3H), 3.81 (s, 3H), 3.84 (s, 3H), 4.04 (q, *J* = 6.9 Hz, 2H), 7.07 (d, *J* = 8.5 Hz, 1H), 7.42 (d, *J* = 2.0, 1H), 7.58 (dd, *J* = 8.5, *J* = 2.0 Hz, 1H); MS (ES+) *m*/*z* 210.9 (M + H) <sup>+1</sup>. Anal. (C<sub>11</sub>H<sub>14</sub>O<sub>4</sub>) C, H.

**4-Ethoxy-3-methoxybenzoic Acid Methyl Ester (42b).** Using the procedure described above for **42a** and a total reaction time of 2 h gave, after recrystallization from hexane, 82% of product as white crystals: <sup>1</sup>H NMR (DMSO- $d_6$ )  $\delta$  1.35 (t, 3H, J = 7.0), 3.81 (s, 3H), 3.82 (s, 3H), 4.09 (q, J = 7.0 Hz, 2H), 7.05 (d, J = 8.5 Hz, 1H), 7.44 (d, J = 1.95 Hz, 1H), 7.56 (dd, J = 8.5, 1.95 Hz, 1H); MS (EI) m/z 210 (M)<sup>+1</sup>.

**5-Ethoxy-4-methoxy-2-nitrobenzoic Acid Methyl Ester** (43a). Concentrated HNO<sub>3</sub> (15 mL) was slowly added to a slurry of 15.0 g (71.4 mmol) of 42a in 45 mL of glacial acetic acid. After an exotherm had subsided, the reaction was reheated to 50-60 °C for 45 min. It was then poured into ice H<sub>2</sub>O and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The extracts were washed with H<sub>2</sub>O and 0.5 M NaOH, dried (MgSO<sub>4</sub>) and evaporated to give 17.8 g (98% crude yield) of product as yellow crystals. An analytical sample was obtained from CH<sub>2</sub>Cl<sub>2</sub>–hexane: mp85–88 °C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  1.36 (t, *J* = 7.0 Hz, 3H), 3.83 (s, 3H), 3.91 (s, 3H), 4.18 (q, 2H, *J* = 7.0 Hz, 2H), 7.30 (s, 1H), 7.63 (s, 1H); MS (ES+) *m*/*z* 256.0 (M + H)<sup>+1</sup>. Anal. (C<sub>11</sub>H<sub>13</sub>-NO<sub>6</sub>) C, H, N.

**4-Ethoxy-5-methoxy-2-nitrobenzoic Acid Methyl Ester** (**43b**). The compound was prepared from **42b** using the procedure described above for **43a** and a reaction time of 1 h. A crude yield of 87% of the product was obtained. A purified sample (light yellow crystals) was obtained by recrystallization from heptane: <sup>1</sup>H NMR (DMSO- $d_6$ )  $\delta$  1.36 (t, J = 7.02 Hz, 3H), 3.83 (s, 3H), 3.92 (s, 3H), 4.18 (q, J = 7.02 Hz, 2H), 7.32 (s, 1H), 7.61 (s, 1H); MS (ES+) m/z 255.8 (M + H)<sup>+1</sup>.

**7-Nitro-2,3-dihydrobenzo[1,4]dioxine-6-carboxylic Acid Methyl Ester (43c).** This compound was prepared from **42c**<sup>27</sup> using the procedure described for **43a** and a reaction time of 3 h at 70 °C. Recrystallization from heptane–toluene gave 91% of product as yellow crystals: <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  3.80 (s, 3H), 4.39 (s, 4H), 7.32 (s, 1H), 7.66 (s, 1H); MS (EI) *m*/*z* 239 (M)<sup>+1</sup>.

2-Amino-5-ethoxy-4-methoxybenzoic Acid Methyl Ester (44a). A mixture of 17.0 g (66.7 mmol) of 43a, 13.1 g (233 mmol) of powdered Fe and 17.7 g (334 mmol) of NH<sub>4</sub>Cl in 95 mL of H<sub>2</sub>O and 245 mL of MeOH was heated at reflux for 4.5 h. More Fe (13.1 g, 233 mmol) was added and the reaction was heated for 2.5 h. Additional amounts of Fe (13.1 g, 233 mmol) and NH<sub>4</sub>Cl (17.7 g, 334 mmol) were then added and heating was continued for 12 h more. The reaction was filtered and the insoluble material was washed with H<sub>2</sub>O and MeOH. The combined filtrate and wash was evaporated and the residue was partitioned between CHCl<sub>3</sub> and H<sub>2</sub>O. The organic material was treated with activated carbon, dried (MgSO<sub>4</sub>), evaporated and dried in vacuo (50 °C) to give 11.0 g (73% crude yield) of product as tan crystals. An analytical sample was prepared by recrystallization from EtOH: mp 120-122 °C; 1H NMR (DMŠO- $d_6$ )  $\delta$  1.26 (t, J = 5.1 Hz, 3H), 3.74 (s, 6H), 3.85 (q, 2H, J = 5.1 Hz), 6.36 (s, 1H), 6.44 (s, 2H), 7.14 (s, 1H); MS(ES+) m/z 225.9 (M + H)<sup>+1</sup>. Anal. (C<sub>11</sub>H<sub>15</sub>NO<sub>4</sub>) C, H, N.

**2-Amino-4-ethoxy-5-methoxybenzoic Acid Methyl Ester (44b).** This compound was prepared from **43b** in a manner similar to **44a**. Additional powdered Fe and NH<sub>4</sub>Cl were added as necessary to drive the reaction to completion. The crude yield was 83% as pink crystals. A purified sample was prepared by recrystallization from Et<sub>2</sub>O: <sup>1</sup>H NMR (DMSO- $d_6$ )  $\delta$  1.34 (t, J = 6.99 Hz, 3H), 3.64 (s, 3H), 3.74 (s, 3H), 3.98 (q, 2H, J = 6.99 Hz), 6.35 (s, 1H), 6.42 (s, 2H), 7.12 (s, 1H); MS (ES+) m/z 226.2 (M + H)<sup>+1</sup>.

**7-Amino-2,3-dihydrobenzo[1,4]dioxine-6-carboxylic Acid Methyl Ester (44c).** This compound was prepared from **43c** in a manner similar to **44a**. Additional powdered Fe and NH<sub>4</sub>Cl were added to complete the reaction. Filtration of the crude product through silica (CHCl<sub>3</sub>) helped to remove colored impurities. Solids washed with MeOH to give a 90% yield of product as tan crystals: <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  3.75 (s, 3H), 4.13 (m, 2H), 4.23 (m, 2H), 6.24 (s, 3H), 7.14 (s, 1H); MS (ES+) *m*/*z* 209.9 (M + H)<sup>+1</sup>.

**6-Ethoxy-7-methoxy-4-oxo-1,4-dihydroquinoline-3-carbonitrile (46a).** A solution of 10.2 g (45.3 mmol) of **44a** and 12.6 mL (10.8 g, 90.7 mmol) of dimethylformamide dimethylacetal in 50 mL of DMF was heated at reflux under N<sub>2</sub> for 2.3 h. Volatile material was removed and the residue was azeotroped twice with toluene and dried in vacuo (25 °C). The formamidine product **45a** was a purple syrup that crystallized on standing.

A solution of 5.32 mL (4.18 g, 102 mmol) of CH<sub>3</sub>CN in 80 mL of THF was added over 15 min to a solution of 40 mL (100 mmol) of 2.5 M n-BuLi in hexane and 60 mL of THF at -78 °C under N<sub>2</sub>. After 20 min, a solution of the crude 45a in 80 mL of THF was added over 0.5 h. The reaction was stirred at -78 °C for 1 h and then guenched with 13 mL (13.7 g, 227 mmol) of glacial acetic acid. The mixture was warmed to 25 °C and volatile material was removed. The residue was slurried with H<sub>2</sub>O, collected and dried. This material was washed twice with CHCl<sub>3</sub> to give 7.95 g of product as yellow crystals. An analytical sample (cream crystals) was obtained by boiling a portion of the crude product in MeOH and drying in vacuo (50 °C): mp > 310 °C; <sup>1</sup>H NMR (DMSO- $d_6$ )  $\delta$  1.38 (t, J = 6.96 Hz, 3H), 3.89 (s, 3H), 4.11 (q, J = 6.96 Hz, 2H), 7.04 (s, 1H), 7.43 (s, 1H), 8.59 (s, 1H); MS (ES+) m/z 243.2 (M + H)<sup>+1</sup>. Anal. ( $C_{13}H_{12}N_2O_3$ ) C, H, N.

**7-Ethoxy-6-methoxy-4-oxo-1,4-dihydroquinoline-3-car-bonitrile (46b).** Starting with **44b** and using the procedure described for **46a** a crude yield of 83% (yellow solid) of the title compound was obtained: <sup>1</sup>H NMR (DMSO- $d_6$ )  $\delta$  1.40 (t,

J=6.96 Hz, 3H), 3.86 (s, 3H), 4.12 (q, J=6.96 Hz, 2H), 7.03 (s, 1H), 7.45 (s, 1H), 8.59 (s, 1H); MS (ES+)  $m\!/z$  245.2 (M + H)^{+1}.

**9-Oxo-2,3,6,9-tetrahydro[1,4]dioxino[2,3-***g***]quinoline-8-carbonitrile (46c).** Starting with **44c** and using the procedure described for **46a** a crude yield of 72% (tan powder) of the title compound was obtained: <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  4.34 (m, 2H), 4.37 (m, 2H), 7.05 (s, 1H), 7.46 (s, 1H), 8.58 (s, 1H); MS (ES+) *m*/*z* 228.8 (M + H)<sup>+1</sup>.

**4-Chloro-6-ethoxy-7-methoxyquinoline-3-carbonitrile (47a).** A slurry of 12.9 g (52.7 mmol) of **46b** in 200 mL of CH<sub>2</sub>Cl<sub>2</sub> under N<sub>2</sub> was treated with 23.1 mL (33.5 g, 263 mmol) of oxalyl chloride and 1 mL of DMF for 2 days. The reaction was then diluted with CHCl<sub>3</sub> and mixed with saturated aqueous NaHCO<sub>3</sub>. The organic layer was separated, dried (MgSO<sub>4</sub>), evaporated and dried in vacuo. The crude yield was 86% of a gray solid: <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  1.43 (t, 3H, *J* = 6.99 Hz), 4.02 (s, 3H), 4.27 (q, 2H, *J* = 6.99 Hz), 7.43 (s, 1H), 7.55 (s, 1H), 8.99 (s, 1H); MS (ES+) *m*/*z* 262.8, 264.8 (M + H)<sup>+1</sup>.

**4-Chloro-7-ethoxy-6-methoxyquinoline-3-carbonitrile (47b).** This compound was prepared from **46b** and oxalyl chloride as previously described. The crude yield was 86% of a gray solid: <sup>1</sup>H NMR (DMSO- $d_6$ )  $\delta$  1.44 (t, 3H, J = 6.96 Hz), 4.02 (s, 3H), 4.30 (q, 2H, J = 6.96 Hz), 7.46 (s, 1H), 7.54 (s, 1H), 8.99 (s, 1H); MS (ES+) m/z 262.8, 264.8 (M + H)<sup>+1</sup>.

**9-Chloro-2,3-dihydro[1,4]dioxino[2,3-g]quinoline-8-carbonitrile (47c).** This compound was prepared from **46c** and POCl<sub>3</sub> as previously described for **4c**. The crude yield was 97% of a tan solid: <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  4.48 (m, 4H), 7.55 (s, 1H), 7.57 (s, 1H), 8.94 (s, 1H); MS (ES+) *m*/*z* 246.8, 248.8 (M + H)<sup>+1</sup>.

**4-(3-Bromophenylamino)-6-ethoxy-7-methoxyquinoline-3-carbonitrile (48).** This compound was prepared from **47a** and 3-bromoaniline as previously described for **8**. Recrystallization from EtOH gave an 86% yield of tan crystals: mp 190– 191.5 °C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  1.40 (t, 3H, *J* = 6.99 Hz), 3.96 (s, 3H), 4.16 (q, 2H, *J* = 6.99 Hz), 7.22 (m, 1H), 7.34 (m, 3H), 7.40 (s, 1H), 7.67 (s, 1H), 8.54 (s, 1H), 9.50 (s, 1H); HRMS (EI) *m/z* calcd for C<sub>19</sub>H<sub>16</sub>BrN<sub>3</sub>O<sub>2</sub> 397.0426, found 397.0429. Anal. (C<sub>19</sub>H<sub>16</sub>BrN<sub>3</sub>O<sub>2</sub>·EtOH) C, H, N.

**4-(3-Bromophenylamino)-7-ethoxy-6-methoxyquinoline-3-carbonitrile (49).** This compound was prepared from **47b** and 3-bromoaniline as previously described for **8**. The crude yield was 73% of a tan solid: <sup>1</sup>H NMR (DMSO- $d_6$ )  $\delta$  1.42 (t, J = 6.93 Hz, 3H), 3.91 (s, 3H), 4.25 (q, J = 6.93 Hz, 2H), 7.22 (m, 1H), 7.33 (m, 3H), 7.40 (s, 1H), 7.68 (s, 1H), 8.54 (s, 1H), 9.54 (s, 1H); HRMS (EI) *m*/*z* calcd for C<sub>19</sub>H<sub>16</sub>Br N<sub>3</sub>O<sub>2</sub> 397.0426, found 397.0436. Anal. (C<sub>19</sub>H<sub>16</sub>BrN<sub>3</sub>O<sub>2</sub>·0.5H<sub>2</sub>O) C, H, N.

**9-(3-Bromophenylamino)-2,3-dihydro[1,4]dioxino[2,3***g*]quinoline-8-carbonitrile (50). This compound was prepared from **47c** and 3-bromoaniline as previously described for **8**. Recrystallization from EtOAc gave a 65% yield of tan crystals: <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  4.42 (m, 4H), 7.21 (m, 1H), 7.33 (m, 3H), 7.41 (s, 1H), 7.86 (s, 1H), 8.50 (s, 1H), 9.57 (s, 1H); HRMS (FAB+) *m*/*z* (M + H)<sup>+1</sup> calcd for C<sub>18</sub>H<sub>13</sub>BrN<sub>3</sub>O<sub>2</sub> 382.0191, found 382.0215. Anal. (C<sub>18</sub>H<sub>12</sub>BrN<sub>3</sub>O<sub>2</sub>·0.3H<sub>2</sub>O) C, H, N.

**[4-(3-Bromophenylamino)-6,7-dimethoxyquinolin-3-yl]methanol (51).** To a stirred mixture of **5** (1.45 g, 3.36 mmol) and 67 mL of toluene at 0 °C was added 1.53 M diisobutylaluminum hydride in toluene (11 mL, 16.8 mmol) during 10 min. After an additional 10 min, the solution was treated with 7 mL of MeOH at 0 °C then stirred at 25 °C for 10 min. The resulting gel was diluted with THF and stirred with Na<sub>2</sub>SO<sub>4</sub>· 10H<sub>2</sub>O for 30 min. Solids were removed by filtration, and the filtrate was concentrated. The residue was recrystallized from CH<sub>2</sub>Cl<sub>2</sub>-hexane to give 0.56 g (43%) of a white solid: mp 176– 180 °C; <sup>1</sup>H NMR (DMSO-*d<sub>6</sub>)*  $\delta$  8.76 (s, 1H), 8.44 (s, 1H), 7.38 (s, 1H), 7.17 (s, 1H), 7.15–6.60 (m, 4H), 5.31 (t, *J* = 5.3 Hz, 1H), 4.49 (d, *J* = 5.3 Hz, 2H), 3.93 (s, 3H), 3.73 (s, 3H); MS (ES+) *m/z* 389.1, 391.1 (M + H)<sup>+1</sup>. Anal. (C<sub>18</sub>H<sub>17</sub>BrN<sub>2</sub>O<sub>3</sub>) C, H, N.

4-(3-Bromophenylamino)-6,7-dimethoxyquinoline-3-

**carbaldehyde (52).** To a stirred solution of **51** (3.20 g, 8.22 mmol) in 82 mL of CH<sub>2</sub>Cl<sub>2</sub> were added NaOAc (0.20 g, 2.5 mmol) and pyridinium chlorochromate (2.65 g, 12.3 mmol). After 2 h at 25 °C the mixture was stirred at 0 °C with 1 N NaOH (50 mL). After filtration through Celite the CH<sub>2</sub>Cl<sub>2</sub> layer was washed with water, dried, and concentrated. The residue was chromatographed on silica gel with CH<sub>2</sub>Cl<sub>2</sub>–EtOAc to give 1.92 g (60%) of a white solid after recrystallization from EtOAc: mp 175–177 °C; <sup>1</sup>H NMR (DMSO-d<sub>∂</sub>)  $\delta$  10.07 (s, 1H), 10.05 (s, 1H), 8.83 (s, 1H), 7.38 (s, 1H), 7.27 (s, 1H), 7.14 (s, 1H), 7.30–7.08 (s, 3H), 3.96 (s, 3H), 3.60 (s, 3H); MS (ES+) m/z 387.2, 389.2 (M + H)<sup>+1</sup>. Anal. (C<sub>18</sub>H<sub>15</sub>BrN<sub>2</sub>O<sub>3</sub>) C, H, N, Br.

**4-(3-Bromophenylamino)-6,7-dimethoxyquinoline-3carboxylic Acid (53).** A mixture of 5 (0.86 g, 2.0 mmol), 10 N NaOH (1.0 mL, 10 mmol), and 20 mL of EtOH was stirred at 25 °C for 2 h. The resulting solution was concentrated to remove EtOH, and the residue was dissolved in H<sub>2</sub>O and acidified with NaH<sub>2</sub>PO<sub>4</sub>. The resulting white solid was filtered off, washed with water, and dried to give 0.81 g (100%) of product: mp 282–285 °C dec; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  14.0 (bs, 1H), 9.12 (s, 1H), 7.64 (s, 1H), 8.30–7.60 (m, 3H), 7.47 (s, 1H), 7.45 (s, 1H), 7.26 (s, 1H), 4.01 (s, 6H); MS (ES+) *m/z* 403.1, 405.1 (M + H)<sup>+1</sup>. Anal. (C<sub>18</sub>H<sub>15</sub>BrN<sub>2</sub>O<sub>4</sub>) C, H, N.

**4-(3-Bromophenylamino)-6,7-dimethoxyquinoline-3carboxylic Acid Amide (54).** A mixture of **53** (4.03 g, 10 mmol), 1,1'-carbonyldiimidazole (3.24 g, 20 mmol), and 100 mL of DMF was stirred at 55 °C for 30 min. The resulting solution was cooled to 0 °C, saturated with NH<sub>3</sub> gas, and stirred at 25 °C for 45 min. The residue obtained after evaporation of solvents was stirred in H<sub>2</sub>O. The solid was filtered, washed with H<sub>2</sub>O, dried, and recrystallized from acetone to give a gray solid (4.0 g, 100%): mp 239–242 °C; <sup>1</sup>H NMR (DMSO-*d<sub>i</sub>*)  $\delta$  10.1 (s, 1H), 8.84 (s, 1H), 8.15 (s, 1H), 7.61 (s, 1H), 7.36 (s, 1H), 7.25–6.80 (m, 5H), 3.94 (s, 6H); MS (ES+) *m/z* 402.1, 404.2 (M + H)<sup>+1</sup>. Anal. (C<sub>18</sub>H<sub>16</sub>BrN<sub>3</sub>O<sub>3</sub>) C, H, N, Br.

**4-(3-Bromophenylamino)-6,7-dihydroxyquinoline-3carbonitrile (55).** A 5.11 g (13.3 mmol) quantity of **8** and 30.74 g (266 mmol) of pyridine hydrochloride were mixed and then heated in an oil bath at 207 °C giving a melt. This was heated for 1 h, then cooled and treated with 100 mL of water. The resulting solid was filtered and digested with 2-methoxy ethanol. This gave 3.0 g (63%) of a gray solid: mp 270–275 °C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  12.09 (s, 1H, broad), 10.86 (s, 1H), 10.49 (s, 1H, broad), 8.94 (s, 1H), 7.94 (s, 1H), 7.68 (s, 1H), 7.57 (m, 2H), 7.48 (m, 2H); MS (ES+) *m*/*z* 356.1, 358.1 (M + H)<sup>+1</sup>. Anal. (C<sub>16</sub>H<sub>10</sub>N<sub>3</sub>)<sub>2</sub>Br·1.0H<sub>2</sub>O) C, H, N.

**4-(3-Bromophenylamino)-6,7-bis(methoxymethoxy)quinoline-3-carbonitrile (56).** This compound was prepared in 32% yield using chloromethyl methyl ether according to the procedure for **6**, except that the reaction was run entirely at 0 °C: mp 169–177 °C (EtOAc); <sup>1</sup>H NMR (DMSO- $d_d$ )  $\delta$  9.92 (s, 1H), 8.37 (s, 1H), 7.80 (bs, 1H), 7.25 (s, 1H), 7.20–6.80 (m, 4H), 5.44 (s, 2H), 5.31(s, 2H), 3.44 (s, 3H); 3.27 (s, 3H); MS (ES+) m/z 444.0, 446.0 (M + H)<sup>+1</sup>. Anal. (C<sub>20</sub>H<sub>18</sub>BrN<sub>3</sub>O<sub>4</sub>) C, H, N.

**4-(3-Bromophenylamino)-6,7-bis(2-methoxyethoxy)quinoline-3-carbonitrile (57).** This compound was prepared in 24% yield using bromoethyl methyl ether according to the procedure for **6**, except the reaction was run at 50 °C: mp 135– 138 °C (MeOH); <sup>1</sup>H NMR (DMSO- $d_{\theta}$ )  $\delta$  9.50 (s, 1H), 8.54 (s, 1H), 7.71 (s, 1H), 7.40 (s, 1H), 7.36–7.05 (m, 4H), 4.33–4.23 (m, 4H), 3.77–3.73 (m, 4H), 3.35–3.30 (m, 6H); MS (ES+) *m*/*z* 472.0, 473.9 (M + H)<sup>+1</sup>. Anal. (C<sub>22</sub>H<sub>22</sub>BrN<sub>3</sub>O<sub>4</sub>) C, H, N.

**8-(3-Bromophenylamino)**[1,3]dioxolo[4,5-g]quinoline-7-carbonitrile (58). A solution of 2.17 g (6.09 mmol) of 55, 0.59 mL (1.18 g, 9.14 mmol) of bromochloromethane, and 2.98 g (9.14 mmol) of  $Cs_2CO_3$  in 20 mL of DMF was stirred and heated in an oil bath at 111 °C for 2 h. The reaction was poured into 75 mL of water and the resulting mixture was extracted with four 50-mL portions of  $CH_2Cl_2$ . The combined extracts were dried over anhydrous MgSO<sub>4</sub>, then concentrated with the addition of hexanes until an oil precipitated. This oil was separated and taken up in ethyl acetate, and this solution was washed with several portion of water, then with brine. After drying over anhydrous MgSO<sub>4</sub>, the solution was evaporated in vacuo to a solid. This solid was suspended in CH<sub>2</sub>Cl<sub>2</sub> and poured into hexanes. The product was collected to give 0.95 g (42%) of a tan solid: mp 201–205 °C; <sup>1</sup>H NMR (DMSO-*d<sub>6</sub>*)  $\delta$  9.44 (s, 1H), 8.57 (s, 1H), 7.74 (s, 1H), 7.33 (m, 4H), 7.16 (m, 1H), 6.28 (s, 2H); MS (ES+) *m*/*z* 368.1, 370.1 (M + H)<sup>+1</sup>. Anal. (C<sub>17</sub>H<sub>10</sub>N<sub>3</sub>O<sub>2</sub>Br) C, H, N, Br.

**4-(3-Bromophenylamino)-3,4-dihydro-2***H***-[1,4]dioxepino[2,3-***g***]quinoline-3-carbonitrile (59). To a stirred mixture of 55 (0.71 g, 2.0 mmol), K\_2CO\_3 (1.10 g, 8.0 mmol), and 8 mL of DMF at 65 °C was added a solution of 1,3-dibromopropane (0.20 mL, 2.0 mmol) during 30 min. After an additional 30 min at 65 °C the DMF was evaporated, and the residue was partitioned between CH<sub>2</sub>Cl<sub>2</sub> and H<sub>2</sub>O. After filtration, the CH<sub>2</sub>-Cl<sub>2</sub> layer was washed with H<sub>2</sub>O, dried, and concentrated. The residue chromatographed on silica gel, eluting the product with 60:30:1 CH<sub>2</sub>Cl<sub>2</sub>-EtOAc-HOAc to give 0.32 g (40%) of white solid: mp 120-130 °C; <sup>1</sup>H NMR (DMSO-***d<sub>0</sub>)* **\delta 9.68 (s, 1H), 8.55 (s, 1H), 8.02 (s, 1H), 7.45-7.12 (m, 5H), 7.35 (s, 1H), 4.40-4.24 (m, 4H), 2.30 (s, 3H), 2.24-2.19 (m, 2H); MS (ES+)** *m***/***z* **396.1, 398.1 (M + H)<sup>+1</sup>. Anal. (C<sub>19</sub>H<sub>14</sub>BrN<sub>3</sub>O·HO<sub>2</sub>CCH<sub>3</sub>) C, H, N.** 

**1-(3-Bromophenylamino)-6,7-dimethoxyisoquinoline-4-carbonitrile (61).** A mixture of 6 mmol (0.65 mL, 1.03 g) of 3-bromoaniline and 5 mmol (1.25 g) of **60**<sup>28</sup> in 10 mL of 2-propanol was stirred and heated at reflux in an oil bath at 114 °C for 23 h. On cooling, the product was collected and washed with cold 2-propanol and ether giving 2.04 g (97%) of the product: mp 291–293 °C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  10.11 (t, J = 0.5 Hz, 1H), 8.42 (s, 1H), 8.06 (d, J = 12.2 Hz, 1H), 8.03 (d, J = 1.6 Hz, 1H), 7.80 (m, 1H), 7.36 (m, 2H), 7.17 (s, 1H), 4.02 (s, 3H), 4.01 (s, 3H); MS (ES+) *m*/*z* 384.1, 386.2 (M + H)<sup>+1</sup>. Anal. (C<sub>18</sub>H<sub>14</sub>N<sub>3</sub>O<sub>2</sub>Br·1.0HCl) C, H, N, Br.

**1-(3-Chlorophenylamino)-6,7-dimethoxyisoquinoline-4-carbonitrile (62).** A mixture of 1.24 g (5 mmol) of **60**<sup>28</sup> and 0.63 mL (0.76 g, 6 mmol) of 3-chloroaniline in 10 mL of 2-methoxyethanol was heated at reflux in an oil bath at 149 °C. for 1 h. On cooling the solid was collected and washed with ether. The salt was stirred for about 2 h with 25 mL of saturated aqueous NaHCO<sub>3</sub>. The resulting solid was filtered, washed with water, and dried giving 1.62 g (95%) of the product: mp 278–280 °C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  9.64 (s, 1H), 8.42 (s, 1H), 7.92 (m, 2H), 7.75 (m, 1H), 7.41 (t, *J* = 8.1 Hz, 1H), 7.16 (m, 2H), 4.00 (s, 3H), 3.99 (s, 3H); MS (ES+) *m*/*z* 340.2 (M + H)<sup>+1</sup>. Anal. (C<sub>18</sub>H<sub>14</sub>N<sub>3</sub>O<sub>2</sub>Cl) C, H, N, Cl.

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