Synthesis and in Vitro Pharmacology of Substituted Quinoline-2,4-dicarboxylic Acids as Inhibitors of Vesicular Glutamate Transport

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The vesicular glutamate transport (VGLUT) system selectively mediates the uptake of L-glutamate into synaptic vesicles. Uptake is linked to an H^+-ATPase that provides coupling among ATP hydrolysis, an electrochemical proton gradient, and glutamate transport. Substituted quinoline-2,4-dicarboxylic acids (QDCs), prepared by condensation of dimethyl ketoglutaconate (DKG) with substituted anilines and subsequent hydrolysis, were investigated as potential VGLUT inhibitors in synaptic vesicles. A brief panel of substituted QDCs was previously reported (Carrigan et al. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2607−2612)[1] and showed that certain substituents led to more potent competitive inhibitors of VGLUT. Using these compounds as leads, an expanded series of QDC analogues were prepared either by condensation of DKG with novel anilines or via aryl-coupling (Suzuki or Heck) to dimethyl 6-bromoquinolinedicarboxylate. From the panel of almost 50 substituted QDCs tested as inhibitors of the VGLUT system, the 6-PhCH=CH-QDC (K_i = 167 μM), 6-$PhCH_2CH_2$-QDC (K_i = 143 μM), 6-(4′-phenylstyryl)-QDC (K_i = 64 μM), and 6-biphenyl-4-yl-QDC (K_i = 41 μM) were found to be the most potent blockers. A preliminary assessment of the key elements needed for binding to the VGLUT protein based on the structure−activity relationships for the panel of substituted QDCs is discussed herein. The substituted QDCs represent the first synthetically derived VGLUT inhibitors and are promising templates for the development of selective transporter inhibitors.

Introduction

L-Glutamate (Figure 1) is the primary excitatory neurotransmitter in the mammalian central nervous system (CNS) and participates in processes that range from neuronal communication to neuropathology.[2,3] L-Glutamate mediates standard fast excitatory signaling and contributes to the higher order processing required in development, plasticity, learning, and memory. Excessive activation of ionotropic glutamate receptors can trigger a number of destructive pathways, including osmotic- and calcium-associated neuronal damage.[4−7] Glutamate-mediated pathology, referred to as excitotoxicity, has been implicated as a contributing mechanism in both acute CNS injury (e.g., stroke, trauma, hypolycemia) and chronic neurodegenerative diseases.[6−13] The ability to elucidate and eventually regulate the actions of glutamate as an excitatory transmitter and as an excitotoxin depends on a detailed understanding of each step (uptake, metabolism, release, storage, etc.) in glutamate-mediated neurotransmission.

It is now well accepted that L-glutamate is sequestered into synaptic vesicles that achieve concentrations of up to 60 mM[13] prior to depolarization-triggered, calcium-dependent release from neuron terminals.[14−16] The K_m values determined for the uptake of glutamate into isolated synaptic vesicles are typically 1−2 mM[17] and differ markedly from the values associated with the high-affinity cellular membrane systems (K_m = 5−50 μM).[18,19] Vesicular glutamate transport is mediated by specific integral proteins that bind and translocate this neurotransmitter into synaptic vesicles. The first of these proteins to be isolated, VGLUT1 was originally cloned as a brain-specific Na^+-dependent inorganic phosphate transporter (BNPI).[20] When rat BNPI cDNA was transfected into pheochromocytoma PC12 cells and the light membranes were isolated, the membranes exhibited a 2−4-fold uptake of glutamate as compared to untransfected cell membranes.[21] Similarly, BON cells (neuroendocrine-serotonin-excreting cell line) that express BNP1 exhibited ATP-dependent uptake of glutamate and also demonstrated a quantal release of glutamate upon excitation.[22]

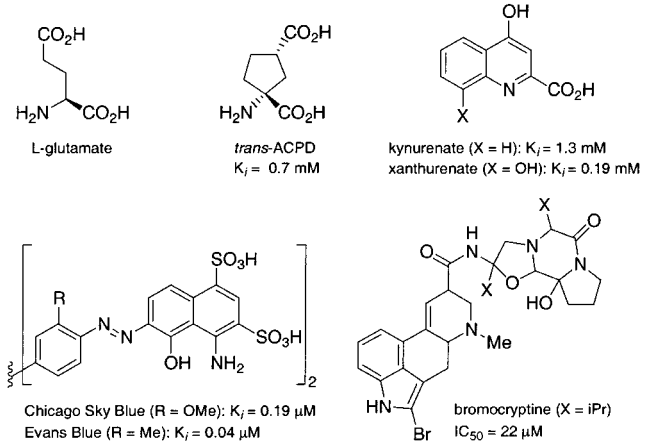

Figure 1. Structures of glutamate and select inhibitors of VGLUT.

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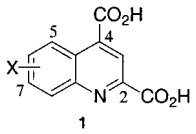

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Figure 2. Structure of substituted QDCs. Substituent = X.

The VGLUT system is clearly distinct from the well-characterized high-affinity excitatory amino acid transport (EAAT) systems present on the plasma membranes of neurons and glia (e.g., EAAC-1, GLT-1, GLAST, and EAAT 1−5)[23,24] that transport glutamate by a sodium-dependent mechanism. Vesicular uptake is coupled to an electrochemical proton gradient generated by a vacuolar-type ATPase (V-ATPase).[14,17] Thus, the driving force for uptake consists of both a $\Delta\psi$ (inside positive) and a ΔpH (inside acidic) that is formed in the presence of permeant chloride anions. Chloride ions also appear to regulate vesicular glutamate uptake by a second mechanism as the anion channel blocker 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) inhibits VGLUT in a manner that is prevented by excess levels of chloride.[25] The substrate binding sites of the EAAT and VGLUT systems also differ pharmacologically. L-Aspartate and D-aspartate are competitive inhibitors of Na^+-dependent cellular uptake yet do not block vesicular transport.[26−28] Conversely, xanthurenate and 7-chlorokynurenate inhibit the uptake of [^{3}H]-L-glutamate into isolated synaptic vesicles[29] but have no effect on the cellular membrane transport of [^{3}H]-D-aspartate into synaptosomes.

To date, known inhibitors of VGLUT vary greatly in structural type, ranging from those with subtle variation in the glutamate molecule to those that are seemingly unrelated to the endogenous substrate. For example, the substituted glutamate analogues *erythro*-4-methyl-L-glutamate ($K_i \approx 0.7$ mM) and 4-methylene-L-glutamate ($K_i \approx 3$ mM) inhibit VGLUT,[30] as do the glutamate receptor agonist and antagonist *trans*-ACPD ($K_i \approx 0.7$ mM)[30] and kynurenate ($K_i \approx 1.3$ mM; Figure 1).[29]

The kynurenate analogues xanthurenate (8-hydroxykynurenate; $K_i \approx 0.19$ mM) and 7-chlorokynurenate ($K_i \approx 0.59$ mM) were slightly more effective as VGLUT inhibitors than kynurenate itself.[29] Some of the more potent inhibitors identified to date include bromocryptine ($IC_{50} \approx 0.022$ mM),[31] the azo dyes Evans Blue ($K_i \approx 0.04\ \mu$M) and Chicago Sky Blue 6B ($K_i \approx 0.19\ \mu$M),[32,33] and Rose Bengal (not shown; $K_i \approx 0.017\ \mu$M).[34] More recently, a family of cytosolic inhibitory protein factors (IPF_α, IPF_β, and IPF_γ) have been identified that potently block glutamate uptake into isolated vesicles with an $IC_{50} = 0.025\ \mu$M.[13] Two marked limitations of currently available VGLUT inhibitors are (1) cross reactivity with other receptor and/or transporter proteins and (2) the fact that the structural motifs that control binding to the VGLUT proteins are not yet clear. Thus, the need for more selective and well-defined inhibitors with which to probe VGLUT function has prompted us to examine the quinoline-2,4-dicarboxylic acids (QDCs) as a novel class of glutamate analogues.

We recently reported that substituted QDCs **1** (Figure 2) competitively inhibit VGLUT (range $K_i \geq 200\ \mu$M).[1] To our knowledge, these are among the first analogues specifically devised to systematically explore the key structural requirements needed for binding to the VGLUT system. In this paper we expand on our preliminary results,[1] introduce a series of novel QDC analogues, and report new structural criteria for inhibition of VGLUT. This information will be important for the design of selective VGLUT inhibitors with which to delineate and regulate the activity of this important transport system.

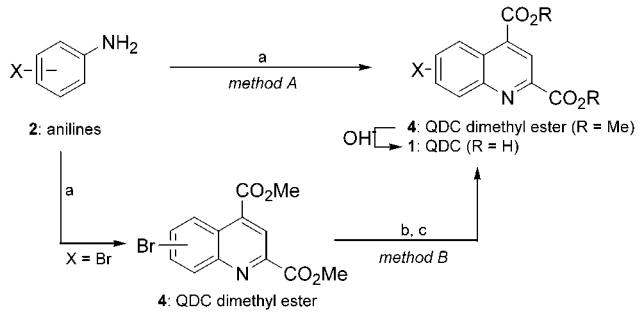
Chemistry Results and Discussion

Inhibitor Design Considerations. Several factors led to the selection of the QDC structure for development as putative inhibitors of VGLUT. First, a basic molecule template was sought that included an embedded glutamate and could be easily manipulated for structural variation. Second, a bicyclic aromatic system was desired in the template since monocyclic analogues are poorer inhibitors of the VGLUT; for example, pyridinecarboxylic acids are far less active than quinolinecarboxylic acids.[29] Consistent with these features, the naphthalene azo dyes incorporate a bicyclic aromatic system and a substituent pattern for amine and acid groups that adequately mimic glutamate. Third, a novel inhibitor class was sought that did not contain the same structural features thought to be important for binding to the various glutamate receptor types; for example, conformational restriction of the aliphatic glutamate backbone (e.g., 2,4-PDC), isostere replacement (AMPA, AP4), or N-alkylation (e.g., NMDA). Fourth, stereochemistry may be an important feature but was given less consideration in this particular study because the potent inhibitors (e.g., azo dyes) contain no stereocenters. Last, the most potent inhibitors reported at VGLUT bear an embedded glutamate with a coplanar arrangement of amino and acid groups, but this conformation differs greatly from the conformation needed to activate glutamate receptors. Thus, it is possible to envision an inhibitor that selectively inhibits vesicular uptake without interacting with glutamate receptors.

The structural templates of the current VGLUT inhibitors (Figure 1) were evaluated and considered for structure−activity studies. However, these compounds were not chosen for further development because these structures are not amenable to substituent variation (azo dyes, ACPD, bromocryptine), cannot be readily synthesized (bromocryptine, ACPD), and/or react elsewhere in the glutamate neurotransmitter system.[35] The QDC template, however, was selected because it contains an embedded glutamate structure (as opposed to the kynurenate and azo dyes) and a fused aromatic ring system. Importantly, the synthesis of QDCs from substituted anilines and dimethyl ketoglutarate[36] (modified Doebner von Miller synthesis) allows for substituent variation at the carbocyclic ring. A preliminary account of QDCs as VGLUT inhibitors has appeared.[1] This work significantly expands the number of compounds designed, prepared, and characterized as inhibitors of the VGLUT system.

Chemical Synthesis. The panel of substituted QDCs used in this study was prepared by two pathways: (a) reaction of a substituted aniline with dimethyl ketoglutaconate (modified Doebner von Miller[37] sequence) to afford dimethyl quinoline-2,4-dicarboxylates (Scheme 1) or (b) application of a Suzuki[38] or Heck Pd-catalyzed cross-coupling reaction[39] between a preformed substi-

Scheme 1[a]



[a] Conditions: (a) $MeO_2CCH{=}CHC(O)CO_2Me$ (3), (b) PdR_4, X (X = electrophile), (c) OH^-.

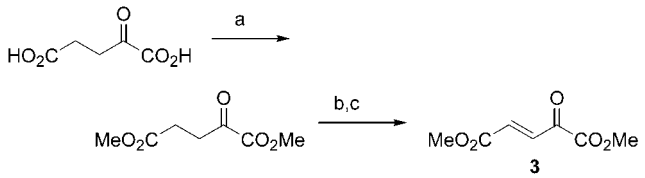
tuted QDC diester and an acceptor molecule (an arylboronic acid or substituted styrene) (Scheme 1). Subsequent hydrolysis of the QDC methyl ester intermediates afforded the target QDCs (Scheme 1).

QDCs **1** are most readily prepared by the acid-promoted reaction of a substituted aniline (**2**) with dimethyl ketoglutaconate (DKG, **3**)[36] to form substituted dimethyl quinoline-2,4-dicarboxylates **4** followed by hydrolysis.[1] The large number of target QDC inhibitors required a large-scale synthesis of dimethyl ketoglutaconate and access to an array of substituted anilines. Limited physical and spectral data were available for **3**,[36] and therefore, key experimental details are provided. In brief, 2-ketoglutaric acid was reacted with methanol and $SOCl_2$ to afford dimethyl ketoglutarate in near-quantitative yield (Scheme 2). Esterification using MeOH/HCl led to an excessive amount of a butyrolactone side product. Bromination (Br_2/CH_2Cl_2) of dimethyl 2-ketoglutarate afforded dimethyl bromo-(keto)glutarate, which following removal of residual HBr and solvent in vacuo, was reacted with triethylamine in an elimination step to afford crystalline **3** in 77% overall yield from dimethyl glutarate. The sequence was amenable for preparations ranging up to 100 g.

Many of the anilines used to prepare the substituted QDCs are commercially available and were used as purchased. However, several substituted anilines containing lipophilic or aromatic substituents required synthesis, and brief details of their preparation are reported here. The alkoxyanilines *p*-*n*-hexyloxyaniline (**2j**), *p*-cyclohexyloxyaniline (**2k**), *p*-(6-hydroxyhexyloxy)-aniline (**2l**), and *p*-(2-phenylethyloxy)aniline (**2p**) were prepared by reaction of sodium *p*-nitrophenolate with hexyl bromide (78%), 6-bromohexanol (99%), cyclohexyl bromide (7%), and 2-bromoethylbenzene (29%), respectively, followed by reduction of the nitro group (10% Pd−C, H_2, MeOH, quantitative). Aryl- and alkyl-tethered anilines were prepared as follows: 3-Nitrobiphenyl was hydrogenated (10% Pd−C, H_2) to afford 3-aminobiphenyl (**2z**) in 99% yield. The Heck reaction[38] was used to couple styrene to 3-nitrobromobenzene or 4-nitrobromobenzene to afford the corresponding *trans*-3-nitrostilbene (24%) or *trans*-4-nitrostilbene (26%), which upon dual hydrogenation of the nitro and alkene groups afforded 3-(2-phenylethyl)aniline (**2cc**; 99%) and 4-(2-phenylethyl)aniline (**2bb**; quantitative).

Substituted dimethyl quinolinedicarboxylates **4** prepared according to Scheme 1 were hydrolyzed to their corresponding QDC diacids **1** and are summarized in Table 1. Yields of the intermediate QDC diesters **4** ranged from poor to moderate (5−60%), but the low yield did not prohibit isolation, characterization, or pharmacologic analysis owing to the accessibility of the starting material. Moreover, the four-step, one-pot synthesis of **4** generally afforded the product in sufficient yield for isolation. As noted earlier, the Doebner von Miller sequence revealed that electron donor substituents (e.g., X = OR) afforded higher yields whereas electron withdrawers (e.g., X = F, NO_2) impeded product formation.[1] Electron donor, meta-substituted anilines reacted with **3** to yield 7-substituted QDC dimethyl esters, whereas halogen substituents predominantly yielded the 5-substituted QDC dimethyl esters.

Scheme 2[a]



[a] Conditions: (a) MeOH, $SOCl_2$, (b) Br_2, (c) Et_3N.

The QDC dimethyl esters were hydrolyzed using either NaOH or LiOH in THF/H_2O and afforded substituted QDC diacids **1** within 2 h. However, when a 5-substituted QDC diester was hydrolyzed using LiOH or NaOH, selective monohydrolysis of the 2-carboxy ester group was observed, suggesting that the steric influence of a substituent at the 5-position greatly reduces the reactivity of the 4-carboxy ester. The substituted QDC dicarboxylate salts resulting from the hydrolysis were neutralized and precipitated by the addition of HCl(aq) to afford the target QDC diacids **1** in nearly quantitative yield. The QDC dimethyl esters **4** and substituted QDCs **1** were fully characterized by elemental and spectral methods.

QDC dimethyl esters containing halogen substituents (**4hh**−**4qq**), alkoxy groups (**4e**−**4v**), phenyl (**4x**−**4aa**), arylalkyl groups (**4bb, 4cc**, **4rr**−**4vv**) and an azo-linked group (**4gg**) were prepared according to Scheme 1 (method A). *o*-Aminophenol, nitroanilines, trifluoromethylanilines, and other anilines bearing strongly electron-withdrawing groups failed to form QDC diesters when reacted with **3**.

Certain QDC analogues were desired that either could not be accessed using method A (Scheme 1) or could be prepared using a more efficient sequence. Since the 5-bromo (**4ll**), 6-bromo (**4mm**), and 8-bromo (**4nn**) QDC dimethyl esters were readily available (the 7-bromo-QDC dimethyl ester was not available), we sought to test whether these structures were suitable for Suzuki- or Heck-type coupling (Scheme 1, method B). Suzuki coupling between phenylboronic acid and **4ll** afforded the 5-Ph-QDC dimethyl ester (**4x**), which was hydrolyzed to yield 5-Ph-QDC (**1x**). 5-Naphthyl (**1dd**), 6-naphthyl (**1ee**), and 8-naphthyl (**1ff**) QDCs were also prepared by Suzuki reactions of **4ll**, **4mm**, or **4nn** with 1-naphthylboronic acid followed by hydrolysis (Scheme 1, method B). Similarly, 6-biphenyl-4-yl-QDC (**1tt**) was synthesized from biphenyl-4-ylboronic acid and **4mm** followed by hydrolysis. One experimental variation to the procedure was that additional increments of the palladium catalyst were needed to ensure complete conversion of the starting materials.

Table 1. Yields and VGLUT Inhibition Data of Substituted QDCs[a]

compd **1a−vv**	X	overall yield (%)	% of control (5 mM)	% of control (250 μM)	K_i(est)[d] (μM)	K_i[e] (μM)
a	H	13	38 ± 4 (4)[f]	100		
b	6-OH	10		100		
c	7-OH	2		100		
d	8-OH	g	2 ± 1 (8)[f]	73 ± 13 (4)	286 ± 117 (3)	
e	6-OCH_3	8	51 ± 2 (6)[f]	100		
f	7-OCH_3	34	48 ± 3 (8)[f]	100		
g	8-OCH_3	31	20 ± 2 (8)[f]	90 ± 7 (2)		
h	6-OCH_2CH_3	23		97 ± 10 (4)		
i	6-OCH_2CH_2OH	18		100		
j	6-$O(CH_2)_5CH_3$	32		79 ± 8 (4)		
k	6-O-cyclohexyl	34		67 ± 1 (2)		
l	6-$O(CH_2)_5CH_2OH$	23		74 ± 3 (2)		
m	6-OPh	17		74 ± 14 (4)		
n	6-OCH_2Ph	14	3 ± 1 (6)[f]	85 ± 21(8)	368 ± 129 (3)	204 ± 66 (3)
o	7-OCH_2Ph	25	1 ± 1 (5)[f]	50 ± 13 (3)		
p	6-OCH_2CH_2Ph	31		37 ± 7 (4)	189 ± 33 (3)	
q	5,7-di-OCH_3	8	15 ± 2 (6)[f]	100		
r	5,8-di-OCH_3	10	62 ± 3 (6)[f]	100		
s	6,7-di-OCH_3	18	58 ± 4 (8)[f]	100		
t	6,8-di-OCH_3	9	40 ± 4 (8)[f]	100		
u	6,7-OCH_2O−	15	24 ± 1 (4)[f]	100		
v	6,7-OCH_2CH_2O−	45	12 ± 2 (4)[f]	100		
w	8-CO_2H	2		100		
x	5-Ph	5[b]		100		
y	6-Ph	10		77 ± 1 (4)		
z	7-Ph	10		91 ± 15 (4)		
aa	8-Ph	24		88 ± 6 (8)		
bb	6-CH_2CH_2Ph	24		47 ± 1 (4)	143 ± 59 (3)	
cc	7-CH_2CH_2Ph	12		29 ± 5 (4)		
dd	5-α-naphthyl	9[b]		64 ± 11 (6)		
ee	6-α-naphthyl	17		53 ± 20 (6)		
ff	8-α-naphthyl	29[b]		70 ± 2 (4)		
gg	6-N=NPh	34	0 ± 0 (4)[f]	31 ± 1 (1)	288 ± 86 (3)	
hh	5-F	15	54 ± 6 (6)[f]	100		
ii	8-F	6	39 ± 2 (8)[f]	100		
jj	6-Cl	17	1 ± 1 (6)[f]	73 ± 6 (4)		
kk	7,8-di-Cl	45	2 ± 1 (4)[f]	nd		
ll	5-Br	11	11 ± 2 (4)[f]	100		
mm	6-Br	9	2 ± 1 (8)[f]	74 ± 7 (4)		
nn	8-Br	23	7 ± 1 (8)[f]	100		
oo	5-I	11		100		
pp	6-I	10	10 ± 1 (4)[f]	87 ± 19 (4)		
qq	6,8-di-Br	6	2 ± 1 (6)[f]	nd		
rr	5-CH=CHPh	10[c]		27 ± 3 (2)		
ss	6-CH=CHPh	6[c]		51 ± 18 (4)	167 ± 38 (6)	129 ± 55 (3)
tt	6-biphenyl-4-yl	4[b]		20 ± 1 (4)	41 ± 9 (3)	95 ± 20 (3)
uu	6-CH=CHPh-Ph	6[c]		37 ± 12 (4)	64 ± 31 (3)	
vv	6-CH=CH-α-naphthyl	5[c]		21 ± 3 (2)		

[a] nd = not determined. Values (% of control) are reported as mean % ± SEM (n) of control uptake (~1500 ± 110 (pmol/min)/mg of protein). [b] Conducted by Suzuki coupling. [c] Conducted by Heck coupling. [d] Estimated from IC_{50} values using the Cheng−Prushoff equation. [e] Derived from Lineweaver−Burk analysis. [f] From ref 1. [g] Prepared by dealkylation of 8-MeO-QDC.

Styryl-substituted QDCs at the 6-position were prepared by a modified Heck reaction (Scheme 1, method B). **4mm** was reacted with styrene or 4-phenylstyrene in dimethoxyethane (DME) or toluene containing Pd-$(OAc)_2$, Ph_3P, and TEA to afford 6-styryl-QDC diester (**4ss**) or 6-(4-phenylstyryl)-QDC diester (**4uu**), respectively. The *trans* geometry of the alkene following these coupling reactions was confirmed by ^1H NMR. 6-Styryl-QDC (**1ss**) and 6-(4-phenylstyryl)-QDC (**1uu**) were obtained by hydrolysis as indicated earlier.

QDC analogues bearing hydroxyl substituents (**1b**−**1d**) were prepared in poor yield by reaction of DKG with *p*-aminophenol or *m*-aminophenol (Scheme 1, method A), which afforded the corresponding 6-OH- and 7-OH-QDC dimethyl esters, respectively. The 8-OH-QDC (**1d**) was prepared by triple dealkylation of 8-methoxy-QDC dimethyl ester with excess boron tribromide (BBr_3).

Pharmacology Results and Discussion

Vesicular Glutamate Transport Assay. The inhibition of VGLUT by compounds **1a**−**1vv** was determined by quantifying the ability to block the uptake of 250 μM [3,4-^{3}H]-L-glutamate into synaptic vesicles isolated from rat forebrain[40] at a 20-fold excess (5 mM) and/or equimolar concentration (250 μM) of inhibitor. Under the assay conditions employed, glutamate transport into the synaptic vesicles was corrected for nonspecific uptake and was linear with respect to both time and protein content (data not shown). QDCs that showed greater than 95% inhibition of uptake at 5 mM were screened at 250 μM with the exception of 8-hydroxy-QDC (**1d**), which was evaluated for specific comparison to xanthurenic acid. The results of these assays are reported in Table 1 as percents of the control (uptake in the absence of inhibitor). Most inhibitors that reduced glutamate uptake through the VGLUT system

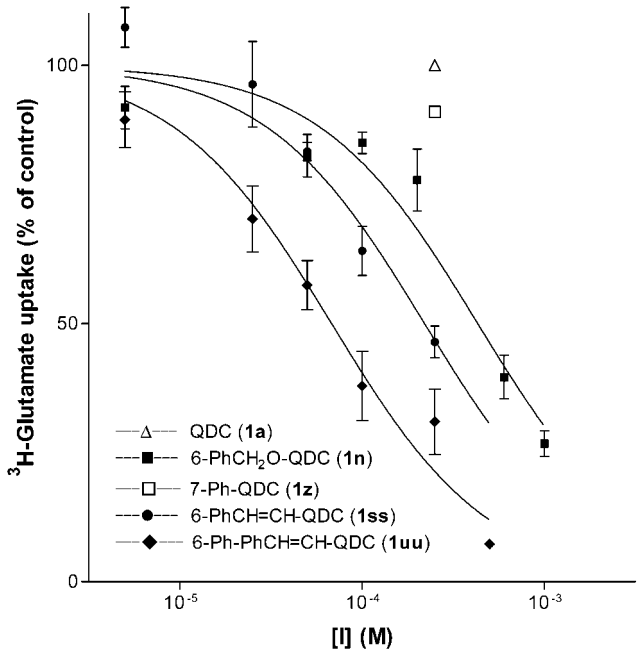


Figure 3. Dose−response profiles (100% for the substituted quinoline-2,4-dicarboxylic acids **1n**, **1ss**, and **1uu**). Single-point data provided for **1a** and **1z** for comparison.

by more than 50% at 250 μM were further examined by dose−response evaluations to determine IC_{50} values. The K_i values were, in turn, estimated using the Cheng−Prusoff equation.[41] Select inhibitors were also evaluated kinetically in greater detail and demonstrated Lineweaver−Burk plots consistent with competitive inhibition. The estimated K_i values and experimentally determined K_i values were in agreement for this subset of compounds (Table 1).

Vesicular Glutamate Transport Pharmacology: Screening. The substituted QDCs listed in Table 1 were evaluated for their ability to block L-glutamate uptake (250 μM) into synaptic vesicles (0% uptake indicates complete inhibition). In some instances, the results acquired with inhibitors at 5 mM were previously reported.[1] Nearly all of the QDCs displayed at least some inhibitory activity at the VGLUT site when tested at 5 mM inhibitor, so most screenings were conducted at 250 μM inhibitor. Representative dose−response curves for 6-PhCH$_2$O-QDC (**1n**), 6-PhCH=CH-QDC (**1ss**), and 6-PhPhCH=CH-QDC (**1uu**) are shown in Figure 3. Point analyses are shown for the parent QDC (**1a**) and 7-Ph-QDC (**1z**), which are less active compounds (Figure 3).

The unsubstituted **1a** (X = H) showed modest activity at 5 mM as an inhibitor (38% uptake) at 5 mM. The effect or contribution of a given substituent ring, therefore, can be preliminarily assessed relative to this parent compound. When a hydroxyl group was employed as substituent (**1b**−**1d**), only the 8-position analogue **1d** showed appreciable inhibitory activity. This result is consistent with the reported difference in activity exhibited between kynurenic acid and xanthurenic acid (Figure 1).[29] Xanthurenic acid has an additional 8-hydroxy group and shows a greater ability to block glutamate uptake into synaptic vesicles. The monomethoxy-QDC analogues **1e**−**1g** were inactive when screened at 250 μM. 8-Methoxy-QDC (**1g**) was less active than **1d**, suggesting that a hydrogen-bonding group at the 8-position may be an important element in VGLUT inhibition. The dimethoxy-QDCs **1q**−**1t** and dioxolane- and dioxane-type QDCs **1u** and **1v** inhibited VGLUT at 5 mM, while the 6,7-dioxo ethers proved to be better VGLUT inhibitors than the corresponding 6,7-dimethoxy-QDC. However, none of the disubstituted ethers showed activity at 250 μM.

5-Fluoro-QDC (**1hh**) and 8-fluoro-QDC (**1ii**) were inactive at 250 μM, and 6-chloro-QDC (**1jj**) showed modest activity. The halogen-substituted QDCs and disubstituted analogues were not pursued in detail because of synthetic limitations, and those showing activity corresponded with structurally similar quinolines that were active at the glutamate and glycine sites on the NMDA receptor.[42]

On the basis of the activity of the alkoxy- and halogen-substituted QDCs, we directly incorporated a phenyl substituent onto the QDC template and examined differences in VGLUT inhibition when the position was varied. The 5-, 6-, 7-, and 8-phenyl-QDCs (**1x**−**1aa**) were all less active than the 7-benzyloxy-QDC (**1o**) and 6-phenethoxy-QDC (**1p**) analogues at 250 μM. However, the assay results indicated that **1y** had the best activity in the series and that **1x** was inactive at 250 μM. The corresponding 5-, 6-, and 8-naphthyl-QDC analogues (**1dd**−**1ff**) were better inhibitors of VGLUT than the phenyl-substituted analogues, and as in the phenyl series, the 6-naphthyl analogue was the most active (uptake blocked to 53% at 250 μM). The naphthyl-substituted QDCs have poor water solubility and were not pursued in great detail. The superior inhibitory activity for QDCs bearing groups at the 6-position was also evidenced in the bromo-QDC series **1ll**−**1nn** and the iodo-QDC compounds **1oo** and **1pp** although the percent glutamate uptake was greater for these substituted QDCs.

Given these results and to limit the scope of QDC analogues to be examined, the 6-position was targeted for further structural modification. Increasing the chain or size of the alkyl ether group attached to the QDC (**1h**−**1l**) led to modest improvement in VGLUT inhibition, although a binding preference was not observed between acyclic (*n*-hexyloxy, **1j**) and cyclic (cyclohexyloxy, **1k**, or phenoxy, **1m**) ether substituents. The addition of a hydroxy group to the distal carbon of an ether chain (**1i** and **1l**) did not improve inhibition. An enhancement in VGLUT inhibition was seen as the 6-substituent was increased in size per the series hydroxy (**1b**) = methoxy (**1e**) < ethoxy (**1h**) < phenoxy (**1m**) = cyclohexyloxy (**1k**) = *n*-hexyloxy (**1j**), suggesting that a lipophilic group improves the interaction between the inhibitor and the VGLUT system. This supposition was examined briefly by constructing and testing the activity of the methylene homologues **1n** and **1p**. Whereas **1m** and **1n** showed comparable inhibition, **1p** was significantly more active as an inhibitor of VGLUT at 250 μM (uptake blocked 37%). The regioisomer **1o** (50% at 250 μM) showed activity midway between those of **1n** and **1p**, indicating that the improved activity found for **1p** may be a combination of lipophilicity and positional requirements of an aromatic substituent within the transporter.

The QDC 8-carboxylic acid analogue (**1w**), prepared to investigate the role of hydrogen bonding and the

importance of ionization at the 8-position, was inactive at 250 μM. Clearly, VGLUT prefers a hydroxy group at the 8-position, since **1d** is a good inhibitor at 250 μM whereas the analogous QDCs bearing carboxylic acid (**1w**), halogen (**1ii**, **1nn**), or phenyl (**1aa**) substituents are poor inhibitors.

Our prior work[1] suggested that QDCs containing an aryl group substituent afforded better VGLUT inhibitors, and possible structural overlaps were sought that may be shared among these potent blockers, and the azo dyes Evans Blue (EB) and Chicago Sky Blue (CSB).[43] To address this, a brief panel of aryl-tethered substituents were synthesized and assayed. 6-(2-Phenylethyl)-QDC (**1bb**) and 7-(2-phenylethyl)-QDC (**1cc**) represent methylene (CH_2) analogues of **1n** and **1o**. The carbon chain analogues **1bb** and **1cc** were more potent VGLUT inhibitors than the corresponding ethers and were consistent in their regiochemical correlation with inhibition; namely, substituents at the 7-position afford slightly greater inhibitory activity. Because the reaction of para-substituted anilines afforded the regioisomerically precise 6-substituted QDCs, we focused our attention on the 6-substituted QDC series.

The azo dyes are among the most potent VGLUT inhibitor class identified to date and include key structural characteristics. EB and CSB are symmetric naphthylamine-2,4-disulfonic acids connected via an azobiphenylyl group (Figure 1). If half of the molecule were presumed responsible for the inhibitory action of the dyes, then the active "half" would appear as depicted in brackets (Figure 1). 6-Azophenyl-QDC (**1gg**) was prepared to mimic half of the EB or CSB structure and found to be an effective VGLUT inhibitor (31% uptake at 250 μM; $K_i = 288$ μM). The greater inhibitory activity of QDC **1gg** relative to many other QDC analogues suggests that the inhibitory action of the dyes is not due solely to the naphthylenedisulfonic acid substituents but is also dependent on the bis(azophenyl) linker and regiospecific placement of the acid moieties. Consistent with this interpretation, greater inhibition was found when the phenyl group was tethered to a QDC via a chain of two or more atoms.

The next structural characteristic we sought to investigate was the importance of the azo linker group present in EB and CSB. The azo (−N═N−) group may bind a complementary group on the VGLUT protein, or the π-bond may be simply acting as a spacer group. To address this, QDC analogues bearing aryl groups tethered by flexible or rigid spacer groups were synthesized and assayed. The two-atom spacer analogues **1n** (85% at 250 μM) and **1bb** (47% at 250 μM) are good inhibitors, showing slightly less activity than **1gg**. The corresponding alkene spacer groups 5-styryl-QDC (**1rr**) and **1ss**, which more closely approximate the azophenyl structural motif, proved to be excellent inhibitors at 250 μM, allowing only 27% and 51% uptake, respectively. Therefore, the basicity or specific chemical nature of the azo group does not appear to be the controlling factor for activity. Instead, the spatial arrangement and/or the two-atom spacer distance of the azo bond are likely to be important. It is possible that the **1rr** analogue shows greater inhibition than **1ss** because the 6-azophenyl group can adopt a *Z*-geometry that places the phenyl group in a common topologic overlap with the phenyl group of the 5-styryl substituent, and this may be the more important geometry for binding.

Three additional phenyl-containing substituents attached to the QDC template were synthesized to further evaluate the features that are responsible for the activity of the azo dyes. 6-Biphenyl-4-yl-QDC (**1tt**), **1uu**, and 6-[2-(β-naphthyl)ethylene]-QDC (**1vv**) were prepared to examine the differences between conjugated two-atom linker groups and a phenyl linker group; the latter places a coplanar restriction on the terminal phenyl substituent. The "inner" phenyl group of **1tt** mimics a spacer group of 2.85 Å (approximately double the length of an alkene bond), and the "external" phenyl group represents the moiety presumed responsible for binding. Assay results at 250 μM indicated that **1tt** is a potent inhibitor and limits glutamate uptake to only 20%, which compares well in inhibitory activity with **1p**. To determine the correlation between linker length and activity, the ethylene-linked QDC analogue **1uu** was screened. QDC analogue **1uu** bears a tandem ethene and phenyl group spacer between the QDC template and the distal phenyl group approximating an overall spacer distance between the QDC template and the distal phenyl of 4.2 Å. Interestingly, **1uu** was only half as effective as **1tt** as an inhibitor. Several possibilities may account for this decrease including (a) the binding boundaries had been exceeded, (b) the geometry of the distal phenyl group is placed in an unfavorable orientation, or (c) the phenyl-tethered analogues exceed the solubility limit in the assay system. The importance of this third possibility was evidenced when **1vv** was tested. Despite effective inhibition of the VGLUT, compound **1vv** proved difficult to solubilize, thereby limiting dose−response analyses.

The next binding characteristic we sought to elucidate was the importance of the distal phenyl group. As indicated in the comparative phenyl (**1x**−**1aa**) and naphthyl (**1dd**−**1ff**) QDC series, the substitution of a naphthyl group for a phenyl afforded greater inhibition of glutamate uptake. Likewise, **1vv** was a significantly more potent inhibitor than the corresponding 6-styryl-QDC, indicating that a planar aromatic group and not a phenyl group was a key element for inhibitory activity. Since **1vv** showed greater inhibition than **1ee** (naphthyl attached to the QDC template), the importance of a spacer group was reinforced.

Vesicular Glutamate Transport Pharmacology: Kinetic Analyses. To better define key structural motifs needed for binding to the VGLUT system, as well as confirm that the mechanism of inhibition is competitive, more detailed kinetic analyses were carried out. K_i values were calculated using the Cheng−Prusoff equation,[41] and IC_{50} values determined from standard dose−response curves as a function of inhibitor concentrations (x−y μM). The inhibitory activity of a select group of analogues was further characterized as a function of both inhibitor and substrate concentrations using Lineweaver−Burk double reciprocal plots (i.e., $1/V$ vs 1/[S]). Such a plot representing the inhibition of glutamate vesicular uptake by **1ss** is shown in Figure 4. In these experiments, the K_m and V_{max} values for the transport of L-glutamate were found to be 1.65 ± 0.3 mM and 18.3 ± 2.2 (pmol/min)/mg of protein, respectively ($n = 7$). In all of these assays, the pattern of

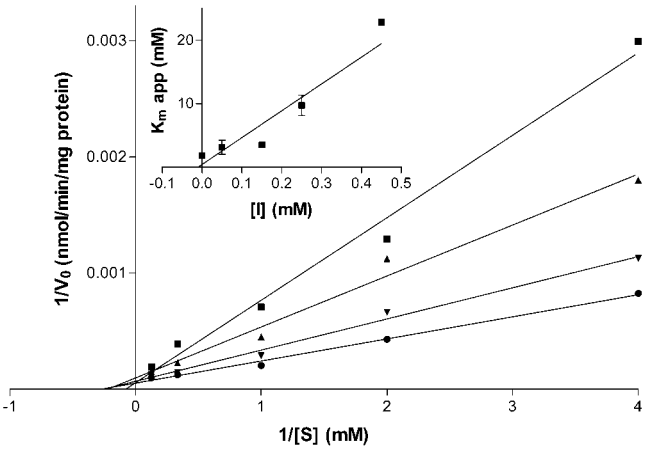


Figure 4. Representative Lineweaver−Burk plot of a single experiment demonstrating competitive inhibition by **1ss** (0.05, 0.15, and 0.25 mM) on the uptake of [^{3}H]-L-glutamate (0.25−8.0 mM) into rat brain synaptic vesicles. The above plots yielded a control $V_{max} = 18.3 \pm 2.2$ (nmol/min)/mg of protein and $K_m = 1.65 \pm 0.3$ mM. The inset shows a replot of K_m(app) vs **1ss** concentration, producing a $K_i = 0.129$ mM.

inhibition observed was consistent with that of a competitive mechanism. Further, the K_i values determined from the Lineweaver−Burk analyses were comparable to those calculated from IC_{50} values (see Table 1).

The K_i(est) found for **1d** was 286 μM, and **1d** is a weaker inhibitor than xanthurenic acid (Figure 1; K_i = 190 μM).[29] 8-Hydroxy-QDC differs from xanthurenic acid by the replacement of a carboxylic acid group (**1d**) for the hydroxyl (xanthurenic acid). Since the QDC template more closely mimics the glutamate structure than the 2-carboxy-4-hydroxyquinolines, it is likely that the 4-carboxylic acid probably plays a minor role in binding to the VGLUT protein.

Estimated K_i values for a small panel of 6-substituted QDC analogues were determined to better define the key structural motifs on the QDC responsible for binding to the transporter. The QDC panel **1n**, **1p**, **1bb**, **1gg**, **1ss**, **1tt**, and **1uu** were selected because each compound contains a 6-positional substituent and a terminal-linked phenyl group as part of the substituent group. The primary differences in the structures were the length and nature of the spacer group. **1n** (−CH_2O−), **1p** (−CH_2CH_2O−), and **1gg** (−N=N−) all contain spacer groups with heteroatom substituents and show modest K_i values ranging from 189 to 368 μM. **1bb** (PhCH_2CH_2–) and **1ss** (PhCH=CH−) differ only in the spacer group flexibility (hybridization at carbon) and are slightly better inhibitors than the heteroatom spacer group compounds. These data support our original supposition (derived from preliminary screen data) that the basicity of the azo linkage or a heteroatom lone pair is not critical for activity. The data from this panel of compounds indicate that the nature of the chemical bonds comprising the spacer group may be important for activity. Markedly better K_i(est) values were found for **1uu** (K_i = 64 μM) and **1tt** (K_i = 41 μM), which contain the biphenylyl substituent with and without the ethene spacer group. Although the incorporation of the biphenyl group improved inhibition of VGLUT as compared to **1y** or **1ss**, lengthening the substituent further, namely, combining an alkene with a biphenylyl group as in **1uu** did not increase the inhibition. **1tt** (K_i = 41 μM) is the strongest QDC-based inhibitor we have identified in the substituted QDC analogue study to date. It is worth noting that the poor water solubility of compounds **1rr**−**1uu** limited the concentration range of the kinetics analyses.

The panel of QDC analogues studied in this paper was limited to monosubstitution on the carbocyclic ring of a QDC template. Although disubstituted QDC analogues could be prepared, yields were poor and unpredictable regiochemically. Work from other laboratories also showed that disubstitution of the carbocyclic ring of quinoline and tetrahydroquinoline carboxylic acids led to enhanced activity at the glycine and NMDA receptor sites,[42,44] and therefore, we sought to suppress potential cross-reactivity at the other receptors. As a result, we sought to optimize inhibition of VGLUT using QDC analogues that varied at a single position. The selectivity for VGLUT is supported, in part, by the following example. **1n**, an analogue that displayed good inhibition of the VGLUT, was found to be completely inactive when tested at 250 μM at the kainate, AMPA, and nonselective glutamate receptor sites (data not shown; MDS Pharma, Bothel, WA). Also important, compound **1n** was inactive when tested at 250 μM as an inhibitor of the Na^+-dependent synaptic transporter (EAAT2). These results suggest that certain QDC analogues may meet the desired criteria as selective inhibitors of the vesicular transport system.

Conclusions

In the present paper, we have shown that changes to the carbocyclic ring substituent on a quinoline-2,4-dicarboxylic acid template afford improved inhibitors of VGLUT. These compounds represent the first class of chemically modified inhibitors targeted to block this transporter. Optimized QDC analogues were shown to be relatively potent competitive inhibitors of VGLUT exhibiting K_i values as low as 41 μM. To put these values in perspective, it must be remembered that VGLUT is considered a "low-affinity" transporter and that the K_m for the endogenous substrate is more than 40-fold greater (i.e., $K_m \approx 1.7$ mM).

Examination of the more potent substituted QDCs and other known VGLUT inhibitors (Figure 1) revealed a number of features that were important for improved binding. First, the coplanar placement of amine and the two acid groups increased inhibition relative to that of glutamate. This rigid positioning of functional groups represents a new conformationally restricted analogue that differs from other inhibitors that bind to other EAA receptors and transporters, for example, ACPD and 2,4-PDC. Second, the heterocyclic nitrogen of the QDCs is less basic than that of glutamate. Therefore, the QDC inhibitors, despite having an embedded glutamate structure, actually bear few physicochemical properties in common with the flexible skeleton and zwitterionic glutamate. Third, QDCs bearing aryl and aryl-containing substituents, particularly those connected at the 6- and 7-positions, show improved inhibition of VGLUT. Particularly promising were QDCs that contained styryl or biphenyl groups as substituents. These inhibitors bear structural resemblance to the symmetric portion of the azo dyes EB and CSB (Figure 1) and possibly

highlight the importance of aromatic substituents and their position and type of attachment and overall intramolecular distance to the embedded glutamate in the design of VGLUT inhibitors. Ornstein[45] recently prepared novel biphenylyl-containing sulfonamides that were found to be AMPA potentiators. Clearly, the biphenylyl substituent is playing an emerging role in the design of targeted glutamate analogues. Although not pursued in detail, we also found that the presence of an 8-hydroxy group confers additional inhibitory strength. 8-Hydroxy-QDC and xanthurenic acid are both more potent inhibitors than the corresponding structure without the 8-hydroxy. It is noteworthy that the azo dyes EB and CSB also bear the equivalent of an 8-hydroxy group on the 1-naphthylamine-2,4-disulfonic acid template. We are currently conducting molecular modeling studies to better define the overall pharmacophore requirements for GVT.

Experimental Section

Unless otherwise noted, reactions were run in flame-dried round-bottom flasks under an argon atmosphere. Anhydrous reagent grade solvents (methylene chloride, CH_2Cl_2; dimethoxyethane, DME; dimethylformamide, DMF; tetrahydrofuran, THF) were used without further purification. Toluene was distilled under calcium hydride prior to use. Aryl and naphthylboronic acids were purchased and used without further purification. The reagents palladium tetrakistriphenylphosphine, palladium acetate, and boron tribromide were used as purchased. Triphenylphosphine (PPh_3) was recrystallized from methanol prior to use. Low-boiling-point, substituted anilines were distilled over NaOH prior to use. Triethylamine (TEA) was distilled from CaH_2 prior to use.

Melting points were determined on a MEL-TEMP II metal block apparatus and are uncorrected. Analytical thin-layer chromatography (TLC) was performed with silica gel (Whatmann) aluminum-backed plates. Flash chromatography was performed on Merck (60 Å) pore silica. Compounds were detected using UV absorption at 254 nm. ^{1}H NMR and ^{13}C NMR spectra were determined on a Varian 400 MHz Unity Plus spectrometer. Chemical shifts are reported in parts per million (ppm, δ) using $CHCl_3$ (7.25 ppm) or DMSO (2.49 ppm) as a reference. High-resolution mass spectrometry (HRMS) was conducted at the Department of Medicinal Chemistry, University of Washington, using an electrospray ionization (ESI) Micromass 70SEQ high-resolution mass spectrometer or a fast atom bombardment (FAB) Finnegan high-resolution mass spectrometer. Elemental analyses for C, H, and N were performed by Midwest Microlab, Indianapolis, IN. QDC diesters and intermediates were identified by elemental analyses. QDC diacids were characterized by HRMS and their purities (>95%) determined by HPLC in two or more solvent systems. Analytical HPLC measurements were run on a Waters 2790 instrument equipped with a Waters 2487 UV detector. Separations were performed with a Waters Xterra RP8 column (3.9 mm × 150 mm, 5 μm). Standard conditions utilized an isocratic, dual-solvent system consisting of solvents A (H_2O with 0.08% TFA) and B (CH_3OH with 0.08% TFA), flow rate 1.5 mL/min, λ = 254 and 300 nm, retention times (t_R) evaluated in minutes. Typical analyses involved two distinct isocratic elutions per compound of interest. Solvent conditions for the isocratic elutions ranged from 30% to 70% B depending on the compound and specific chromatographic properties. In certain instances, peak identity was confirmed by analysis with a Micromass LCT ESI mass spectrometer interfaced with the HPLC system. ^{1}H NMR and ^{13}C NMR spectra are consistent with the assigned structures.

General Procedure for the Synthesis of 4-Alkoxyanilines 2j, 2k, 2l, and 2p from *p*-Nitrophenol. To a solution of sodium 4-nitrophenolate (1.0 equiv) in DMF was added the alkyl bromide (1.0 equiv). The reaction mixture was heated to 100 °C for 48 h. The DMF solution was diluted (20-fold) with water and the mixture extracted with CH_2Cl_2. The organic layer was then extracted with 2 M NaOH. The organic layer was then dried over Na_2SO_4 and evaporated to afford the alkoxynitrobenzene analogue. The nitrobenzene analogue was dissolved in methanol and purged with argon. Palladium (10%) on carbon (1% by mass of starting material) was added to the solution, and the mixture was purged with argon and then saturated with hydrogen under balloon pressure. The reaction was stirred until it was complete as monitored by TLC (2−8 h). The mixture was filtered through Celite and evaporated to give the product. Note: Compounds **2bb**, **2cc**, and **2tt** were also prepared by reduction of the corresponding nitro precursor according to the procedure indicated above.

4-Hexyloxyaniline (2j). Reaction of sodium 4-nitrophenolate with hexyl bromide affords 1-hexyloxy-4-nitrobenzene as a light yellow oil (78%): ^{1}H NMR ($CDCl_3$) δ 8.18 (d, J = 9.2 Hz, 2 H), 6.93 (d, J = 9.2 Hz, 2 H), 4.03 (t, J = 6.4 Hz, 2 H), 1.81 (m, J = 6.4 Hz, 2 H), 1.46 (m, J = 6.4 Hz, 2 H), 1.36−1.31 (m, 4 H), 0.92−0.88 (m, 3 H); ^{13}C NMR ($CDCl_3$) δ 153.3, 140.9, 117.5, 116.7, 69.6, 63.9, 33.7, 30.4, 26.9, 26.6. 1-Hexyloxy-4-nitrobenzene was reduced to 4-hexyloxyaniline and isolated as a light red oil (99% yield). Anhydrous HCl gas was bubbled through the product in CH_2Cl_2 to form the HCl salt, which was recrystallized from EtOAc: ^{1}H NMR ($CDCl_3$) δ 6.73 (d, J = 8.8 Hz, 2H), 6.63 (d, J = 8.8 Hz, 2H), 3.87 (t, J = 6.8 Hz, 2H), 3.38 (br s, 2H), 1.73 (m, J = 6.8 Hz, 2H), 1.43 (m, 2H), 1.36−1.30 (m, 4H), 0.89 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 153.4, 140.8, 117.5, 116.7, 69.7, 32.7, 30.5, 26.8, 23.7, 15.1. Anal. ($C_{12}H_{20}ClNO$) C, H, N.

4-Cyclohexyloxyaniline (2k). Reaction of sodium 4-nitrophenolate with cyclohexyl bromide affords a white solid (7%). ^{1}H NMR ($CDCl_3$): δ 8.15 (dd, J = 7.2 Hz, J = 2.2 Hz, 2 H); 6.92 (dd, J = 2.2 Hz, J = 7.2 Hz, 2 H); 4.39−4.34 (m, 1 H); 1.99−1.96 (m, 2 H); 1.82−1.78 (m, 2 H); 1.60−1.51 (m, 3 H); 1.43−1.31 (m, 3 H). 1-Cyclohexyloxy-4-nitrobenzene was reduced to 4-cyclohexyloxyaniline and isolated as a light red oil (100% yield). ^{1}H NMR ($CDCl_3$): δ 6.75 (d, J = 8.8 Hz, 2H), 6.61 (d, J = 8.8 Hz, 2H), 4.07−4.00 (m, 1H), 1.96−1.93 (m, 2H), 1.81−1.74 (m, 2H), 1.56−1.41 (m, 3H), 1.36−1.24 (m, 3H). ^{13}C NMR ($CDCl_3$): δ 151.7, 141.2, 119.4, 119.1, 117.4, 114.6, 33.1, 26.8, 24.9.

4-(6-Hydroxyhexyloxy)aniline (2l). Reaction of sodium 4-nitrophenolate with 6-bromohexanol affords 6-(4-nitrophenoxy)hexan-1-ol as a light yellow oil (99% yield). ^{1}H NMR ($CDCl_3$): δ 8.17 (d, J = 8.8 Hz, 2H), 6.91 (d, J = 8.8 Hz, 2H), 4.03 (t, J = 6.6 Hz, 2H), 3.64 (q, J = 6.6 Hz, 2H), 1.85−1.78 (m, 2H), 1.62−1.40 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 165.3, 142.4, 127.0, 115.4, 69.8, 63.8, 33.6, 30.0, 26.8, 26.8, 26.5. Anal. ($C_{12}H_{17}NO_4$) C, H, N. 4-(6-Hydroxyhexyloxy)nitrobenzene was reduced to 4-(6-hydroxyhexyloxy)aniline and isolated as a light red oil (100% yield). Anhydrous HCl gas was bubbled through the product in CH_2Cl_2 to precipitate out the HCl salt. The salt was recrystallized from EtOAc/hexane: ^{1}H NMR ($CDCl_3$) δ 6.72 (d, J = 8.6 Hz, 2H), 6.62 (d, J = 8.6 Hz, 2H), 3.87 (t, J = 6.4 Hz, 2H), 3.63 (t, J = 6.4 Hz, 2H),1.74 (m, J = 6.4 Hz, 2H), 1.58 (m, J = 6.4 Hz, 2H), 1.50−1.36 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 153.3, 140.9, 117.5, 116.7, 69.6, 63.9, 33.7, 30.4, 26.9, 26.6. Anal. ($C_{12}H_{20}ClNO_2$) C, H, N.

4-(β-Phenylethyloxy)aniline (2p). Reaction of sodium 4-nitrophenolate with (β-bromoethyl)benzene affords 1-(β-phenylethyloxy)-4-nitrobenzene as a light yellow oil (29% yield): ^{1}H NMR ($CDCl_3$) δ 8.17 (d, J = 9.0 Hz, 2H), 7.37−7.26 (m, 5H), 6.93 (d, J = 9.0 Hz, 2H), 4.26 (t, J = 7.0 Hz, 2H), 3.15 (t, J = 7.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 165.0, 142.5, 138.6, 130.1, 129.7, 127.9, 127.0, 115.6, 70.5, 36.6. Anal. ($C_{14}H_{13}NO_3$) C, H, N. 1-(β-Phenylethyloxy)-4-nitrobenzene was reduced to 4-(β-phenylethyloxy)aniline and isolated as a light red oil (100% yield). The hydrochloride salt was submitted for elemental analysis: ^{1}H NMR ($CDCl_3$) δ 7.37−7.25 (m, 5H), 6.78 (d, J = 8.8 Hz, 2H), 6.67 (d, J = 8.8 Hz, 2H), 4.13 (t, J = 7.2 Hz, 2H), 3.73 (m s, 2H), 3.10 (t, J = 7.2 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 153.2, 140.7, 139.6, 130.2, 129.6, 127.5, 117.7, 116.9, 70.5, 37.0. Anal. ($C_{14}H_{16}ClNO$) C, H, N.

4-(β-Phenylethyl)aniline (2bb). 4-Nitrostilbene was reduced to 4-(β-phenylethyl)aniline as a light purple oil (99% yield). Anhydrous HCl gas was bubbled through the product in CH_2Cl_2 to precipitate out the HCl salt. The salt was recrystallized in EtOAc and submitted for elemental analysis: ^{1}H NMR ($CDCl_3$) δ 7.30−7.25 (m, 2H), 7.20−7.17 (m, 3H), 7.08 (t, J = 7.2 Hz, 1H), 6.63 (d, J = 8.0 Hz, 1H), 6.56−6.54 (m, 2H), 3.79 (br s, 2H), 2.92−2.88 (m, 2H), 2.85−2.80 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 147.1, 144.2, 143.2, 130.3, 126.9, 120.1, 116.5, 114.0, 39.0, 38.9. Anal. ($C_{14}H_{16}$ ClN) C, H, N.

3-(β-Phenylethyl)aniline (2cc). 3-Nitrostilbene was reduced to 3-(β-phenylethyl)aniline as a light purple oil (99% yield). Anhydrous HCl gas was bubbled through the product in CH_2Cl_2 to precipitate out the HCl salt. The salt was recrystallized in EtOAc: ^{1}H NMR ($CDCl_3$) δ 7.30 (t, J = 7.3 Hz, 2H), 7.22−7.19 (m, 3H), 7.10 (t, J = 7.3 Hz, 1H), 6.64 (d, J = 7.3 Hz, 1H), 6.56−6.55 (m, 2H); 3.71 (br s, 2H), 2.94−2.89 (m, 2H), 2.87−2.82 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 147.3, 144.2, 143.0, 130.4, 129.5, 129.4, 127.0, 120.0, 116.5, 114.0, 39.1, 38.9. Anal. ($C_{14}H_{16}$ ClN) C, H, N.

3-Aminobiphenyl (2tt). 3-Nitrobiphenyl was reduced to 3-aminobiphenyl as a light red oil (99%): ^{1}H NMR ($CDCl_3$) δ 7.60 (d, J = 7.6 Hz, 2H), 7.45 (t, J = 7.6 Hz, 2H), 7.36 (t, J = 7.6 Hz, 1H), 7.26 (t, J = 7.6 Hz, 1H), 7.03 (d, J = 7.6 Hz, 1H), 6.94 (s, 1H), 6.71 (dd, J = 2.8, 7.6 Hz, 1H), 3.62 (br s, 2H); ^{13}C NMR ($CDCl_3$) δ 147.7, 143.6, 142.5, 130.8, 129.8, 128.3, 128.2, 118.9, 115.3, 115.1.

Dimethyl 2-Ketoglutaconate (3). Thionyl chloride (4.0 mL, 0.027 mol) was added dropwise to a solution of glutaric acid (4.0 g, 0.027 mole) dissolved in 40 mL of methanol at −5° C. Immediately after addition, the methanol was removed in vacuo at room temperature to give dimethyl glutarate as a light yellow oil (4.76 g, quantitative): ^{1}H NMR ($CDCl_3$) δ 3.86 (s, OCH_3, 3 H), 3.67 (s, OCH_3, 3 H), 3.14 (t, J = 6.6 Hz, $-CH_2$-, 1 H), 2.66 (t, J = 6.6 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 192.2, 172.4, 160.9, 53.1, 52.0, 34.2, 27.4. To a solution of dimethyl glutarate (1.03 g, 5.92 mmol) dissolved in 30 mL of methylene chloride was added bromine (0.46 mL, 8.9 mmol) dissolved in 2 mL of CH_2Cl_2 at reflux. The solution was stirred at reflux for 3 h, after which the solvent and residual HBr were evaporated to yield dimethyl bromoglutarate as an orange oil (1.47 g, 5.8 mmol, 98%): ^{1}H NMR ($CDCl_3$) δ 5.34 (q, J = 9.1, 6.2 Hz, 1 H), 3.90 (s, $-OCH_3$, 3 H), 3.66 (s, $-OCH_3$, 3 H), 3.28 (dd, J = 17.4, 9.1 Hz, 1 H), 3.01 (dd, J = 17.4, 6.2 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 184.4, 170.1, 160.0, 53.6, 52.4, 39.6, 37.4. Triethylamine (5.0 mmol, 0.71 mL) was added to a solution of dimethyl bromoglutarate (4.6 mmol, 1.16 g) in 30 mL of diethyl ether. After being stirred for 0.5 h, the mixture was filtered through a pad of silica gel two times to remove the TEA·HBr salts. The ether solution was evaporated to give a bright yellow solid (616 mg, 3.6 mmol, 79%) of dimethyl 2-ketoglutaconate: ^{1}H NMR ($CDCl_3$) δ 7.63 (d, J = 15.8 Hz, 1H), 6.98 (d, J = 15.8 Hz, 1H), 3.94 (s, 3H), 3.85 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 182.1, 165.0, 160.7, 135.6, 134.1, 53.6, 52.4.

General Procedure for the Synthesis of Quinoline-2,4-dicarboxylic Acids by Condensation of an Aniline with DKG Followed by Hydrolysis. A solution of DKG (1.2 equiv) was added to a solution of aniline (1.0 equiv) in CH_2Cl_2, and the resulting solution was stirred for 0.5 h. A catalytic amount (0.2 equiv) of toluenesulfonic acid (unless otherwise noted) was then added, and the solution was refluxed for 24 h. The reaction mixture was extracted with saturated $NaHCO_3$ three times followed by extraction with 0.1 M HCl. The organic layer was separated and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the crude product was then chromatographed. Analytical samples were prepared by a recrystallization from ethyl acetate/hexanes. The diester was dissolved in a 50:50 solvent solution of THF and H_2O. To this solution was added an excess of hydroxide solution (LiOH or NaOH). The reaction mixture was stirred until it was finished, monitored by TLC. The THF/H_2O solution was removed in vacuo. The product was dissolved in H_2O and precipitated out with 6 M HCl (pH 2). The product was then triturated with water and filtered to obtain the product.

General Procedure for the Synthesis of Dimethyl Quinoline-2,4-dicarboxylates Using Heck/Suzuki Coupling (1x, 1y, 1ff, 1ss, 1tt, 1uu, 1vv). The aryl halide (1.0 equiv) and palladium tetrakistriphenylphosphine [$Pd(PPh_3)_4$] (0.05−0.5 equiv) were dissolved in DME, and the resulting solution was stirred for 15 min. To the solution was added the arylboronic acid (1.5 equiv) in a minimal amount of EtOH followed by 2 M Na_2CO_3 (2.0 equiv) solution. The reaction mixture was then heated to 90 °C for 24 h. The reaction mixture was filtered through Celite,[7] and the DME was removed in vacuo. The product was redissolved in CH_2Cl_2 and extracted with saturated Na_2CO_3 solution, followed by a water wash. The organic layer was then dried (Na_2SO_4) and filtered, and the solvent was removed in vacuo to afford the crude product, which was chromatographed on silica gel.

Quinoline-2,4-dicarboxylic Acid (1a). Aniline (**2a**) affords the unsubstituted QDC dimethyl ester, which was chromatographed (hexanes/EtOAc, 3:1) to isolate a light beige solid (16% yield): mp 128−129 °C; ^{1}H NMR ($CDCl_3$) δ 8.84 (dd, J = 1.4, 8.0 Hz, 1H), 8.69 (s, 1H), 8.37 (d, J = 8.0 Hz, 1 H), 7.85 (dt, J = 1.4, 7.0 Hz, 1H), 7.77 (dt, J = 1.6, 7.0 Hz, 1H), 4.11(s, OCH_3, 3H), 4.07 (s, OCH_3, 3H); ^{13}C NMR ($CDCl_3$) 166.0, 165.3, 148.5, 147.4, 136.2, 131.2, 130.6, 130.4, 126.3, 125.5, 122.3, 54.4, 53.0. Anal. ($C_{13}H_{11}NO_4$) C, H, N. Dimethyl quinoline-2,4-dicarboxylate (**4a**) (29 mg, 0.118 mmol) was hydrolyzed to yield a light orange powder (21 mg, 81% yield): mp 244−245 °C (decarboxylation); ^{1}H NMR (DMSO-d_6) δ 8.79 (d, J = 8.4 Hz, 1H), 8.47 (s, 1H), 8.24 (d, J = 8.4 Hz, 1H), 7.91 (t, J = 8.4 Hz, 1H), 7.85 (t, J = 8.4 Hz, 1H), 3.38 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 167.4, 166.2, 148.9, 148.3, 137.6, 131.2, 131.0, 130.5, 126.0, 125.9, 122.0; HRMS (FAB) m/z calcd for $C_{11}H_7NO_4$ (M^+) 217.0375, found 217.0375; HPLC 99% (t_R = 2.8 min, 60:40; t_R = 1.7 min, 40:60).

6-Hydroxyquinoline-2, 4-dicarboxylic Acid (1b). 4-Aminophenol (**2b**) (75 mg, 0.69 mmol) was converted to the product, which was chromatographed ($CHCl_3$) to give a light yellow solid (34 mg, 19% yield): mp 214−216 °C; ^{1}H NMR ($CDCl_3$) δ 8.69 (s, 1H), 8.29 (d, J = 3.2 Hz, 1H), 8.26 (d, J = 9.6 Hz, 1H), 7.46 (dd, J = 3.2, 9.6 Hz, 1H), 4.08 (s, 3H), 4.03 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 167.2, 166.3, 161.0, 145.1, 144.9, 134.2, 134.1, 129.1, 125.3, 123.7, 107.7, 54.4, 54.1; TLC R_f 0.23 (hexanes/EtOAc, 1:1). Anal. ($C_{13}H_{11}NO_5$) C, H, N. Dimethyl 6-hydroxyquinoline-2,4-dicarboxylate (**4b**) (10 mg, 0.038 mmol) was hydrolyzed to yield an orange solid (4 mg, 50% yield): ^{1}H NMR (DMSO-d_6) δ 10.74 (br s, 1H), 8.42 (s, 1H), 8.15 (d, J = 2.6 Hz, 1H), 8.08 (d, J = 9.6 Hz, 1H), 7.47 (dd, J = 2.6, 9.6 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 168.8, 167.5, 160.5, 146.0, 145.0, 135.5, 133.9, 129.2, 125.0, 123.6, 108.0; HRMS m/z calcd for $C_{11}H_7NO_5$ (M^+) 233.0324, found 233.0322; HPLC 99% (t_R = 1.6 min, 40:60; t_R = 2.6 min, 60:40).

7-Hydroxyquinoline-2,4-dicarboxylic Acid (1c). 3-Aminophenol (**2c**) (300 mg, 2.75 mmol) was converted to the product, which was chromatographed (CH_2Cl_2) to yield a yellow solid (18 mg, 3% yield): mp 245−246 °C; ^{1}H NMR (DMSO-d_6) δ 8.57 (d, J = 9.6 Hz, 1H), 8.24 (s, 1H), 7.44 (m, 2H), 3.99 (s, 1H), 3.98 (s, 3H), 3.95 (s, 3H), 1.23 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 167.3, 166.3, 161.0, 151.6, 149.0, 137.2, 128.2, 125.0, 121.1, 120.0, 112.6, 54.6, 54.3; HRMS (FAB) m/z calcd for $C_{13}H_{11}NO_5$ (M^+) 261.0637, found 261.0630. Dimethyl 7-hydroxyquinoline-2,4-dicarboxylate (**4c**) (10 mg, 0.04 mmol) was hydrolyzed to yield a bright orange solid (4 mg, 50%): ^{1}H NMR (DMSO-d_6) δ 10.75 (br s, 1H), 8.61 (d, J = 8.8 Hz, 1H), 8.20 (s, 1H), 7.47 (d, J = 2.4 Hz, 1H), 7.40 (dd, J = 2.4, 8.8 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 168.8, 167.4, 160.9, 151.5, 149.9, 138.5, 128.3, 124.5, 121.2, 119.9, 112.5; HRMS (ESI) m/z calcd for $C_{11}H_6NO_5$ (M − H) 232.0246, found 232.0240; HPLC 99% (t_R = 1.8 min, 60:40; t_R = 1.5 min, 40:60).

8-Hydroxyquinoline-2,4-dicarboxylic Acid (1d). To a solution of dimethyl 8-methoxyquinoline-2,4-dicarboxylate (**4g**) (50 mg, 0.18 mmol) in CH_2Cl_2 (5 mL) was added dropwise 1 M BBr_3 (1.09 mL, 1.09 mmol) in CH_2Cl_2. After the reaction was stirred overnight, CH_2Cl_2 (10 mL) was added to the reaction followed by ice−water (20 mL). The organic solvent was evaporated off at room temperature. To the aqueous

solution was added concentrated NaOH (0.5 mL), and the solution was filtered to remove the solid impurities. The product was then precipitated out by the addition of 6 M HCl and chilled at 0 °C. The solid was triturated (1 mL H_2O) and washed (2 × 1 mL of H_2O) to yield a light orange powder (12 mg, 29% yield): ^{1}H NMR (DMSO-d_6) δ 10.33 (s, 1H), 8.45 (s, 1H), 8.18 (d, J = 8.3 Hz, 1H), 7.71 (t, J = 8.3 Hz, 1H), 7.25 (d, J = 8.3 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 168.4, 166.2, 155.7, 145.6, 139.4, 139.3, 133.5, 128.2, 122.5, 117.0, 113.8; HRMS (FAB) m/z calcd for $C_{11}H_7NO_5$ (M^+) 233.0324, found 233.0331; HPLC 97% (t_R = 5.1 min, 60:40; t_R = 2.1 min, 40:60).

6-Methoxyquinoline-2,4-dicarboxylic Acid (1e). *p*-Anisidine (**2e**) was converted to 6-methoxy-QDC dimethyl ester using BF_3·OEt_2 as the acid catalyst. The product was chromatographed (hexanes/EtOAc, 3:1) to yield a light beige solid (10.5% yield): mp 152−156 °C; ^{1}H NMR ($CDCl_3$) δ 8.69 (s, 1H), 8.27 (d, J = 2.7 Hz, 1H), 8.21 (d, J = 9.2 Hz, 1H), 7.46 (dd, J = 2.7, 9.2 Hz, 1H), 4.07 (s, 3H), 4.03 (s, 3H), 3.98 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.2, 165.5, 161.1, 145.1, 144.6, 133.1, 132.7, 128.4, 123.9, 123.1, 103.0, 55.7, 53.2, 52.7. Anal. ($C_{14}H_{13}NO_5$) C, H, N. Dimethyl 6-methoxyquinoline-2,4-dicarboxylate (**4e**) (50 mg, 0.18 mmol) was hydrolyzed to yield a yellow product (36 mg, 80% yield): ^{1}H NMR (DMSO-d_6) δ 8.48 (s, 1H), 8.24 (d, J = 3.0 Hz, 1H), 8.13 (d, J = 9.4 Hz, 1H), 7.57 (dd, J = 3.0, 9.4 Hz, 1H), 3.92 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 168.6, 167.4, 161.6, 147.1, 145.8, 136.2, 133.7, 128.9, 124.8, 123.9, 105.1, 57.2; HRMS (ESI) m/z calcd for $C_{12}H_{10}NO_5$ (M + H) 248.0559, found 248.0550; HPLC 99% (t_R = 2.7 min, 60:40; t_R = 6.6 min, 70:30).

7-Methoxyquinoline-2,4-dicarboxylic Acid (1f). *m*-Anisidine (**2f**) was converted to the 7-methoxy-QDC dimethyl ester, which was chromatographed (hexanes/EtOAc, 3:1) to afford a light yellow solid (40% yield): mp 148−149 °C; ^{1}H NMR ($CDCl_3$) δ 8.75 (d, J = 9.4 Hz, 1H), 8.55 (s, 1H), 7.68 (d, J = 2.9 Hz, 1H), (dd, J = 9.4, 2.9 Hz, 1H), 4.10 (s, 3H), 4.05 (s, 3H), 3.37 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.1, 161.3, 159.8, 150.6, 147.5, 135.8, 126.5, 124.0, 121.9, 120.2, 108.4, 55.8, 53.4, 52.9. Anal. ($C_{14}H_{13}NO_5$) C, H, N. Dimethyl 7-methoxyquinoline-2,4-dicarboxylate (**4f**) (50 mg, 0.18 mmol) was hydrolyzed to yield a yellow product (38 mg, 84% yield): ^{1}H NMR (DMSO-d_6) δ 8.69 (d, J = 9.0 Hz, 1H), 8.31 (s, 1H), 7.59 (d, J = 2.4 Hz, 1H), 7.48 (dd, J = 2.4, 9.0 Hz, 1H), 3.96 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 168.6, 167.4, 162.2, 151.6, 150.2, 138.4, 128.2, 124.5, 122.3, 120.8, 109.8, 57.2; HRMS (FAB) m/z calcd for $C_{12}H_9NO_5$ (M^+) 247.0481, found 247.0477; HPLC 99% (t_R = 2.2 min, 50:50; t_R = 1.6 min, 30:70).

8-Methoxyquinoline-2,4-dicarboxylic Acid (1g). *o*-Anisidine (**2g**) was converted to the 8-methoxy-QDC dimethyl ester, which was chromatographed (hexanes/EtOAc, 3:1) to afford a light yellow solid (43% yield): mp 155−156 °C; ^{1}H NMR ($CDCl_3$) δ 8.72 (s, 1H), 8.37 (d, J = 8.5 Hz, 1H), 7.70 (app t, J = 8.5 Hz, 1H), 7.16 (d, J = 8.5 Hz, 1H), 4.11 (s, 3H), 4.18 (s, 3H), 4.06 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.1, 165.4, 156.1, 146.0, 141.0, 136.1, 131.1, 127.5, 122.8, 117.0, 108.4, 56.3, 53.3, 53.0. Anal. ($C_{14}H_{13}NO_5$) C, H, N. Dimethyl 8-methoxyquinoline-2,4-dicarboxylate (**4g**) was hydrolyzed to yield a yellow product (33 mg, 73% yield): ^{1}H NMR (DMSO-d_6) δ 8.37 (s, 1H), 8.21 (d, J = 7.6 Hz, 1H), 7.67 (t, J = 7.6 Hz, 1H), 7.28 (d, J = 7.6 Hz, 1H), 3.98 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 167.6, 166.2, 155.7, 148.1, 139.7, 139.2, 130.0, 126.5, 121.4, 117.0, 108.9, 55.8; HRMS (ESI) m/z calcd for $C_{12}H_{10}NO_5$ (M + H) 248.0559, found 248.0556; HPLC 99% (t_R = 3.2 min, 60:40; t_R = 1.7 min, 40:60).

6-Ethoxyquinoline-2,4-dicarboxylic Acid (1h). 4-Ethoxyaniline (**2h**) was converted to dimethyl 6-ethoxyquinoline-2,4-dicarboxyate. Chromatography (hexanes/EtOAc, 4:1) afforded a white solid (26% yield): mp 138−139 °C; ^{1}H NMR ($CDCl_3$) δ 8.69 (s, 1H), 8.25 (d, J = 3.0 Hz, 1H), 8.21 (d, J = 9.4 Hz, 1H), 7.44 (dd, J = 3.0, 9.4 Hz, 1H), 4.22 (q, J = 7.0 Hz, 2H), 4.07 (s, 3H), 4.03 (s, 3H), 1.49 (t, J = 7.0 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 167.3, 166.6, 161.6, 146.1, 145.5, 134.1, 133.8, 129.6, 125.2, 124.3, 104.6, 65.2, 54.3, 53.8, 15.6. Anal. ($C_{15}H_{15}NO_5$) C, H, N. Dimethyl 6-ethoxyquinoline-2,4-dicarboxylate (**4h**) (25 mg, 0.08 mmol) was hydrolyzed to yield a yellow solid (21 mg, 88% yield): ^{1}H NMR (DMSO-d_6) δ 8.48 (s, 1H), 8.23 (d, J = 3.0 Hz, 1H), 8.13 (d, J = 9.6 Hz, 1H), 7.56 (dd, J = 3.0, 9.6 Hz, 1H), 4.20 (q, J = 7.0 Hz, 2H), 1.42 (t, J = 7.0 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 168.6, 167.4, 160.9, 147.0, 145.7, 136.2, 133.7, 129.0, 125.0, 123.9, 105.7, 65.4, 16.0; HRMS (ESI) m/z calcd for $C_{13}H_{12}NO_5$ (M + H) 262.0716, found 262.0720; HPLC 99% (t_R = 6.3 min, 60:40; t_R = 2.3 min, 40:60).

6-(2-Hydroxyethoxy)quinoline-2, 4-dicarboxylic Acid (1i). 2-(4-Aminophenyl)ethanol (**2i**) was converted to dimethyl 6-(2-hydroxyethoxy)quinoline-2,4-dicarboxylate. Chromatography (hexanes/EtOAc, 1:2) afforded a light yellow solid (20% yield): mp 171−173 °C; ^{1}H NMR ($CDCl_3$) δ 8.71 (s, 1H), 8.31 (d, J = 2.6 Hz, 1H), 8.24 (d, J = 9.2 Hz, 1H), 7.49 (dd, J = 2.6, 9.2 Hz, 1H), 4.29 (t, J = 4.2 Hz, 2H), 4.08 (s, 3H), 4.06 (t, J = 4.2 Hz, 2H), 4.04 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 167.2, 166.5, 161.2, 146.3, 145.9, 134.3, 133.9, 129.4, 124.9, 124.3, 105.0, 70.8, 62.3, 54.3, 53.9. Anal. ($C_{15}H_{15}NO_6$) C, H, N. Dimethyl 6-(2-hydroxyethoxy)quinoline-2,4-dicarboxylate (**4i**) (25 mg, 0.08 mmol) was hydrolyzed to yield a bright yellow solid (22 mg, 92% yield): ^{1}H NMR (DMSO-d_6) δ 8.49 (s, 1H), 8.25 (d, J = 3.2 Hz, 1H), 8.15 (d, J = 9.2 Hz, 1H), 7.60 (dd, J = 3.2, 9.2 Hz, 1H), 4.16 (t, J = 5.2 Hz, 2H), 3.81 (t, J = 5.2 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 178.0, 168.6, 167.4, 161.0, 147.1, 145.7, 136.1, 133.8, 128.9, 124.9, 123.9, 105.9, 71.6, 60.8; HRMS (ESI) m/z calcd for $C_{13}H_{12}NO_6$ (M + H) 278.0665, found 278.0658; HPLC 96% (t_R = 2.3 min, 60:40; t_R = 1.8 min, 50:50).

6-Hexyloxyquinoline-2,4-dicarboxylic Acid (1j). 4-Hexyloxyaniline (**2j**) was converted to dimethyl 6-hexyloxyquinoline-2,4-dicarboxylate. Chromatography (hexanes/EtOAc, 95:5) afforded a yellow solid (36% yield): mp 49−50 °C; ^{1}H NMR ($CDCl_3$) δ 8.08 (s, 1H), 8.24 (d, J = 2.4, Hz, 1H), 8.19 (d, J = 8.8 Hz, 1H), 7.44 (dd, J = 2.4, 8.8 Hz, 1H), 4.12 (t, J = 6.6 Hz, 2H), 4.06 (s, 3H), 4.02 (s, 3H), 1.84 (q, J = 6.6 Hz, 2H), 1.49 (q, J = 2.4 Hz, 2H), 1.37−1.32 (m, 4H), 0.89 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 167.3, 166.6, 161.8, 146.1, 145.4, 134.1, 133.7, 129.6, 125.3, 124.2, 104.6, 69.6, 54.2, 53.8, 32.6, 30.0, 26.8, 23.6, 15.1. Anal. ($C_{19}H_{23}NO_5$) C, H, N. Dimethyl 6-hexyloxyquinoline-2,4-dicarboxylate (**4j**) (20 mg, 0.06 mmol) was hydrolyzed to yield a white solid (17 mg, 89% yield): ^{1}H NMR (DMSO-d_6) δ 8.35 (s, 1H), 8.27 (d, J = 2.8 Hz, 1H), 8.08 (d, J = 9.2 Hz, 1H), 7.24 (dd, J = 2.8, 9.2 Hz, 1H), 4.07 (t, J = 7.1 Hz, 2H), 1.77 (q, J = 7.1 Hz, 2H), 1.43 (q, J = 7.1 Hz, 2H), 1.31−1.28 (m, 4H), 0.86−0.83 (m, 3H); ^{13}C NMR (DMSO-d_6) δ 169.4, 167.8, 160.3, 145.5, 133.4, 129.0, 124.5, 123.2, 116.8, 116.7, 106.2, 69.5, 32.5, 30.0, 26.7, 23.6, 15.4; HRMS (ESI) m/z calcd for $C_{17}H_{19}NO_5$ (M + Na) 340.1161, found 340.1165; m/z calcd for $C_{17}H_{18}NO_5$ (M + H) 316.1185, found 316.1178; HPLC 98% (t_R = 8.5 min, 50:50; t_R = 25.6 min, 40:60).

6-Cyclohexyloxyquinoline-2,4-dicarboxylic Acid (1k). 4-Cyclohexyloxyaniline (**2k**) was converted to dimethyl 6-cyclohexyloxyquinoline-2,4-dicarboxylate. Chromatography (hexanes/EtOAc, 4: 1) afforded a light yellow solid (53% yield): mp 49−50 °C; ^{1}H NMR ($CDCl_3$) δ 8.70 (s, 1H), 8.29 (d, J = 2.6 Hz, 1H), 8.22 (d, J = 9.2 Hz, 1H), 7.45 (dd, J = 2.6, 9.2 Hz, 1H), 4.53 (m, J = 4.0 Hz, 1H), 4.07 (s, 3H), 4.04 (s, 3H), 2.09−2.03 (m, 2H), 1.86−1.78 (m, 2H), 1.68−1.59 (m, 3H); 1.51−1.32 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 167.4, 166.7, 160.5, 145.9, 145.3, 134.0, 133.9, 125.8, 124.2, 119.8, 118.4, 106.0, 76.9, 54.3, 53.8, 32.4, 26.6, 24.6; HRMS (ESI) m/z calcd for $C_{19}H_{22}NO_5$ (M + H) 344.1498, found 344.1487. Dimethyl 6-cyclohexyloxyquinoline-2,4-dicarboxylate (**4k**) (8 mg, 0.02 mmol) was hydrolyzed to yield a light yellow solid (5 mg, 65% yield): ^{1}H NMR (DMSO-d_6) δ 8.46 (s, 1H), 8.24 (d, J = 2.6 Hz, 1H), 8.11 (d, J = 9.2 Hz, 1H), 7.56 (dd, J = 2.6, 9.2 Hz, 1H), 4.52 (q, J = 4.6 Hz, 1H), 2.01 (m, 2H), 1.72 (m, 2H), 1.53−1.14 (m, 6H); ^{13}C NMR (DMSO-d_6) δ 168.6, 167.4, 159.7, 147.0, 125.5, 136.0, 133.8, 128.9, 125.2, 123.8, 107.3, 76.8, 32.5, 26.5, 24.6; HRMS (ESI) m/z calcd for $C_{17}H_{18}NO_5$ (M + H) 316.1185, found 316.1178; HPLC 99% (t_R = 12.9 min, 50:50; t_R = 5.3 min, 40:60).

6-(6-Hydroxyhexyloxy)quinoline-2,4-dicarboxylic Acid (1l). 6-(4-Aminophenoxy)hexan-1-ol (**2l**) was converted to dimethyl 6-(6-hydroxyhexyloxy)quinoline-2,4-dicarboxylate. Chromatography (hexanes/EtOAc, 1:2) afforded a light yellow

solid (34% yield): mp 104−105 °C; ^{1}H NMR ($CDCl_3$) δ 8.70 (s, 1H), 8.26 (d, J = 2.4 Hz, 1H), 8.20 (d, J = 9.8 Hz, 1H), 7.45 (dd, J = 2.4, 9.8 Hz, 1H), 4.15 (t, J = 6.7 Hz, 2H), 4.07 (s, 3H), 4.03 (s, 3H), 3.66 (t, J = 6.7 Hz, 2H), 1.88 (m, J = 6.7 Hz, 2H), 1.63−1.43 (m, 8H); ^{13}C NMR ($CDCl_3$) δ 167.4, 166.6, 161.7, 146.1, 145.4, 134.1, 133.8, 129.6, 125.3, 124.2, 104.6, 69.5, 63.9, 54.3, 33.7, 30.0, 26.9, 26.6; HRMS (ESI) *m/z* calcd for $C_{19}H_{22}NO_5$ (M + H) 362.1604, found 362.1602. Dimethyl 6-(6-hydroxyhexyloxy)quinoline-2,4-dicarboxylate (**4l**) (20 mg, 0.05 mmol) was hydrolyzed to yield a beige solid (13 mg, 68% yield): ^{1}H NMR (DMSO-d_6) δ 8.47 (s, 1H), 8.22 (d, J = 2.6 Hz, 1H), 8.11 (d, J = 9.0 Hz, 1H), 7.56 (dd, J = 2.6, 9.0 Hz, 1H), 4.12 (t, J = 6.4 Hz, 2H); 3.38 (t, J = 6.4 Hz, 2H), 1.79 (m, J = 6.8 Hz, 2H), 1.46−1.34 (m, 6H); ^{13}C NMR (DMSO-d_6) δ 168.6, 167.4, 161.0, 145.7, 136.1, 133.7, 129.0, 125.0, 123.9, 105.7, 69.6, 62.1, 34.0, 30.0, 26.9, 26.8; HRMS (ESI) *m/z* calcd for $C_{17}H_{20}NO_6$ (M + H) 334.1291, found 334.1287; HPLC 98% (t_R = 4.4 min, 50:50; t_R = 2.4 min, 40:60).

6-Phenoxyquinoline-2,4-dicarboxylic Acid (1m). 4-Aminobiphenyl ether (**2m**) (400 mg, 2.16 mmol) was converted to dimethyl 6-phenoxyquinoline-2,4-dicarboxylate. Chromatography (hexanes/EtOAc, 4:1) yielded a white solid (168 mg, 23% yield): mp 124−125 °C; ^{1}H NMR ($CDCl_3$) δ 8.68 (s, 1H), 8.31 (d, J = 8.4 Hz, 1H), 8.30 (s, 1H), 7.57 (d, J = 8.4 Hz, 1H), 7.43 (t, J = 7.6 Hz, 2H), 7.23 (t, J = 7.6 Hz, 1H), 7.13 (d, J = 7.6 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ166.9, 166.4, 160.4, 156.5, 146.8, 146.6, 135.4, 134.3, 131.2, 128.9, 125.9, 124.9, 124.2, 121.3, 117.3, 111.2, 54.4, 53.8; TLC R_f 0.21 (hexanes/EtOAc, 3:1). Anal. ($C_{19}H_{15}NO_5$) C, H, N. Dimethyl 6-phenoxyquinoline-2,4-dicarboxylate (**4m**) (35 mg, 0.104 mmol) was hydrolyzed to yield a white solid (26 mg, 76% yield): ^{1}H NMR (DMSO-d_6) δ 8.48 (s, 1H), 8.26 (d, J = 9.4 Hz, 1H), 8.24 (d, J = 3.4 Hz, 1H), 7.69 (dd, J = 3.4, 9.4 Hz, 1H), 7.49 (t, 8.1 Hz, 2H), 7.27 (t, J = 8.1 Hz, 1H), 7.21 (d, J = 8.1 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 168.2, 167.3, 159.8, 156.6, 148.3, 146.4, 136.8, 134.5, 131.9, 128.5, 126.5, 125.3, 123.9, 121.6, 111.3; HRMS (ESI) *m/z* calcd for $C_{17}H_{12}NO_5$ (M + H) 310.0715, found 310.0718; HPLC 97% (t_R = 8.5 min, 50:50; t_R = 3.9 min, 40:60).

6-Benzyloxyquinoline-2,4-dicarboxylic Acid (1n). 4-Benzyloxyaniline (**2n**) (694 mg, 3.47 mmol) was converted to dimethyl 6-benzyloxyquinoline-2,4-dicarboxylate. Chromatography (hexanes/EtOAc, 3: 1) afforded a light yellow solid (14% yield): mp 184−186 °C dec; ^{1}H NMR ($CDCl_3$) δ 8.70 (s, 1H), 8.39 (d, J = 2.8 Hz, 1H), 8.23 (d, J = 8.8 Hz, 1H), 7.54−7.49 (m, 3H), 7.41 (t, J = 6.8 Hz, 2H), 7.35 (d, J = 6.8 Hz, 1H), 5.2 (s, 2H), 4.07 (s, 3H), 4.03 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.2, 165.5, 160.2, 145.2, 144.7, 135.9, 133.4, 132.8, 128.7, 128.4, 128.7, 127.9, 127.5, 124.2, 123.2, 104.2, 70.5, 53.2, 52.7; HRMS (FAB) *m/z* calcd for $C_{20}H17NO_5$ (M^+) 351.1107, found 351.1103. Dimethyl 6-benzyloxyquinoline-2,4-dicarboxylate (**4n**) (25 mg, 0.0712 mmol) was hydrolyzed to yield light yellow needles (24 mg, 96% yield): mp 184−186 °C dec; ^{1}H NMR (DMSO-d_6) δ 8.70 (d, J = 9.6 Hz, 1H), 8.30 (s, 1H), 7.67 (d, J = 2.6 Hz, 1H), 7.55 (dd, J = 2.6, 9.6 Hz, 1H), 7.51 (d, J = 6.8 Hz, 1H), 7.42−7.33 (m, Ar-H, 5H), 5.33 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 168.6, 167.4, 161.1, 151.5, 150.4, 138.2, 137.9, 130.1, 129.6, 129.3, 129.2, 128.3, 124.8, 122.4, 120.9, 111.1, 71.2; HRMS (FAB) *m/z* calcd for $C_{18}H_{13}NO_5$ (M^+) 323.0794, found 323.0790; HPLC 97% (t_R = 2.3 min, 50:50; t_R = 4.3 min, 70:30).

7-Benzyloxyquinoline-2,4-dicarboxylic Acid (1o). 3-Benzyloxyaniline (**2o**) was converted to dimethyl 7-benzyloxyquinoline-2,4-dicarboxylate. Chromatography (hexanes/EtOAc, 3:1) afforded a light yellow solid (26%): mp 184−186 °C; ^{1}H NMR ($CDCl_3$) δ 8.70 (s, 1H), 8.39 (d, J = 3.0 Hz, 1H), 8.23 (d, J = 8.8 Hz, 1H), 7.53 (dd, J = 3.0, 8.8 Hz, 1H), 7.49 (d, J = 6.8 Hz, 1H), 7.42−7.34 (m, 4H), 5.23 (s, 2H), 4.07 (s, 3H), 4.03 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.2, 165.5, 160.2, 145.2, 144.7, 135.9, 133.2, 132.8, 128.7, 128.4, 127.9, 127.4, 124.2, 123.2, 104.2, 70.5, 53.2, 52.7. Anal. ($C_{20}H_{17}NO_5$) C, H, N. Dimethyl 7-benzyloxyquinoline-2,4-dicarboxylate (**4o**) (25 mg, 0.07 mmol) was hydrolyzed to yield a gold-colored solid (24 mg, 96% yield): mp 149−150 °C; ^{1}H NMR (DMSO-d_6) δ 8.69 (d, J = 9.2 Hz, 1H), 8.24 (s, 1H), 7.64 (d, J = 2.8 Hz, 1H), 7.53−7.50 (m, 3H), 7.40 (t, J = 7.0 Hz, 2H), 7.33 (t, J = 7.0 Hz, 1H), 5.32 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 168.8, 167.5, 161.0, 151.3, 150.5, 138.0, 130.1, 129.6, 129.4, 128.5, 124.5, 122.4, 120.7, 110.9, 71.2; HRMS (FAB) *m/z* calcd for $C_{18}H_{13}NO_5$ (M^+) 323.0794, found 323.0794; HPLC 99% (t_R = 7.1 min, 50:50; t_R = 3.8 min, 40:60).

6-(β-Phenylethyloxy)quinoline-2,4-dicarboxylic Acid (1p). 4-(β-Phenylethyloxy)aniline (**2p**) was converted to dimethyl 6-(β-phenylethyloxy)quinoline-2,4-dicarboxylate. Chromatography (hexanes/EtOAc, 4:1) afforded a white solid (34% yield): mp 109−110 °C; ^{1}H NMR ($CDCl_3$) δ 8.70 (s, 1H), 8.28 (d, J = 2.6 Hz, 1H), 8.21 (d, J = 9.2 Hz, 1H), 7.46 (dd, J = 2.6, 9.2 Hz, 1H), 7.33 (dd, J = 1.2, 4.8 Hz, 2H), 7.32 (s, 2H), 7.25 (m, 1H), 4.37 (t, J = 7.0 Hz, 2H), 4.07 (s, 3H), 4.02 (s, 3H), 3.18 (t, J = 7.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 167.3, 166.6, 161.4, 146.2, 145.6, 138.9, 134.2, 133.8, 130.2, 129.6, 129.5, 127.7, 125.3, 124.2, 104.7, 70.2, 54.3, 53.8, 36.6. Anal. ($C_{21}H_{19}NO_5$) C, H, N. Dimethyl 6-(β-phenylethyloxy)quinoline-2,4-dicarboxylate (**4p**) (25 mg, 0.07 mmol) was hydrolyzed to yield a white solid (22 mg, 92% yield): ^{1}H NMR (DMSO-d_6) δ 8.49 (s, 1H), 8.28 (d, J = 2.8 Hz, 1H), 8.13 (d, J = 9.6 Hz, 1H), 7.57 (dd, J = 2.8, 9.6 Hz, 1H), 7.38 (d, J = 6.8 Hz, 2H), 7.33 (t, J = 6.8 Hz, 2H), 7.24 (t, J = 6.8 Hz, 1H), 4.37 (t, J = 6.8 Hz, 2H), 3.14 (t, J = 6.8 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 168.6, 167.4, 160.7, 147.1, 145.8, 139.5, 136.1, 133.8, 130.6, 129.9, 128.9, 128.0, 124.9, 124.0, 105.9, 70.3, 36.1; HRMS (ESI) *m/z* calcd for $C_{19}H_{16}NO_5$ (M + H) 338.1029, found 338.1032; HPLC 96% (t_R = 13.9 min, 50:50; t_R = 5.7 min, 40:60).

5,7-Dimethoxyquinoline-2,4-dicarboxylic Acid (1q). 3,5-Dimethoxyaniline (**2q**) was converted to dimethyl 5,7-dimethoxyquinoline-2,4-dicarboxylate. Chromatography (hexanes/EtOAc, 2:1) yielded a bright yellow product (17% yield): mp 133−136 °C; ^{1}H NMR ($CDCl_3$) δ 7.92 (s, 1H), 7.25 (d, J = 2.2 Hz, 1H), 6.63 (d, J = 2.2 Hz, 1H), 4.04 (s, 3H), 3.97 (s, 3H), 3.92 (s, 3H), 3.91 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.3, 166.3, 163.2, 156.0, 151.5, 149.0, 139.9, 117.4, 114.3, 102.7, 102.5, 57.7, 57.0, 54.3, 53.8. Anal. ($C_{15}H_{15}NO_6$) C, H, N. Dimethyl 5,7-dimethoxyquinoline-2,4-dicarboxylate (**4q**) (50 mg, 0.16 mmol) was hydrolyzed to yield a bright yellow solid (23 mg, 48% yield): ^{1}H NMR (DMSO-d_6) δ 7.65 (s, 1H), 7.12 (d, J = 2.4 Hz, 1H), 6.78 (d, J = 2.4 Hz, 1H), 3.90 (s, 3H), 3.86 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 171.3, 167.6, 163.1, 156.8, 152.3, 151.0, 142.6, 116.7, 113.1, 102.4, 58.0, 57.2; HRMS (FAB) *m/z* calcd for $C_{13}H_{11}NO_6$ (M^+) 277.0586, found 277.0576; HPLC 96% (t_R = 3.8 min, 50:50; t_R = 2.5 min, 40:60).

5,8-Dimethoxyquinoline-2,4-dicarboxylic Acid (1r). 2,5-Dimethoxyaniline (**2r**) was converted to dimethyl 5,8-dimethoxyquinoline-2,4-dicarboxylate. Chromatography (hexanes/EtOAc, 3:1) afforded a dark brown solid (15% yield): mp 186−187 °C; ^{1}H NMR ($CDCl_3$) δ 8.16 (s, 1H), 7.06 (d, J = 8.8 Hz, 1H), 6.98 (d, J = 8.8 Hz, 1H), 4.06 (s, 6H), 4.02 (s, 3H), 3.94 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.1, 166.3, 151.4, 148.6, 147.6, 141.2, 140.1, 120.2, 119.3, 109.6, 109.2, 58.0, 57.3, 54.3, 53.9. Anal. ($C_{15}H_{15}NO_6$) C, H, N. Dimethyl 5,8-dimethoxyquinoline-2,4-dicarboxylate (**4r**) (50 mg, 0.16 mmol) was hydrolyzed to yield a yellow solid (33 mg, 69% yield): ^{1}H NMR (DMSO-d_6) δ 7.87 (s, 1H), 7.16 (d, J = 8.8 Hz, 1H), 7.02 (d, J = 8.8 Hz, 1H), 3.92 (s, 3H), 3.80 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 171.2, 167.6, 151.2, 151.0, 149.1, 142.4, 140.8, 119.4, 118.1, 110.6, 109.8, 58.1, 57.4; HRMS (ESI) *m/z* calcd for $C_{13}H_{11}NO_6$ (M + H) 278.0665, found 278.0665; HPLC 99% (t_R = 2.1 min, 60:40; t_R = 1.5 min, 40:60).

6,7-Dimethoxyquinoline-2,4-dicarboxylic Acid (1s). 3,4-Dimethoxyaniline (**2s**) was converted to dimethyl 6,7-dimethoxyquinoline-2,4-dicarboxylate, which was triturated with EtOAc to yield a dark green-brown solid (26% yield): mp 186−188 °C; ^{1}H NMR ($CDCl_3$) δ 8.61 (s, 1H), 8.32 (s, 1H), 7.63 (s, 1H), 4.06 (br, 6H), 4.02 (s, 3H), 4.02 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.4, 165.6, 153.2, 146.4, 144.7, 132.6, 123.6, 121.2, 109.1, 103.0, 56.4, 56.3, 53.2, 52.7. Anal. ($C_{15}H_{15}NO_6$) C, H, N. Dimethyl 6,7-dimethoxyquinoline-2,4-dicarboxylate (**4s**) (50 mg, 0.16 mmol) was hydrolyzed to yield bright yellow needles (32 mg, 70% yield): ^{1}H NMR (DMSO-d_6) δ 8.36 (s, 1H), 8.22 (s, 1H), 7.56(s, 1H), 3.96 (s, 3H), 3.93 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 167.8, 166.5, 153.3, 152.7, 146.2, 146.0, 134.4, 122.8,

120.7, 109.1, 101.8, 56.4, 56.2; HRMS (ESI) *m/z* calcd for $C_{13}H_{11}NO_6$ (M + H) 278.0665, found 278.0667; HPLC 99% (t_R = 9.2 min, 60:40; t_R = 2.6 min, 40:60).

6,8-Dimethoxyquinoline-2,4-dicarboxylic Acid (1t). 2,4-Dimethoxyaniline (**2t**) was converted to dimethyl 6,8-dimethoxyquinoline-2,4-dicarboxylate. Chromatography (EtOAc) afforded a light yellow solid (12.6% yield): mp 160−162 °C; ^{1}H NMR ($CDCl_3$) δ 8.73 (s, 1H), 7.87 (d, J = 2.4 Hz, 1H), 6.76 (d, J = 2.4 Hz, 1H), 4.04 (s, 3H), 4.03 (s, 3H), 4.02 (s, 3H), 3.97 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.3, 165.8, 162.2, 157.1, 142.3, 137.9, 133.1, 129.4, 123.4, 102.1, 95.5, 56.4, 55.8, 53.1, 52.7. Anal. ($C_{15}H_{15}NO_6$) C, H, N. Dimethyl 6,8-dimethoxyquinoline-2,4-dicarboxylate (**4t**) (50 mg, 0.16 mmol) was hydrolyzed to yield bright yellow needles (32 mg, 70% yield): mp 235−236 °C dec; ^{1}H NMR (DMSO-d_6) δ 8.46 (s, 1H), 7.78 (d, J = 2.2 Hz, 1H), 6.95 (d, J = 2.2 Hz, 1H), 3.98 (s, 3H), 3.91 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 168.8, 167.5, 162.6, 158.6, 145.4, 138.6, 136.0, 129.7, 124.5, 103.6, 96.7, 57.5, 57.1. Anal. ($C_{13}H_{11}NO_6 \cdot H_2O$) C, H, N.

[1,3]Dioxolo[4,5-*g*]quinoline-6,8-dicarboxylic Acid (1u). Benzo[1,3]dioxolo-5-ylamine (**2u**) was converted to dimethyl [1,3]dioxolo[4,5-*g*]quinoline-6,8-dicarboxylate following chromatography (hexanes/EtOAc, 3:1) to afford a white solid (20% yield): mp 217−218 °C; ^{1}H NMR ($CDCl_3$) δ 8.58 (s, 1H), 8.25 (s, 1H), 7.63 (s, 1H), 6.19 (s, 2H), 4.07 (s, 3H), 4.03 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.2, 165.5, 151.4, 147.7, 145.0, 133.9, 125.0, 121.2, 106.9, 102.5, 101.1, 72.9, 53.2, 52.8. Anal. ($C_{14}H_{11}NO_6$) C, H, N. Dimethyl [1,3]dioxolo[4,5-*g*]quinoline-6,8-dicarboxylate (**4u**) (50 mg, 0.17 mmol) was hydrolyzed to a light fluffy foam (37 mg, 77% yield): mp 256 °C dec; ^{1}H NMR (DMSO-d_6) δ 8.33 (s, 1H), 8.11 (s, 1H), 7.51 (s, 1H), 6.29 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 168.7, 167.4, 152.8, 152.4, 148.5, 147.2, 136.5, 125.4, 121.8, 107.4, 104.5, 101.9; HRMS (FAB) *m/z* calcd for $C_{12}H_7NO_6$ (M^+) 261.0237, found 261.0267; HPLC 99% (t_R = 4.0 min, 70:30; t_R = 2.2 min, 50:50).

2,3-Dihydro[1,4]dioxino[2,3-g]quinoline-7,9-dicarboxylic Acid (1v). 2,3-Dihydrobenzo[1,4]dioxin-6-ylamine (**2v**) was converted to dimethyl 2,3-dihydro[1,4]dioxino[2,3-*g*]quinoline-7,9-dicarboxylate. Chromatography (hexanes/EtOAc, 3:1) afforded a light beige solid (52% yield): mp 173−174 °C; ^{1}H NMR ($CDCl_3$) δ 8.53 (s, 1H), 8.30 (s, 1H), 7.76 (s, 1H), 4.40 (s, 4H), 4.05 (s, 3H), 4.01 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 167.3, 160.5, 148.9, 146.6, 146.4, 134.9, 124.1, 122.1, 116.6, 111.5, 65.6, 65.4, 54.3, 53.9. Anal. ($C_{15}H_{13}NO_6$) C, H, N. Dimethyl 2,3-dihydro-[1,4]dioxino[2,3-*g*]quinoline-7,9-dicarboxylate (**4v**) (50 mg, 0.165 mmol) was hydrolyzed to give a yellow solid (43 mg, 90% yield): ^{1}H NMR (DMSO-d_6) δ 8.32 (s, 1H), 8.24 (s, 1H), 7.58 (s, 1H), 4.42 (s, 4H); ^{13}C NMR (DMSO-d_6) δ 168.7, 167.5, 149.0, 148.7, 147.9, 146.4, 136.2, 123.5, 121.7, 115.9, 111.4, 66.0, 65.8; Anal. ($C_{13}H_9NO_6 \cdot {}^5/_4H_2O$) C, H, N.

Quinoline-2,4,8-tricarboxylic Acid (1w). Methyl anthranilate (**2w**) (471 mg, 3.12 mmol) was converted to trimethyl quinoline-2,4,8-tricarboxylate. Chromatography (hexanes/CH_2Cl_2/MeOH, 8:1:1) and overnight storage of the resulting fractions for 12 h led to light yellow needles (20 mg, 2% yield): mp 115−116 °C; ^{1}H NMR ($CDCl_3$) δ 9.00 (dd, J = 1.5, 8.2 Hz, 1H), 8.72 (s, 1H), 8.17 (dd, J = 1.5, 8.2 Hz, 1H), 7.78 (dd, J = 1.5, 8.2 Hz, 1H), 4.07 (s, 3H), 4.06 (s, 3H), 4.06 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 168.5, 166.8, 166.2, 149.4, 146.9, 137.3, 134.0, 132.6, 130.3, 130.0, 127.3, 123.7, 54.4, 54.1, 53.9. Anal. ($C_{15}H_{13}NO_6$) C, H, N. Trimethyl quinoline-2,4,8-tricarboxylate (**4w**) (10 mg, 0.035 mmol) was hydrolyzed to give a white solid (6 mg, 68%): mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 9.04 (d, J = 8.6 Hz, 1H), 8.67 (d, J = 8.6 Hz, 1H), 8.58 (s, 1H), 8.05 (t, J = 8.6 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 167.8, 167.5, 165.5, 148.6, 146.2, 141.5, 136.5, 132.2, 131.7, 127.5, 127.4, 123.5; HRMS (FAB) *m/z* calcd for $C1_2H_8NO_6$ (M + H) 262.0352 found 262.0358; HPLC 93% (t_R = 2.0 min, 70:30; t_R = 2.2 min, 50:50).

5-Phenylquinoline-2,4-dicarboxylic Acid (1x). Dimethyl 6-bromoquinoline-2,4-dicarboxylate (55 mg, 0.17 mmol) and phenylboronic acid (40 mg, 0.25 mmol) were coupled using 10 mg (0.004 mmol) of palladium tetrakistriphenylphosphine to afford dimethyl 5-phenylquinoline-2,4-dicarboxylate. Chromatography (hexanes/EtOAc, 3:1) yielded a beige solid (17 mg, 31% yield): mp 162−163 °C; ^{1}H NMR ($CDCl_3$) δ 9.09 (d, J = 2.0 Hz, 1H), 8.71 (s, 1H), 8.41 (d, J = 8.8 Hz, 1H), 8.11 (dd, J = 8.8, 2.0 Hz, 1H), 7.76 (d, J = 7.2 Hz, 2H), 7.51 (t, J = 7.2 Hz, 2H), 7.43 (t, J = 7.2 Hz, 1H), 4.11 (s, 3H), 4.07 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 167.1, 166.4, 149.1, 148.2, 144.1, 140.9, 136.8, 133.8, 132.7, 131.4, 130.1, 129.5, 129.0, 128.8, 127.7, 124.2, 123.9, 54.5, 54.0. Anal. ($C_{19}H_{15}NO_4 \cdot {}^1/_2H_2O$) C, H, N. Dimethyl 5-phenylquinoline-2,4-dicarboxylate (**4x**) (25 mg, 0.08 mmol) was hydrolyzed to yield a bright yellow solid (18 mg, 75% yield): ^{1}H NMR (DMSO-d_6) δ 8.74 (dd, J = 2.0, 8.0 Hz, 1H), 8.43 (s, 1H), 7.93−7.86 (m, 2H), 7.69 (d, 6.8 Hz, 2H), 7.47 (t, J = 7.6 Hz, 2H), 7.40 (t, J = 7.6 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 168.8, 167.4, 149.5, 146.9, 142.8, 140.2, 139.2, 132.5, 132.4, 131.0, 129.2, 128.8, 127.4, 126.5, 122.8; HRMS (FAB) *m/z* calcd for $C_{17}H_{11}NO_4$ (M^+) 293.0688, found 293.0694; HPLC 99% (t_R = 3.8 min, 40:60; t_R = 7.8 min, 50:50).

6-Phenylquinoline-2,4-dicarboxylic Acid (1y). Dimethyl 6-bromo-2,4-quinolinedicarboxylate (55 mg, 0.17 mmol) and phenylboronic acid (40 mg, 0.25 mmol) were coupled using palladium tetrakis(triphenylphosphine) (0.010 g; 0.004 mmol) to afford dimethyl 6-phenylquinoline-2,4-dicarboxylate. Chromatography (hexanes/EtOAc, 3:1) yielded a beige solid (17 mg, 31%): ^{1}H NMR ($CDCl_3$) δ 9.09 (d, J = 2.0 Hz, 1 H), 8.71 (s, 1 H), 8.41 (d, J = 8.8 Hz, 1 H), 8.11 (dd, J = 8.8 Hz, J = 2.0 Hz, 1 H), 7.76 (d, J = 7.2 Hz, 2 H), 7.51 (t, J = 7.2 Hz, 2 H), 7.43 (t, J = 7.2 Hz, 1 H), 4.11 (s, 3 H), 4.07 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 167.1, 166.4, 149.1, 148.2, 144.1, 140.9, 136.8, 133.8, 132.7, 131.4, 130.1, 129.5, 129.0, 128.8, 127.7, 124.2, 123.9, 54.5, 54.0; TLC R_f 0.22 (hexanes/EtOAc, 3:1). Anal. ($C_{19}H_{15}NO_4 \cdot {}^1/_2H_2O$) C, H, N. Dimethyl 6-phenylquinoline-2,4-dicarboxylate (**4y**) (16 mg, 0.050 mmol) was hydrolyzed to yield a light yellow solid (12 mg, 82%): ^{1}H NMR (DMSO-d_6) δ 9.05 (d, J = 2.0 Hz, 1H), 8.50 (s, 1H), 8.31 (d, J = 8.6 Hz, 1H), 8.24 (dd, J = 2.0, 8.6 Hz, 1H), 7.81 (d, J = 7.3 Hz, 2H), 7.55 (t, J = 7.3 Hz, 2H), 7.46 (t, J = 7.3 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 168.5, 167.3, 149.9, 148.9, 142.9, 140.7, 138.5, 132.7, 131.5, 130.8, 130.0, 128.9, 127.4, 124.5, 123.7; HRMS (FAB) *m/z* calcd for $C_{17}H_{11}NO_4$ (M^+) 293.0688, found 293.0693; HPLC 99% (t_R = 4.0 min, 40:60; t_R = 8.8 min, 50:50).

7-Phenylquinoline-2,4-dicarboxylic Acid (1z). 3-Aminobiphenyl (**2z**) (275 mg, 1.49 mmol) was converted to dimethyl 7-phenylquinoline-2,4-dicarboxylate. Chromatography (hexanes/EtOAc, 4:1) afforded a yellow solid (80 mg, 14%): mp 153−154 °C; ^{1}H NMR ($CDCl_3$) δ 8.94 (d, J = 8.8 Hz, 1 H), 8.70 (s, 1H), 8.61 (d, J = 1.6 Hz, 1 H), 8.07 (dd, J = 9.6, 2.0 Hz, 2 H), 7.80 (dd, J = 3.4 Hz, 1 H), 7.49 (dt, J = 3.4 Hz, 3 H), 4.13 (s, 3 H), 4.10 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 167.1, 166.4, 150.1, 149.0, 144.2, 140.2, 136.9, 131.1, 130.3, 129.5, 128.5, 127.1, 126.5, 123.5, 54.6, 54.0; TLC R_f 0.12 (hexanes/EtOAc, 3:1). Anal. ($C_{19}H_{15}NO_4$) C, H, N. Dimethyl 7-phenylquinoline-2,4-dicarboxylate (**4z**) (20 mg, 0.06 mmol) was hydrolyzed to yield a light yellow solid (13 mg, 68% yield): ^{1}H NMR (DMSO-d_6) δ 8.90 (d, J = 9.0 Hz, 1H), 8.49 (d, J = 2.0 Hz, 1H), 8.47 (s, 1H), 8.23 (dd, J = 2.0, 9.0 Hz, 1H), 7.93 (d, J = 7.2 Hz, 2H), 7.56 (t, J = 7.2 Hz, 2H), 7.47 (t, J = 7.2 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 168.4, 167.3, 150.6, 149.9, 143.4, 139.9, 138.5, 130.8, 130.7, 130.1, 128.8, 128.7, 127.8, 126.2, 123.0; HRMS (FAB) *m/z* calcd for $C_{17}H_{11}NO_4$ (M^+) 293.0688, found 293.0688; HPLC 99% (t_R = 4.1 min, 40:60; t_R = 8.9 min, 50:50).

8-Phenylquinoline-2,4-dicarboxylic Acid (1aa). 2-Aminobiphenyl (**2aa**) (400 mg, 2.37 mmol) was converted to dimethyl 8-phenylquinoline-2,4-dicarboxylate. Chromatography (100% CH_2Cl_2) afforded a light yellow solid (230 mg, 30%): mp 136−137 °C; ^{1}H NMR ($CDCl_3$) δ 8.81 (dd, J = 1.4, 8.8 Hz), 8.66 (s, 1 H), 7.90 (dd, J = 1.4, 7.4 Hz, 1 H), 7.83 (d, J = 7.4 Hz, 1 H), 7.81−7.78 (m, 2 H), 7.50 (t, J = 6.8 Hz, 2 H), 7.43 (t, J = 7.2 Hz, 1 H), 4.07 (s, 3 H), 3.99 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ: 167.4, 166.6, 148.0, 147.2, 143.2, 139.5, 137.4, 132.3, 131.2, 129.0, 128.8, 127.6, 125.9, 123.1, 54.1, 54.0; TLC R_f 0.15 (hexanes/EtOAc, 4:1), R_f 0.34 (CH_2Cl_2). Anal. ($C_{19}H_{15}NO_4$) C, H, N. Dimethyl 8-phenylquinoline-2,4-dicarboxylate (**4aa**) (20 mg, 0.06 mmol) was hydrolyzed to yield a yellow solid (15 mg, 80% yield): ^{1}H NMR (DMSO-d_6) δ 8.76 (dd, J = 1.6, 8.0 Hz,

1H), 8.46 (s, 1H), 7.94−7.88 (m, 2H), 7.71 (d, J = 6.8 Hz, 2H), 7.49 (t, J = 6.8 Hz, 2H), 7.42 (t, J = 6.8 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 168.8, 167.4, 149.6, 142.8, 140.2, 139.3, 132.8, 132.5, 131.3, 129.3, 129.0, 127.3, 126.5, 122.8; HRMS (FAB) m/z calcd for $C_{17}H_{11}NO_4$ (M^+) 293.0688, found 293.0687; HPLC 99% (t_R = 3.8 min, 40:60; t_R = 7.9 min, 50:50).

6-Phenylethylquinoline-2,4-dicarboxylic Acid (1bb). 4-Phenylethylaniline (**2bb**) (109 mg, 0.63 mmol) was converted to dimethyl 6-phenylethylquinoline-2,4-dicarboxylate. Chromatography (hexanes/EtOAc, 4:1) gave a light orange solid (61 mg, 33% yield): mp 128−129 °C; ^{1}H NMR ($CDCl_3$) δ 8.65 (s, 1H), 8.62 (d, J = 1.6 Hz, 1H), 8.26 (d, J = 9.0 Hz, 1H), 7.66 (dd, J = 1.6, 9.0 Hz, 1H), 7.29−7.25 (m, 2H), 7.21−7.12 (m, 3H), 4.09 (s, 3H), 4.04 (s, 3H), 3.16 (dd, J = 8.8, 10.6 Hz, 2H), 3.03 (dd, J = 8.8, 10.6 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 167.2, 166.5, 148.6, 147.7, 145.8, 142.1, 136.3, 133.3, 132.1, 129.5, 127.5, 127.2, 125.0, 123.5, 54.4, 53.9, 39.5, 38.5. Anal. ($C_{21}H_{19}NO_4$) C, H, N. Dimethyl 6-phenylethylquinoline-2,4-dicarboxylate (**4bb**) (20 mg, 0.06 mmol) was hydrolyzed to yield an orange solid (14 mg, 74% yield): ^{1}H NMR (DMSO-d_6) δ 8.56 (d, J = 1.6 Hz, 1H), 8.41 (s, 1H), 8.13 (d, J = 8.8 Hz, 1H), 7.83 (dd, J = 1.6, 8.8 Hz, 1H), 7.24 (d, J = 4.4 Hz, 4H), 7.17−7.24 (m, 1H), 3.13 (dd, J = 9.4, 2.8 Hz, 2H), 2.97 (dd, J = 9.4, 2.8 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 168.6, 167.4, 149.2, 148.3, 145.4, 142.6, 138.0, 133.7, 131.8, 130.0, 129.8, 127.5, 127.0, 125.4, 123.1, 39.0, 38.1; HRMS (ESI) m/z calcd for $C_{19}H_{16}NO_4$ (M + H) 322.1079, found 322.1073; HPLC 99% (t_R = 4.7 min, 40:60; t_R = 10.7 min, 50:50).

7-Phenylethylquinoline-2,4-dicarboxylic acid (1cc). 3-(β-Phenylethyl)aniline (**2cc**) was converted to dimethyl 7-phenylethylquinoline-2,4-dicarboxylate. Chromatography (CH_2Cl_2) afforded a light peach-colored solid (18% yield): mp 103−104 °C; ^{1}H NMR ($CDCl_3$) δ 8.76 (d, J = 8.8 Hz, 1H), 8.65 (s, 1H), 8.19 (s, 1H), 7.59 (dd, J = 1.8, 8.8 Hz, 1H), 7.29 (d, J = 7.6 Hz, 1H), 7.26 (d, J = 1.8 Hz, 1H), 7.20 (t, J = 7.6 Hz, 2H), 4.11 (s, 3H), 4.07 (s, 3H), 3.18 (dd, J = 8.4, 2.8 Hz, 2H), 3.07 (dd, J = 8.4, 2.8 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 167.2, 166.5, 150.5, 148.5, 145.8, 142.0, 136.8, 133.2, 130.8, 129.5, 127.3, 126.4, 125.9, 122.8, 54.4, 54.0, 38.8, 38.1. Anal. ($C_{21}H_{19}NO_4$) C, H, N. Dimethyl 7-phenylethylquinoline-2,4-dicarboxylate (**4cc**) (20 mg, 0.06 mmol) was hydrolyzed to yield an orange solid (13 mg, 69% yield): ^{1}H NMR (DMSO-d_6) δ 8.67 (d, J = 9.0 Hz, 1H), 8.38 (s, 1H), 8.00 (d, J = 3.7 Hz, 1H), 7.73 (dd, J = 3.7, 9.0 Hz, 1H), 7.23 (d, J = 4.4 Hz, 4H), 7.14 (m, J = 4.4 Hz, 1H), 3.14 (t, J = 7.2 Hz, 2H), 3.01 (t, J = 7.2 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 168.6, 167.4, 149.9, 149.7, 146.0, 142.6, 138.4, 133.1, 131.0, 130.4, 130.0, 129.8, 127.5, 126.8, 125.4, 122.4, 38.1, 37.7; HRMS (ESI) m/z calcd for $C_{19}H_{14}NO_4$ (M − H) 320.0923, found 320.0924; HPLC 96% (t_R = 4.7 min, 40:60; t_R = 10.7 min, 50:50).

5-(1-Naphthyl)quinoline-2,4-dicarboxylic Acid (1dd). Dimethyl 5-bromoquinoline-2,4-dicarboxylate (**4ll**) (168 mg, 0.5185 mmol) and palladium tetrakistriphenylphosphine (0.026 mmol, 30 mg) were dissolved in 10 mL of DME and stirred for 0.5 h. To the solution was added naphthalene α-boronic acid (0.78 mmol, 134 mg) followed by 518 μL of 2 M Na_2CO_3. The reaction mixture was held at reflux for 48 h, cooled to room temperature, filtered through Celite, and flushed with CH_2Cl_2 and the solvent removed to afford dimethyl 5-(1-naphthyl)-quinoline-2,4-dicarboxylate. Chromatography (hexanes/EtOAc, 4:1) yielded a light yellow solid (109 mg, 57%): mp 78−79 °C; ^{1}H NMR ($CDCl_3$) δ 8.97 (d, J = 8.8 Hz, 1 H), 8.75 (s, 1 H), 8.55 (d, J = 1.2 Hz, 1 H), 7.98−7.92 (m, 4 H); 7.61−7.52 (m, 3 H), 7.46 (dt, J = 1.2 Hz, J = 8.8 Hz, 1 H); 4.13 (s, 3 H), 4.11 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 167.1, 166.4, 149.9, 149.0, 144.5, 139.4, 137.1, 134.9, 134.2, 132.7, 132.2, 129.7, 129.6, 128.6, 127.6, 127.2, 126.7, 126.6, 126.4, 123.4, 54.6, 54.1; TLC R_f 0.33 (hexanes/EtOAc, 3:1). Anal. ($C_{23}H_{17}NO_4$) C, H, N. Dimethyl 5-(1-naphthyl)quinoline-2,4-dicarboxyate (**4dd**) (20 mg, 0.06 mmol) was hydrolyzed to yield a white solid (79% yield): ^{1}H NMR (DMSO-d_6) δ 8.93 (d, J = 9.0 Hz, 1 H), 8.51 (s, 1 H), 8.28 (d, J = 2.0 Hz, 1 H), 8.06−8.04 (m, 2 H), 7.99 (dd, J = 9.0, 2.0 Hz, 1 H), 7.84 (d, J = 8.4 Hz, 1 H), 7.67−7.61 (m, 2 H), 7.58 (t, J = 7.4 Hz, 1 H), 7.52 (t, J = 7.4 Hz, 1 H); ^{13}C NMR (DMSO-d_6) δ 168.5, 167.3, 150.6, 149.5, 143.8, 139.3, 138.7, 135.0, 133.8, 132.1, 130.1, 129.1, 128.4, 127.8, 127.3, 126.5, 126.2, 123.2; HRMS (ESI) m/z calcd for $C_{21}H_{12}NO_4$ (M − H) 342.0766, found 342.0773; HPLC 99% (t_R = 8.2 min, 40:60; t_R = 24.5 min, 50:50).

6-(1-Naphthyl)quinoline-2,4-dicarboxylic Acid (1ee). 1-(4-Aminophenyl)naphthalene (**2ee**) (238 mg, 1.09 mmol) was converted to dimethyl 6-(1-naphthyl)quinoline-2,4-dicarboxylate. Chromatography (hexanes/EtOAc, 4:1) yielded a bright yellow solid (51 mg, 24%): mp 216−217 °C; ^{1}H NMR ($CDCl_3$) δ 8.97 (d, J = 1.8 Hz, 1 H), 8.76 (s, 1 H), 8.49 (d, J = 8.8 Hz, 1 H), 8.03 (dd, J = 1.8, 8.8 Hz, 1 H), 7.98−7.95 (m, 2 H), 7.90 (d, J = 8.0 Hz, 1 H), 7.62−7.47 (m, 4 H), 4.17 (s, 3 H), 4.05 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 167.1, 166.4, 149.0, 148.5, 144.3, 140.0, 134.9, 134.4, 132.4, 131.9, 129.7, 129.6, 128.8, 127.7, 127.4, 127.2, 126.6, 126.5, 123.8; TLC R_f 0.24 (hexanes/EtOAc, 3:1). Anal. ($C_{23}H_{17}NO_4$) C, H, N. Dimethyl 6-(1-naphthyl)-quinoline-2,4-dicarboxylate (**4ee**) was hydrolyzed to yield a white solid (86% yield): ^{1}H NMR (DMSO-d_6) δ 9.91 (d, 2.0 Hz, 1 H), 8.54 (s, 1 H), 8.36 (d, J = 8.8 Hz, 1 H), 8.04 (dd, J = 2.0, 6.4 Hz, 3 H), 7.80 (d, J = 8.8 Hz, 1 H), 7.64 (t, J = 7.2 Hz, 1 H), 7.58 (dd, J = 1.2, 6.8 Hz, 1 H), 7.53 (dd, J = 1.2, 9.2 Hz, 1 H), 7.50 (d, J = 6.4 Hz, 1 H); ^{13}C NMR (DMSO-d_6) δ 168.45, 167.3, 150.2, 148.8, 143.1, 140.0, 138.4, 134.9, 134.3, 132.2, 132.1, 130.1, 130.0, 129.1, 128.4, 127.8, 127.7, 127.2, 127.1, 126.5, 123.7; HRMS (FAB) m/z calcd for $C_{21}H_{13}NO_4$ (M^+) 343.0845, found 343.0851; HPLC 99% (t_R = 7.4 min, 40:60; t_R = 22.1 min, 50:50).

8-(1-Naphthyl)quinoline-2,4-dicarboxylic acid (1ff). Dimethyl 8-bromoquinoline-2,4-dicarboxylate (**4nn**) (107 mg, 0.33 mmol) and naphthylboronic acid were coupled using palladium tetrakistriphenylphosphine (0.019 g, 0.02 mmol) to form dimethyl 8-(1-naphthyl)quinoline-2,4-dicarboxylate. Chromatography (hexanes/EtOAc, 4:1) yielded a light yellow solid (120 mg, 98%): mp 140−142 °C; ^{1}H NMR ($CDCl_3$) δ 8.92 (dd, J = 2.0 Hz, 8.0 Hz, 1 H), 8.63 (s, 1 H), 7.95−7.84 (m, 4 H), 7.60 (t, J = 7.0 Hz, 1 H), 7.55 (dd, J = 2.0, 6.8 Hz, 1 H), 7.44 (dt, J = 2.0, 7.0 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 167.5, 166.5, 148.5, 148.4, 142.7, 137.9, 137.2, 134.6, 133.9, 130.8, 129.8, 129.2, 129.1, 127.5, 127.4, 126.7, 126.5, 126.4, 126.1, 123.2, 54.0, 53.9; TLC R_f 0.31 (hexanes/EtOAc, 3:1). Anal. ($C_{23}H_{17}NO_4$) C, H, N. Dimethyl 8-(1-naphthyl)quinoline-2,4-dicarboxylate (**4ff**) was hydrolyzed to afford a white solid (66%): ^{1}H NMR (DMSO-d_6) δ 8.88 (d, J = 8.4 Hz, 1 H), 8.43 (s, 1 H), 8.00 (dd, J = 2.8, 8.4 Hz, 2 H), 7.98 (t, J = 7.2 Hz, 1 H), 7.88 (dd, J = 1.6, 7.2 Hz, 1 H), 7.62 (t, J = 7.2 Hz, 1 H), 7.49 (d, J = 7.2 Hz, 1 H), 7.48 (d, J = 7.2 Hz, 1 H), 7.31 (t, J = 8.8 Hz, 1 H), 7.23 (d, J = 8.8 Hz, 1 H); ^{13}C NMR (DMSO-d_6) δ 168.8, 167.1, 149.9, 148.3, 142.2, 139.3, 138.7, 134.6, 134.1, 133.8, 131.0, 129.8, 129.6, 129.3, 127.5, 127.1, 127.0, 126.7, 122.9; HRMS (ESI) m/z calcd for $C_{21}H_{14}NO_4$ (M + H) 344.0923, found 344.0915; HPLC 98% (t_R = 5.5 min, 40:60; t_R = 14.1 min, 50:50).

6-Phenylazoquinoline-2,4-dicarboxylic Acid HCl Salt (1gg). 6-Phenylazoaniline (**2gg**) (250 mg, 1.27 mmol) was converted to dimethyl 6-phenylazoquinoline-2,4-dicarboxylate by refluxing the reaction for 4 days with no addition of catalyst. TsOH was then added, and the resulting solution was refluxed for an additional 12 h. Chromatography (CH_2Cl_2) afforded an orange-red solid (154 mg, 35% yield): mp 175−177 °C; ^{1}H NMR ($CDCl_3$) δ 9.44 (d, J = 2.0 Hz, 1H), 8.75 (s, 1H), 8.44−8.36 (m, 3H), 8.01 (dd, J = 2.0, 8.4 Hz, 1H), 7.56−7.52 (m, 3H), 4.12 (s, 3H), 4.11 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.8, 166.2, 153.6, 153.4, 150.8, 149.3, 138.0, 133.5, 133.0, 132.3, 130.3, 128.0, 126.6, 126.3, 124.4, 124.1, 122.9, 54.6, 54.2. Anal. ($C_{19}H_{15}N_3O_4$) C, H, N. Dimethyl 6-phenylazoquinoline-2,4-dicarboxylate (**4gg**) (50 mg, 0.14 mmol) was hydrolyzed to yield a dark red solid, which was precipitated as the HCl salt (46 mg, 96% yield): ^{1}H NMR (DMSO-d_6) δ 9.41 (d, J = 2.0 Hz, 1H), 8.56 (s, 1H), 8.36 (d, J = 9.2 Hz, 1H), 8.33 (dd, J = 9.2 Hz, 1H), 8.01−7.98 (m, 3H), 7.64−7.62 (m, 3H); ^{13}C NMR (DMSO-d_6) δ 168.2, 167.1, 153.5, 152.7, 151.3, 150.7, 129.4, 136.0, 133.9, 133.8, 131.2, 127.7, 126.5, 124.5, 124.2, 122.8; HRMS (ESI) m/z calcd for $C_{17}H_{12}N_3O_4$ (M + H) 322.0828, found

322.0835; HPLC 97% (t_R = 4.1 min, 40:60; t_R = 7.8 min, 50:50).

5-Fluoroquinoline-2,4-dicarboxylic Acid (1hh). 3-Fluoroaniline (**2hh**) (400 mg, 3.60 mmol) was converted to dimethyl 5-fluoroquinoline-2,4-dicarboxylate using $BF_3(OEt)_2$ as the acid catalyst. Chromatography (hexanes/EtOAc, 3:1) yielded fluffy white crystals (278 mg, 29% yield): mp 119−120 °C; ^{1}H NMR ($CDCl_3$) δ 8.92 (dd, J = 6.0, 9.8 Hz, 1H), 8.66 (s, 1H), 7.99 (dd, J = 2.4, 9.8 Hz, 1H), 7.58−7.53 (m, 1H), 4.10 (s, 3H), 4.06 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.8, 166.1, 165.6, 163.1, 151.0, 150.9, 149.7, 137.1, 129.1, 129.0, 124.4, 122.8, 122.0, 121.7, 115.7, 54.5, 54.1. Anal. ($C_{13}H_{10}NO_4F$) C, H, N. Dimethyl 5-fluoroquinoline-2,4-dicarboxylate (**4hh**) (50 mg, 0.19 mmol) was hydrolyzed to yield a white solid (24 mg, 50% yield): ^{1}H NMR (DMSO-d_6) δ 8.89 (dd, J = 9.6, 6.2 Hz, 1H), 8.44 (s, 1H); 8.01 (dd, J = 2.8, 9.6 Hz, 1H), 7.80 (dt, J = 2.8, 9.6 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 168.3, 167.1, 165.4, 162.9, 151.3, 150.7, 150.6, 139.0, 130.1, 130.0, 124.3, 122.6, 121.9, 121.7, 115.3, 115.2; HRMS (FAB) m/z calcd for $C_{11}H_6NO_4$ (M^+) 235.0281, found 235.0289; HPLC 99% (t_R = 2.0 min, 40:60; t_R = 2.7 min, 50:50).

8-Fluoroquinoline-2,4-dicarboxylic Acid (1ii). 2-Fluoroaniline (**2ii**) (83 mg, 0.75 mmol) was converted to dimethyl 8-fluoroquinoline-2,4-dicarboxylate using $BF_3(OEt)_2$ as the acid catalyst. Chromatography (hexanes/EtOAc, 3:1) yielded off-white crystals (15 mg, 8% yield): mp 141−144 °C; ^{1}H NMR ($CDCl_3$) δ 8.77 (s, 1H), 8.66 (d, J = 8.4 Hz, 1H), 7.73 (m, 1H), 7.55 (m, 1H), 4.11 (s, 3H), 4.09 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.8, 166.1, 160.8, 158.2, 148.8, 137.1, 131.4 (J = 9.1 Hz), 128.7, 124.3, 122.5 (d, J = 4.0 Hz), 54.6, 54.2. Anal. ($C_{13}H_{10}NO_4F$) C, H, N. Dimethyl 8-fluoroquinoline-2,4-dicarboxylate (**4ii**) (50 mg, 0.19 mmol) was hydrolyzed to yield a white powder (35 mg, 73% yield): ^{1}H NMR (DMSO-d_6) δ 8.57 (d, J = 8.4 Hz, 1H), 8.50 (s, 1H), 7.84−7.72 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 168.2, 167.0, 160.5, 157.9, 150.3, 139.7, 139.6, 136.8, 131.8, 131.7,128.4, 124.0, 123.1, 116.3, 116.1; HRMS (ESI) m/z calcd for $C_{11}H_7NO_4F$ (M + H) 236.0359, found 236.0361; HPLC 99% (t_R = 2.2 min, 50:50; t_R = 3.2 min, 60:40).

6-Chloroquinoline-2,4-dicarboxylic Acid (1jj). 4-Chloroaniline (**2jj**) (50 mg, 0.39 mmol) was converted to dimethyl 6-chloroquinoline-2,4-dicarboxylate. Chromatography (hexanes/EtOAc, 7:3) yielded an oil (44 mg, 40% yield): ^{1}H NMR ($CDCl_3$) δ 8.85 (d, J = 1.9 Hz, 1 H), 8.64 (s, 1 H), 8.20 (d, J = 9.5 Hz, 1 H), 7.71 (dd, J = 1.9, 9.5 Hz, 1 H), 4.06 (s, 3 H), 4.02 (s, 3 H); ^{13}C ($CDCl_3$) δ 165.3, 164.8, 147.5, 146.8, 136.8, 134.6, 132.5, 131.6, 126.7, 124.6, 123.1, 53.4, 52.9; HRMS m/z calcd for $C_{13}H_{11}NO_4Cl$ 280.0377, found 280.0378. Dimethyl 6-chloroquinoline-2,4-dicarboxylate (**4jj**) (40 mg, 0.14 mmol) was hydrolyzed to yield a white powder following addition of HCl (15 mg, 43% yield): ^{1}H NMR (DMSO) δ 8.90 (d, J = 1.5 Hz, 1 H), 8.52 (s, 1 H), 8.24 (d, J = 8.9 Hz, 1 H), 7.94 (dd, J = 1.5, 8.9 Hz, 1 H); ^{13}C (DMSO) δ 167.0, 166.0, 149.5, 146.9, 136.3, 135.3, 133.1, 131.7, 126.7, 125.0, 123.3; HRMS m/z calcd for $C_{11}H_7NO_4Cl$ 252.0064, found 252.0053; HPLC 99% (t_R = 2.6 min, 40:60; t_R = 4.1 min, 50:50).

7,8-Dichloroquinoline-2,4-dicarboxylic Acid (1kk). 2,3-Dichloroaniline (**2kk**) (100 mg, 0.62 mmol) was converted to dimethyl 7,8-dichloroquinoline-2,4-dicarboxylate. Chromatography (hexanes/EtOAc, 7:3) yielded an oil (116 mg, 60% yield): ^{1}H NMR ($CDCl_3$) δ 8.70 (d, J = 9.6 Hz, 1 H), 8.65 (s, 1 H), 7.71 (d, J = 9.6 Hz, 1 H), 4.07 (s, 1 H), 4.03 (s, 1 H); ^{13}C ($CDCl_3$) δ 165.3, 164.8, 148.8, 145.8, 136.6, 135.6, 133.4, 131.5, 126.0, 124.6, 123.0, 53.6, 53.3; HRMS m/z calcd for $C_{13}H_{10}NO_4Cl_2$ 313.9987, found 313.9980. Dimethyl 7,8-dichloroquinoline-2,4-dicarboxylate (**4kk**) (70 mg, 0.22 mmol) was hydrolyzed to yield a white powder following addition of HCl (49 mg, 78% yield): ^{1}H NMR (DMSO) δ 8.76 (d, J = 9.5 Hz, 1 H), 8.36 (s, 1 H), 7.89 (d, J = 9.5 Hz, 1 H); ^{13}C (DMSO) δ 167.2, 166.0, 151.7, 144.7, 133.5, 132.2, 131.2, 129.4, 126.7, 125.8, 121.3; HRMS m/z calcd for $C_{11}H_6NO_4Cl_2$ 285.9674, found 285.9666; HPLC 99% (t_R = 3.2 min, 40:60; t_R = 5.8 min, 50:50).

5-Bromoquinoline-2,4-dicarboxylic Acid (1ll). 3-Bromoaniline (**2ll**) (200 mg, 1.16 mmol) was converted to dimethyl 5-bromoquinoline-2,4-dicarboxylate. Chromatography (hexanes/EtOAc, 4:1) afforded a white solid (76 mg, 20% yield): mp 154−156 °C; ^{1}H NMR ($CDCl_3$) δ 8.78 (d, J = 9.2 Hz, 1H), 8.77 (s, 1H), 8.57 (d, J = 2.4 Hz, 1H), 7.84 (dd, J = 2.4, 9.6 Hz, 1H), 4.11 (s, 3H), 4.07 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.2, 166.1, 150.3, 149.6, 137.2, 134.8, 134.4, 128.1, 126.2, 126.1, 123.7, 54.6, 54.2. Anal. ($C_{13}H_{10}NO_4$) C, H, N. Dimethyl 5-bromoquinoline-2,4-dicarboxylate (**4ll**) (50 mg, 0.15 mmol) was hydrolyzed to give a yellow solid (27 mg, 56% yield): ^{1}H NMR (DMSO-d_6) δ 8.76 (d, J = 9.4 Hz, 1H), 8.49 (s, 1H), 8.46 (d, J = 1.8 Hz, 1H), 7.99 (dd, J = 1.8, 9.4 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 168.1, 167.0, 151.3, 150.1, 138.9, 134.5, 133.7, 129.1, 125.9, 125.7, 123.6; HRMS (FAB) m/z calcd for $C_{13}H_{11}BrNO_6$ (M^+) 294.9480 (^{79}Br) and 296.9460 (^{81}Br), found 294.9488 (^{79}Br) and 296.9472 (^{81}Br); HPLC 99% (t_R = 2.3 min, 40:60; t_R = 3.6 min, 50:50).

6-Bromoquinoline-2,4-dicarboxylic Acid (1mm). 4-Bromoaniline (**2mm**) (300 mg, 1.74 mmol) was converted to dimethyl 6-bromoquinoline-2,4-dicarboxylate. Chromatography (CH_2Cl_2) afforded a white solid (60 mg, 11% yield): mp 158−160 °C; ^{1}H NMR ($CDCl_3$) δ 9.11 (d, J = 2.2 Hz, 1H), 8.71 (s, 1H), 8.21 (d, J = 9.0 Hz, 1H), 7.92 (dd, J = 2.2, 9.0 Hz, 1H), 4.10 (s, 3H), 4.07 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.6, 166.1, 148.8, 148.3, 135.9, 135.4, 133.7, 129.1, 128.3, 126.7, 124.3, 54.6, 54.2. Anal. ($C_{13}H_{10}BrNO_4$) C, H, N. Dimethyl 6-bromoquinoline-2,4-dicarboxylate (**4mm**) (50 mg, 0.15 mmol) was hydrolyzed to give a yellow solid (38 mg, 79% yield): ^{1}H NMR (DMSO-d_6) δ 9.08 (d, J = 2.2 Hz, 1H), 8.52 (s, 1H), 8.17 (d, J = 9.0 Hz, 1H), 8.06 (dd, J = 2.2, 9.0 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 168.0, 167.0, 150.6, 148.1, 137.3, 135.3, 134.1, 129.3, 128.2, 125.4, 124.3; HRMS (FAB) m/z calcd for $C_{11}H_6BrNO_4$ (M^+) 294.9480 (^{79}Br) and 296.9460 (^{81}Br), found 294.9482 (^{79}Br) and 296.9462 (^{81}Br); HPLC 99% (t_R = 2.4 min, 40:60; t_R = 4.1 min, 50:50).

8-Bromoquinoline-2,4-dicarboxylic Acid (1nn). 2-Bromoaniline (**2nn**) (300 mg, 1.74 mmol) was converted to dimethyl 8-bromoquinoline-2,4-dicarboxylate. Chromatography (hexanes/EtOAc, 4:1) yielded a white solid (210 mg, 37% yield): mp 149−150 °C; ^{1}H NMR ($CDCl_3$) δ 8.82 (dd, J = 1.2, 8.3 Hz, 1H), 8.71 (s, 1H), 8.18 (dd, J = 1.2, 8.3 Hz, 1H), 7.59 (t, J = 8.3 Hz, 1H), 4.10 (s, 3H), 4.06 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.7, 166.1, 149.2, 146.7, 137.9, 135.5, 131.6, 128.8, 127.9, 126.4, 124.2, 54.5, 54.2; TLC R_f 0.33 (hexanes/EtOAc, 3:1); HRMS (ESI) m/z calcd for $C_{13}H_{11}NO_4Br$ (M + H) 323.9871, found 323.9889. Anal. Found: C, 48.04; H, 3.14; N, 4.23. Calcd for $C_{13}H_{10}BrNO_4$: C, 48.17; H, 3.11; N, 4.32. Dimethyl 8-bromoquinoline-2,4-dicarboxylate (**4nn**) (50 mg, 0.15 mmol) was hydrolyzed to give a yellow solid (31 mg, 64% yield): ^{1}H NMR (DMSO-d_6) δ 8.73 (d, J = 9.6 Hz, 1H), 8.44 (s, 1H), 8.41 (d, J = 1.8 Hz, 1H), 7.94 (dd, J = 1.8, 9.6 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 168.3, 167.1, 151.4, 150.1, 139.6, 134.3, 133.6, 129.2, 125.9, 125.5, 123.4; HRMS (ESI) m/z calcd for $C_{11}H_7NO_4Br$ (M + H) 295.9558, found 295.9557; HPLC 99% (t_R = 2.7 min, 40:60; t_R = 4.5 min, 50:50).

5-Iodoquinoline-2,4-dicarboxylic Acid (1oo). 3-Iodoaniline (**2oo**) (500 mg, 2.28 mmol) was converted to dimethyl 5-iodoquinoline-2,4-dicarboxylate. Chromatography (hexanes/EtOAc, 4:1) afforded a light yellow solid (200 mg, 24% yield): mp 183−185 °C; ^{1}H NMR ($CDCl_3$) δ 8.74 (d, J = 1.8 Hz, 1H), 8.65 (s, 1H), 8.55 (d, J = 9.4 Hz, 1H), 7.94 (dd, J = 1.8, 9.4 Hz, 1H), 4.08 (s, 3H), 4.04 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.6, 166.1, 150.2, 149.2, 141.1, 140.0, 137.2, 127.8, 126.5, 123.9, 98.2, 54.6, 54.2. Anal. ($C_{13}H_{10}INO_4$) C, H, N. Dimethyl 5-iodoquinoline-2,4-dicarboxylate (**4oo**) (50 mg, 0.13 mmol) was hydrolyzed to a light beige solid powder (22 mg, 46% yield): ^{1}H NMR (DMSO-d_6) δ 8.64 (d, J = 2.0 Hz, 1H), 8.57 (d, J = 8.8 Hz, 1H), 8.48 (s, 1H), 8.11 (dd, J = 1.6, 8.8 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 168.2, 167.0, 150.8, 150.1, 140.1, 139.7, 138.8, 128.6, 126.1, 123.6, 99.7; HRMS (FAB) m/z calcd for $C_{11}H_6INO_4$ (M^+) 342.9342, found 342.9334; HPLC 99% (t_R = 2.9 min, 40:60; t_R = 4.9 min, 50:50).

6-Iodoquinoline-2,4-dicarboxylic Acid (1pp). 4-Iodoaniline (**2pp**) (500 mg, 2.28 mmol) was converted to dimethyl 6-iodoquinoline-2,4-dicarboxylate, which was triturated from

MeOH to afford a light beige solid (116 mg, 14% yield): mp 146−147 °C; ^{1}H NMR ($CDCl_3$) δ 9.31 (d, J = 2.0 Hz, 1H), 8.69 (s, 1H), 8.09 (dd, J = 2.0, 9.2 Hz, 1H), 8.04 (d, J = 9.2 Hz, 1H), 4.09 (s, 3H), 4.06 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.6, 166.1, 149.0, 148.6, 140.7, 135.8, 135.7, 133.5, 128.6, 124.1, 99.2, 54.6, 54.2. Anal. ($C_{13}H_{10}INO_4$) C, H, N. Dimethyl 6-iodoquinoline-2,4-dicarboxylate (**4pp**) was hydrolyzed to yield a white solid: ^{1}H NMR (DMSO-d_6) δ 9.26 (d, J = 2.0 Hz, 1H), 8.46 (s, 1H), 8.17 (dd, J = 2.0, 8.6 Hz, 1H), 7.97 (d, J = 8.6 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 166.6, 165.9, 165.1, 149.3, 148.3, 141.1, 136.0, 135.5, 133.7, 123.9, 100.4; HRMS (ESI) m/z calcd for $C_{11}H_7NO_4I$ (M + H) 343.9416, found 343.9420; HPLC 99% (t_R = 2.9 min, 40:60; t_R = 5.0 min, 50:50).

6,8-Dibromoquinoline-2,4-dicarboxylic Acid (1qq). 2,4-Dibromoaniline (**2qq**) (1.00 g, 3.98 mmol) was converted to dimethyl 6,8-dibromoquinoline-2,4-dicarboxylate. Chromatography (CH_2Cl_2) afforded a white crystalline solid (280 mg, 18%) that was insoluble at concentrations sufficient for NMR analysis: mp 246−247 °C. Anal. ($C_{13}H_9Br_2NO_4$) C, H, N. Dimethyl 6,8-dibromoquinoline-2,4-dicarboxylate (**4qq**) (20 mg, 0.06 mmol) was hydrolyzed to yield a white solid: ^{1}H NMR (DMSO-d_6) δ 9.05 (d, J = 2.2 Hz, 1 H), 8.54 (s, 1 H), 8.49 (d, J = 2.2 Hz, 1 H); ^{13}C NMR (DMSO-d_6) δ 167.7, 166.8, 151.3, 145.3, 138.3, 138.0, 129.3, 129.2, 128.7, 125.1, 124.7; HRMS (ESI) m/z calcd for $C_{11}H_6NO_4Br_2$ (M + H) 373.8664, found 373.8666; HPLC 98% (t_R = 4.3 min, 40:60; t_R = 8.6 min, 50:50).

5-[(*E*)-2-Phenylethenyl]quinoline-2,4-dicarboxylic Acid (1rr). **4ll** (50 mg, 0.15 mmol) and styrene were converted to dimethyl 5-[(*E*)-2-phenylethenyl]quinoline-2,4-dicarboxylate. Chromatography (hexanes/EtOAc, 5:1) yielded a bright green-yellow solid (32 mg, 60% yield): mp 122−124 °C; ^{1}H NMR ($CDCl_3$) δ 8.80 (d, J = 9.2 Hz, 1H), 8.61 (s, 1H), 8.35 (d, J = 1.8 Hz, 1H), 7.94 (dd, J = 1.8 Hz, 9.2 Hz, 1H), 7.56 (d, J = 7.0 Hz, 2H), 7.39 (t, J = 7.0 Hz, 2H), 7.32−7.22 (m, 3H), 4.09 (s, 3H), 4.05 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 167.0, 166.4, 150.3, 148.9, 140.7, 137.6, 136.7, 135.8, 133.1, 129.9, 129.5, 128.1, 128.0, 126.9, 126.8, 123.0, 54.5, 54.0. Anal. ($C_{21}H_{17}NO_4 \cdot {}^1/_4H_2O$) C, H, N. Dimethyl 5-[(*E*)-2-phenylethenyl]quinoline-2,4-dicarboxylate (**4rr**) (20 mg, 0.06 mmol) was hydrolyzed to yield a bright orange solid (16 mg, 84% yield): ^{1}H NMR (DMSO-d_6) δ 8.76 (d, J = 8.8 Hz, 1H), 8.38 (s, 1H), 8.31 (d, J = 2.0 Hz, 1H), 8.18 (dd, J = 2.0, 8.8 Hz, 1H), 7.69 (d, J = 7.4 Hz, 2H), 7.56 (d, J = 8.8 Hz, 1H), 7.41 (t, J = 7.4 Hz, 2H), 7.31 (t, J = 7.4 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 168.7, 167.3, 149.0, 147.8, 138.8 134.4, 133.4, 132.1, 130.5, 130.1, 129.5, 128.7, 128.5, 128.3, 127.1, 126.1, 124.1, 123.4; HRMS (ESI) m/z calcd for $C_{19}H_{11}NO_3$ (M − OH_2) 301.0739 found 301.0750; HPLC 99% (t_R = 5.9 min, 40:60; t_R = 14.9 min, 50:50).

6-[(*E*)-2-Phenylethenyl]quinoline-2,4-dicarboxylic Acid (1ss). **4ll** (50 mg, 0.15 mmol) and styrene were converted to dimethyl 6-[(*E*)-2-phenylethenyl]quinoline-2,4-dicarboxylate. Chromatography (hexanesEtOAc, 5:1) yielded a bright green-yellow solid (32 mg, 60% yield): mp 203−204 °C; ^{1}H NMR ($CDCl_3$) δ 8.87 (d, J = 1.8 Hz, 1H), 8.68 (s, 1H), 8.31 (d, J = 8.8 Hz, 1H), 8.10 (dd, J = 1.8, 8.8 Hz, 1H), 7.58 (d, J = 6.8 Hz, 2H), 7.40 (t, J = 6.8 Hz, 2H), 7.34−7.31 (m, 3H), 4.10 (s, 3H), 4.08 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 167.1, 166.4, 149.5, 147.8, 140.5, 137.7, 136.3, 133.3, 132.9, 132.6, 129.9, 129.5, 129.2, 128.8, 128.0, 124.8, 124.0, 54.4, 54.0. Anal. ($C_{21}H_{17}NO_4 \cdot {}^1/_4H_2O$) C, H, N. Dimethyl 6-[(*E*)-2-phenylethenyl]quinoline-2,4-dicarboxylate (**4ss**) (20 mg, 0.06 mmol) was hydrolyzed to yield a dark red solid (16 mg, 84% yield): ^{1}H NMR (DMSO-d_6) δ 8.83 (d, J = 2.0 Hz, 1H), 8.46 (s, 1H), 8.34 (dd, J = 2.0, 8.8 Hz, 1H), 8.21 (d, J = 8.8 Hz, 1H), 7.73 (d, J = 6.8 Hz, 2H), 7.63−7.51 (m, 2H), 7.56 (d, J = 3.6 Hz, 2H), 7.43 (t, J = 7.6 Hz, 2H), 7.33 (t, J = 7.6 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 168.5, 167.3, 149.3, 140.1, 138.2, 138.1, 133.6, 133.4, 133.0, 132.9, 132.4, 130.3, 130.2, 129.9, 129.3, 128.6, 127.6, 125.5, 125.4, 123.6; HRMS (ESI) m/z calcd for $C_{19}H_{12}NO_4$ (M − H) 318.0766 found 318.0772; HPLC 96% (t_R = 6.7 min, 40:60; t_R = 18.1 min, 50:50).

6-Biphenyl-4-ylquinoline-2,4-dicarboxylic Acid (1tt). **4mm** (57 mg, 0.176 mmol) and biphenyl-4-ylboronic acid (52 mg, 0.26 mmol) were coupled to form dimethyl 6-biphenyl-4-ylquinoline-2,4-dicarboxylate. Chromatography (hexanes/EtOAc, 4:1) yielded a bright green-yellow solid (34 mg, 48% yield): mp 169−172 °C; ^{1}H NMR ($CDCl_3$) δ 9.15 (d, J = 1.8 Hz, 1H), 8.72 (s, 1H), 8.42 (d, J = 9.0 Hz, 1H), 8.15 (dd, J = 1.8, 9.0 Hz, 1H), 7.85 (d, J = 7.90 Hz, 2H), 7.74 (d, J = 7.90, 2H), 7.66 (d, J = 7.6 Hz, 2H), 7.47 (t, J = 7.6 Hz, 2H), 7.37 (t, J = 7.6 Hz, 1H), 4.11 (s, 3H), 4.08 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 167.1, 166.4, 149.1, 148.2, 143.5, 142.4, 141.4, 139.7, 136.7, 132.8, 131.4, 130.0, 129.1, 128.8, 128.1, 127.8, 124.1, 123.9, 54.5, 54.0. Anal. ($C_{25}H_{19}NO_4 \cdot {}^3/_2H_2O$) C, H, N. Dimethyl 6-biphenyl-4-ylquinoline-2,4-dicarboxylate (**4tt**) (15 mg, 0.04 mmol) was hydrolyzed to yield a red solid (11 mg, 76% yield): ^{1}H NMR (DMSO-d_6) δ 9.14 (s, 1H), 8.52 (s, 1H), 8.33 (s, 2H), 7.94 (d, J = 8.0 Hz, 2H), 7.88 (d, J = 8.0 Hz, 2H), 7.77 (d, J = 7.6 Hz, 2H), 7.51 (t, J = 7.6 Hz, 2H), 7.40 (t, J = 7.6 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 168.5, 167.3, 149.9, 148.9, 142.3, 141.7, 140.9, 139.6, 138.6, 132.7, 131.3, 130.6, 129.4, 129.3, 129.0, 128.2, 127.4, 124.3, 123.8; HRMS (ESI) m/z calcd for $C_{23}H_{14}NO_4$ (M − H) 368.0923 found 368.0919; HPLC 94% (t_R = 4.7 min, 30:70; t_R = 13.2 min, 40:60).

6-[(*E*)-2-[1,1′-Biphenyl]-4-ylethenyl]quinoline-2,4-dicarboxylic Acid (1uu). **4mm** (50 mg, 0.15 mmol) and 4-phenylstyrene were coupled to form dimethyl 6-[(*E*)-2-[1,1′-biphenyl]-4-ylethenyl]quinoline-2,4-dicarboxylate. Chromatography (hexanes/EtOAc, 5:1) yielded a bright green-yellow solid (32 mg, 60% yield): mp 169−172 °C; ^{1}H NMR ($CDCl_3$) δ 8.89 (d, J = 1.6 Hz, 1H), 8.69 (s, 1H), 8.33 (d, J = 8.8 Hz, 1H), 8.13 (dd, J = 8.8, 1.6 Hz, 1H), 7.67−7.63 (m, 6H), 7.46 (t, J = 2.0 Hz, 2H), 7.39−7.34 (m, 3H), 4.10 (s, 3H), 4.09 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 167.1, 166.4, 149.6, 147.8, 142.3, 141.5, 140.5, 136.7, 136.2, 132.8, 132.6, 129.9, 129.2, 128.8, 128.6, 128.5, 128.0, 124.8, 124.1, 54.4, 54.0. Anal. ($C_{27}H_{21}NO_4 \cdot {}^1/_8H_2O$) C, H, N. Dimethyl 6-[(*E*)-2-[1,1′-biphenyl]-4-ylethenyl]quinoline-2,4-dicarboxylate (**4uu**) (20 mg, 0.05 mmol) was hydrolyzed to yield a dark red solid (18 mg, 95% yield): ^{1}H NMR (DMSO-d_6) δ 8.83 (br s, 1H), 8.43 (s, 1H), 8.34 (d, J = 9.8 Hz, 1H), 8.20 (d, J = 9.8 Hz, 1H), 7.82 (d, J = 8.2 Hz, 2H), 7.72 (d, J = 8.2 Hz, 4H), 7.60 (d, J = 7.1 Hz, 2H), 7.46 (t, J = 7.1 Hz, 2H), 7.36 (t, J = 7.1 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 168.5, 167.3, 149.3, 141.4, 141.0, 140.2, 138.2, 137.3, 132.9, 132.4, 130.5, 129.5, 129.4, 129.2, 128.5, 128.1, 127.6, 125.5, 123.6; HRMS (ESI) m/z calcd for $C_{25}H_{18}NO_4$ (M + H) 396.1236, found 396.1236; HPLC 99% (t_R = 6.7 min, 30:70; t_R = 15.9 min, 40:60).

6-(2-Vinylnaphthyl)quinoline-2,4-dicarboxylic Acid (1vv). Dimethyl 6-bromoquinoline-2,4-dicarboxylate (40 mg, 0.12 mmol) and 2-vinylnaphthalene (27 mg, 0.17 mmol) were converted to dimethyl 6-(2-vinylnaphthyl)quinoline-2,4-dicarboxylate using the Suzuki coupling. Chromatography (MeOH/CH_2Cl_2, 1:99) afforded a bright yellow solid (32 mg, 65%): mp 184−185 °C; ^{1}H NMR ($CDCl_3$) δ 8.91 (d, J = 2.0 Hz, 1H), 8.68 (s, 1 H), 8.33 (d, J = 9.2 Hz, 1H), 8.14 (dd, J = 2.0, 9.2 Hz, 1H), 7.92 (s, 1H), 7.86−7.77 (m, 4H), 7.53−7.41 (m, 4H), 4.10 (s, 3H), 4.09 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 167.1, 166.4, 149.6, 147.8, 140.5, 136.2, 135.2, 134.7, 134.5, 133.3, 132.6, 129.6, 129.2, 129.1, 128.7, 127.6, 127.5, 124.8, 124.4, 124.1, 54.4, 54.0. Anal. ($C_{25}H_{19}NO_4$) C, H, N. Dimethyl 6-(2-vinylnaphthyl)-quinoline-2,4-dicarboxylate (**4vv**) (25 mg, 0.03 mmol) was hydrolyzed to yield a dark red solid (18 mg, 78%): ^{1}H NMR (DMSO-d_6) δ 8.87 (d, J = 1.6 Hz, 1H), 8.45 (s, 1H), 8.37 (dd, J = 1.6, 9.0 Hz, 1H), 8.22 (d, J = 9.0 Hz, 1H), 8.14 (s, 1H), 7.99 (d, J = 7.2 Hz, 1H), 7.92 (d, J = 9.0 Hz, 1H), 7.91 (t, J = 9.0 Hz, 2H), 7.70 (s, 2H), 7.51 (m, 7.2 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 178.0, 168.5, 167.3, 149.3, 140.2, 138.2, 135.8, 133.4, 132.5, 129.9, 129.6, 129.5, 129.2, 128.8, 128.1, 127.9, 127.7, 125.5, 125.4, 123.6; HRMS (FAB) m/z calcd for $C_{23}H_{16}NO_4$ (M + H) 370.1079, found 370.1089; HPLC 94% (t_R = 5.4 min, 30:70; t_R = 16.1 min, 40:60).

Isolation of Synaptic Vesicles. Synaptic vesicles were isolated from the forebrains of male Sprague−Dawley rats (200−300 g) as described.[39] The rats were sacrificed, and their cerebrums were rapidly removed and minced with scissors in an ice-cold solution consisting of 0.32 M sucrose, 1.0 mM sodium bicarbonate, 1.0 mM magnesium acetate, and 0.5 mM

calcium acetate (pH 7.2). The minced cerebrums were homogenized (motorized Potter-Elvejhem, Teflon/glass (Wheaton)) and centrifuged (12000g_{max}, 15 min, Sorval SS-34 rotor), after which the resulting pellet was resuspended in a 6.0 mM Tris−maleate buffer (pH 8.1). Following incubation (45 min) at 4 °C, the suspension was centrifuged (43000g_{max}, 15 min, Sorval SS-34 rotor.) The collected supernatant was recentrifuged (222000g_{max}, 55 min, Beckman Ti 60 rotor) and the resulting pellet resuspended by homogenation in 0.32 M sucrose, 1.0 mM sodium bicarbonate, and 1.0 mM dithiothreitol (pH 7.2). Synaptic vesicles prepared in this manner and stored at −80 °C exhibited similar levels of glutamate uptake activity for at least two weeks.

Assay of Vesicular Glutamate Transport. The uptake of glutamate into synaptic vesicles was quantified using a modification of the procedure described.[39] Synaptic vesicles were resuspended by vortexing in a buffer containing 5.0 mM $MgCl_2$, 375 mM sucrose, and 5.0 mM *N*-(2-hydroxyethyl)-piperazine-*N*-(2-ethanesulfonic acid) (HEPES) (pH 7.4) and maintained at 4 °C. The vesicles (80 μL, 100−150 μg of protein) were preincubated for 5 min at 30 °C. Uptake was initiated by addition of a concentrated stock solution (20 μL, 30 °C) that yielded a final assay mixture of 0.25 mM [3,4-^{3}H]-L-glutamate, 2.0 mM ATP, 4.0 mM $MgCl_2$, 4.0 mM KCl, 300 mM sucrose, and 5.0 mM HEPES (pH 7.4). QDCs were added to the assays simultaneously with the [^{3}H]-L-glutamate. Following a 1.5 min incubation, uptake was terminated by the addition of 3.0 mL of ice-cold 150 mM KCl followed immediately by rapid vacuum filtration (10 psi) through Millipore HAWP filters (25 mm, 0.45 μm). Assay tubes and filters were sequentially rinsed twice more with 3.0 mL of ice-cold 150 mM KCl. The filters were transferred to 5.0 mL glass scintillation vials, and 3.5 mL of Liquiscint scintillation (National Diagnostics) was added to each. Radioactivity retained on the filters was quantified by liquid scintillation counting (LSC, Beckman LS 6500). Nonspecific uptake, binding, or leakage was corrected for by subtracting [^{3}H]-L-glutamate accumulated in the absence of ATP. Under the assay conditions employed, glutamate uptake into the synaptic vesicles was linear with respect to both time and protein content.[46] Lineweaver−Burk plots and associated kinetic parameters were estimated by computer analysis (k_{cat} kinetic program, BioMetallics) with weighting based on constant relative error. K_i values were estimated on the basis of a replot of K_m(app) values.

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