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Synthesis and SAR of 5-, 6-, 7- and 8-Aza Analogues of 3-Aryl-4-hydroxyquinolin-2(1*H*)-one as NMDA/Glycine Site Antagonists

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Abstract—A series of 5-, 6-, 7- and 8-aza analogues of 3-aryl-4-hydroxyquinolin-2(1*H*)-one was synthesized and assayed as NMDA/glycine receptor antagonists. The in vitro potency of these antagonists was determined by displacement of the glycine site radioligand [^{3}H]5,7-dicholorokynurenic acid ([^{3}H]DCKA) in rat brain cortical membranes. Selected compounds were also tested for functional antagonism using electrophysiological assays in *Xenopus* oocytes expressing cloned NMDA receptor (NR) 1A/2C subunits. Among the 5-, 6-, 7-, and 8-aza-3-aryl-4-hydroxyquinoline-2(1*H*)-ones investigated, 5-aza-7-chloro-4-hydroxy-3-(3-phenoxyphenyl)quinolin-2-(1*H*)-one (**13i**) is the most potent antagonist, having an IC_{50} value of 110 nM in [^{3}H]DCKA binding and a K_b of 11 nM in the electrophysiology assay. Compound **13i** is also an active anticonvulsant when administered systemically in the mouse maximum electroshock-induced seizure test (ED_{50} = 2.3 mg/kg, IP).

Introduction

Excessive stimulation of *N*-methyl-D-aspartate (NMDA) receptors, a subclass of ionotropic glutamate receptors, is known to cause neurodegeneration.[1] Channel gating at NMDA receptors is regulated by an allosteric strychnine-insensitive glycine binding site. Occupation of this site by an agonist, either glycine or D-serine, is necessary for channel gating by the excitatory neurotransmitter glutamate.[2] Considerable effort has gone into the development of antagonists for the NMDA receptor glycine site with the aim of finding therapies for a variety of CNS disorders.

Several classes of potent glycine site antagonists have been reported. Examples include 4-hydroxy-3-(3-phenoxyphenyl)quinoline-2(1*H*)-ones such as **A**,[3] 1,2,3,4-tetrahydroquinoline-2,3,4-trione-3-oximes such as **B**,[4] substituted 1,4-dihydroquinoxaline-2,3-diones (QX) such as licostinel (**C**),[5] 7-chloro-6-methyl-5-nitro QX (**D**),[6] 4-hydroxy-3-nitroquinolin-2(1*H*)-ones such as **E**,[7] and 5-(*N*-oxyaza)-7-substituted-1,4-dihydroquinoxaline-2,3-diones such as **F**[8] (Chart 1). Herein, we report on the synthesis and SAR of 5-, 6-, 7- and 8-aza-3-aryl-4-hydroxyquinolin-2(1*H*)-ones as NMDA receptor glycine site antagonists patterned after the prototypic quinolinone **A**.[3]

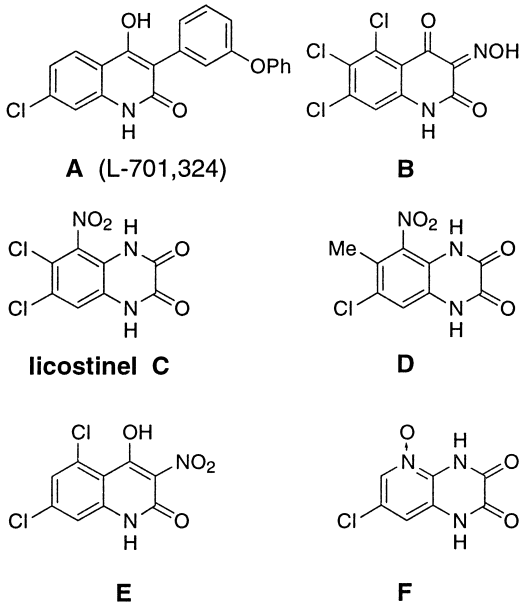

Chart 1.

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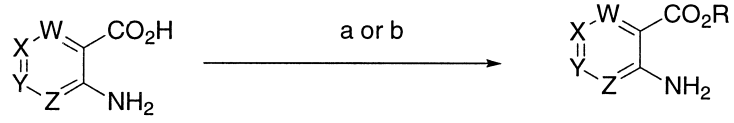
Chemistry

The synthesis of aza 4-hydroxy-3-phenylquinolin-2(1*H*)-ones (**13a–q**) is shown in Scheme 1. Treatment of aza-amino acids **1–5** with diazomethane or refluxing ethanol containing sulfuric acid provided the corresponding ester **6–10**. Ester **11** was prepared from ester **10** by chlorination in HCl containing 30% H_2O_2. Acylation of **6–11** in turn with the appropriate acid chloride in 1,2-dichloroethane in the presence of triethylamine afforded the corresponding amides **12a–q**. Intramolecular cyclization of each amide with two equiv of potassium hexamethyldisilazide (KHDMS) in THF afforded the desired azaquinolinones **13a–q**.

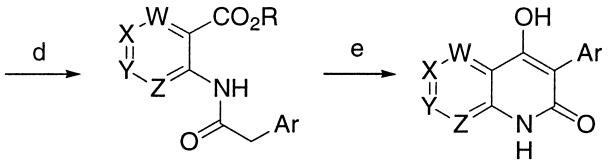
3-Aminopicolinic acid (**1**) was prepared from commercially available quinolinic acid **14** as shown in Scheme 2. Acid **14** was heated in acetic anhydride and then allowed to react with acetamide to form quinolinimide **15**.[9] Imide **15** underwent a Hofmann rearrangement to yield 3-aminopicolinic acid (**1**) in 10% yield.[10]

3-Amino-5-chloropicolinic acid (**2**) was prepared from commercially available 2-amino-5-chloropyridine (**16**) as shown in Scheme 3. Nitration of pyridine **16** with concentrated nitric acid and sulfuric acid at 60 °C gave 2-amino-5-chloro-3-nitropyridine (**17**) in 67% yield.[11] Diazotitation followed by bromination with hydrobromic acid provided 2-bromo-5-chloro-3-nitropyridine (**18**) in 65% yield. Reaction of **18** with Cu(I) cyanide afforded 5-chloro-2-cyano-3-nitropyridine (**19**) in 92% yield based on recovered compound **18**. Hydrogenation of **19** in the presence of Raney Ni in 95% ethanol with concomitant hydrolysis afforded 3-amino-5-chloropicolinamide (**20**) in 95% yield. Hydrolysis of **20** with concentrated hydrochloric acid gave 3-amino-5-chloropicolinic acid (**2**) as its HCl salt.



1 W = N, X = C-H, Y = C-H, Z = C-H
2 W = N, X = C-H, Y = C-Cl, Z = C-H
3 W = C-H, X = N, Y = C-H, Z = C-H
4 W = C-H, X = C-H, Y = N, Z = C-H
5 W = C-H, X = C-H, Y = C-H, Z = N

6 W = N, X = C-H, Y = C-H, Z = C-H, R = Me
7 W = N, X = C-H, Y = C-Cl, Z = C-H, R = Et
8 W = C-H, X = N, Y = C-H, Z = C-H, R = Et
9 W = C-H, X = C-H, Y = N, Z = C-H, R = Et
10 W = C-H, X = C-H, Y = C-H, Z = N, R = Et
c
11 W = C-H, X = C-Cl, Y = C-H, Z = N, R = Et



12 a-q **13 a-q**

a W = N, X = C-H, Y = C-H, Z = C-H, Ar = C_6H_5
b W = N, X = C-H, Y = C-Cl, Z = C-H, Ar = C_6H_5
c W = N, X = C-H, Y = C-Cl, Z = C-H, Ar = *p*-ClC_6H_4
d W = N, X = C-H, Y = C-Cl, Z = C-H, Ar = *m*-ClC_6H_4
e W = N, X = C-H, Y = C-Cl, Z = C-H, Ar = *o*-ClC_6H_4
f W = N, X = C-H, Y = C-Cl, Z = C-H, Ar = *m*-$MeOC_6H_4$
g W = N, X = C-H, Y = C-Cl, Z = C-H, Ar = *m*-MeC_6H_4
h W = N, X = C-H, Y = C-Cl, Z = C-H, Ar = *m*-$NO_2C_6H_4$
i W = N, X = C-H, Y = C-Cl, Z = C-H, Ar = *m*-$PhOC_6H_4$
j W = N, X = C-H, Y = C-Cl, Z = C-H, Ar = 3-thienyl
k W = N, X = C-H, Y = C-Cl, Z = C-H, Ar = 2-thienyl
l W = C-H, X = N, Y = C-H, Z = C-H, Ar = *m*-$PhOC_6H_4$
m W = C-H, X = C-H, Y = N, Z = C-H, Ar = *m*-$PhOC_6H_4$
n W = C-H, X = C-H, Y = C-H, Z = N, Ar = *m*-$PhOC_6H_4$
o W = C-H, X = C-H, Y = C-H, Z = N, Ar = C_6H_5
p W = C-H, X = C-Cl, Y = C-H, Z = N, Ar = *m*-$PhOC_6H_4$
q W = C-H, X = C-Cl, Y = C-H, Z = N, Ar = C_6H_5

Scheme 1. (a) CH_2N_2/MeOH; (b) EtOH/H_2SO_4/reflux; (c) Concd HCl, 30% H_2O_2, 60°, 94%; (d) $ArCH_2COCl/Et_3N/CH_2Cl_2$/rt; (e) (i) KHMDS/THF/−78 °C to rt; (ii) H_3O^+.

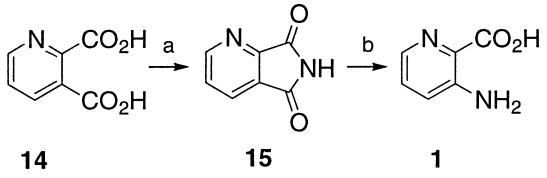
It is noteworthy that several attempts to hydrolyze 5-chloro-2-cyano-3-nitropyridine (**19**) failed to give the desired product. For example, treatment of compound **19** with $MeOH/H_2SO_4$ or 6 N HCl under reflux returned only starting material. Hydrolysis of **19** in methanol or ethanol containing aqueous sodium hydroxide resulted in denitration giving 5-chloro-2-cyano-3-methoxypyridine (**21a**) and 5-chloro-2-cyano-3-ethoxypyridine (**21b**) in 65 and 55% yield, respectively.

4-Aminonicotinic acid (**3**) and amino acid **4** were synthesized as depicted in Scheme 4. Nitration of 3-picoline-1-oxide (**22**) with 70% nitric acid in sulfuric acid gave nitro compound **23** in 46% yield. Oxidation of the methyl group of **23** afforded carboxylic acid **24** in 26% yield, which was then hydrogenated to give 4-aminonicotinic acid (**3**) in 40% yield. Amino acid **4** was prepared by effecting a Hofmann rearrangement on commercially available 3,4-pyridinedicarboximide (**25**).

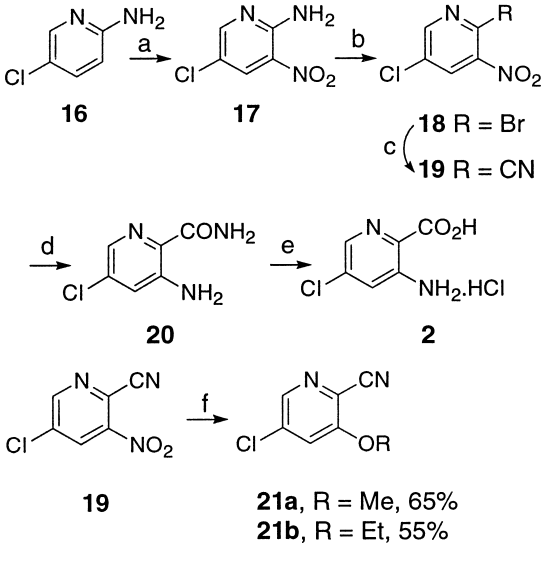
5-Aza-7-chloro-4-hydroxy-3-nitroquinolin-2-one (**28**) was prepared by acetylation of amine **7** with acetic anhydride to form amide **26**, cyclization of **26** with KHDMS, and then nitration with concentrated nitric acid in acetic acid (Scheme 5).

Pharmacology

The affinity of compounds for the NMDA receptor glycine site was measured by displacement of [^{3}H]5,7-dichlorokynurenic acid ([^{3}H]DCKA) binding to rat brain cortical membranes (Table 1).[8,12] For selected compounds, potencies at the NMDA receptor glycine site and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors were determined electrophysiologically in *Xenopus* oocytes expressing recombinant rat NR1A/2C receptors and rat brain poly(A)+ RNA, respectively (Table 2). Apparent antagonist dissociation constants (K_b values) were estimated by assuming competitive inhibition and assaying suppression of membrane current responses elicited by fixed concentrations of agonist: 1 μm glycine and 100 μm glutamate for NMDA receptors; 10 μm AMPA for AMPA receptors. Anticonvulsant activity of selected compounds was measured in a mouse maximum electroshock-induced seizure (MES) model.[8]



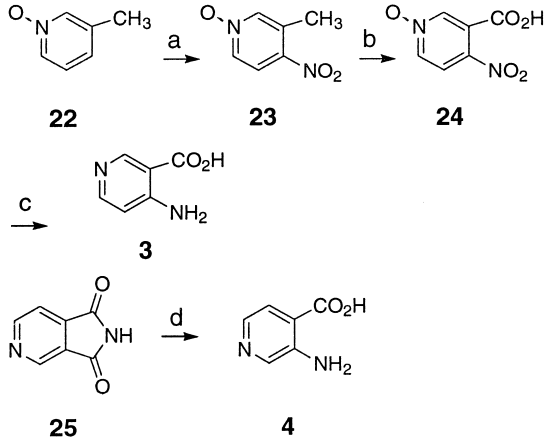
Scheme 2. (a) (i) $(CH_3CO)_2O/120\,^\circ C$; (ii) CH_3CONH_2/reflux, 56%; (b) 10% NaOH/NaOBr/rt to 85 °C, 10%.



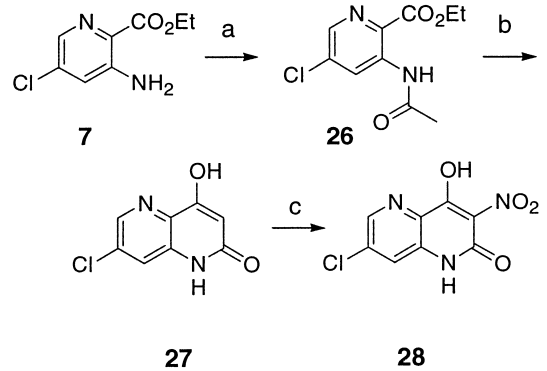
Scheme 3. (a) $HNO_3/H_2SO_4/60\,^\circ C$, 67%; (b) $NaNO_2/HBr/Br_2$, 65%; (c) CuCN/185 °C, 92%; (d) Raney Ni/H_2/95% EtOH, 95%; (e) concd HCl, reflux, 85%; (f) ROH/10% NaOH/rt.

Results and Discussion

The SAR of aza analogues of 3-aryl-4-hydroxyquinolin-2(1*H*)-ones as antagonists at the NMDA/glycine site is given in Table 1. Compound **13b** is much more potent than **13a**, demonstrating the importance of having a chlorine atom in the 7-position of the 5-aza analogues for high potency. Compound **13b** is 10-fold more potent than 5-aza-3-nitro analogue **28**, indicating that a phenyl



Scheme 4. (a) 70% HNO_3/H_2SO_4/0–10 °C, 46%; (b) concd H_2SO_4/$Na_2Cr_2O_7$/rt, then 20–40 °C, 26%; (c) 10% Pd/C, $NH_4OH/H_2O/H_2$, 40%; (d) NaOBr/10% NaOH/85 °C, 79%.



Scheme 5. (a) $(CH_3CO)_2O$/dioxane, 65%; (b) KHDMS/THF, then H_3O^+, 78%; (c) HNO_3/AcOH/60 °C, 35%.

group is favored over a nitro group in the 3-position in this series. However, analogue **28** is 20-fold less potent than its carbocyclic analogue **29**. Apparently, 5-aza substitution is detrimental to potency in the 3-nitro series.

Introducing a chlorine atom (**13d**), a methyl group (**13g**), a methoxy group (**13f**) or a nitro group (**13h**) in the *meta*-position of the 3-phenyl ring of **13b** has little effect on potency while adding a chlorine atom at either the *ortho*- (**13e**) or *para*- (**13c**) position results in an 8- and 4-fold drop in potency, respectively. This suggests there may be bulk tolerance specifically at the *meta*-position of the 3-phenyl ring. Consistent with this notion and with the observations of Kulagowski et al.[3] in the non-aza series, introduction of a *m*-phenoxy group (**13i**) increased potency 30-fold to 0.11 μM, rendering **13i** the most potent compound in the 5-aza series, and among all the aza analogues of **A** investigated. Either a 2- (**13k**) or 3-thienyl group (**13j**) may be substituted for the phenyl group in **13b** without much change in potency.

Moving the nitrogen atom from the 5-postion in **13i** to the 6-position and deleting the chlorine atom gave 6-aza analogue **13l**, which has an IC_{50} value of 20 μM. It seems likely that the 180-fold drop in potency results at least partially from the change in the position of the nitrogen atom to the 6-position. Aza analogues containing a chlorine atom adjacent to the nitrogen atom were not prepared owing to the potential lability of the chlorine atom toward nucleophilic substitution and long term stability concerns about such antagonists. The 7-aza analogue **13m** (IC_{50} 5.4 μM) is more potent than **13l,** but is still significantly less potent than **13i**. The 8-aza analogues **13n–q** derived from nicotinic acid all have low potencies. Introduction of a 6-chloro substitution (**13p** versus **13n** and **13q** versus **13o**) does not improve activity significantly. Apparently, the unshared electrons of the nitrogen atom in the 8-position are responsible for the poor binding, although the chlorine atom is also not optimally positioned in **13p**,**q**.

Values from the [^{3}H]DCKA binding assay indicate that **13i** is 5-fold less potent than the prototypic quinolinone **A** (IC_{50} in a [^{3}H]L-689,560 binding assay = 2 nM[3]). To directly compare these compounds and to get a measure of the functional activity in the series, we tested selected compounds in oocyte electrophysiological assays (Table 2). All the compounds tested were functional antagonists. The overall trend in potency ran in parallel with the [^{3}H]DCKA binding assay, that is **13i** was the most potent compound tested while **13l** and **13p** were the least potent. However, apparent affinities calculated from the electrophysiological assay ranged from 3- to 30-fold lower than in the binding assays. In particular, the K_b for **13i** was 11 nM as compared to 110 nM in the binding assay. The prototypic compound **A** had a K_b of 3.5 nM, which was only three times more potent than **13i**. Interestingly, the kinetics of the blockade and wash were distinctly slow for **13i** and **A** as compared to quinoxaline-2,3-dione antagonists such as licostinel (**C**), taking min to equilibrate to steady-state levels and tens of min to wash out.

Compounds in Table 2 were also tested for inhibition of rat brain AMPA receptors expressed in oocytes. Unlike a number of classes of NMDA glycine site antagonists,[4–6] the aza-3-aryl-4-hydroxyquinolinones were weak or inactive as AMPA receptor antagonists. This is consistent with the pharmacology of the prototype compound **A**.[3]

Quinolinone **A** is reported to be active as an anticonvulsant in vivo.[3] Although we have not tested compound **A** ourselves in vivo, we have tested compound **13i** administered systemically in the mouse maximum electroshock-induced seizure (MES) test. In this assay, compound **13i** was active as an anticonvulsant (ED_{50} = 2.3 mg/kg, IP), indicating that 5-aza derivative **13i** likely penetrates the blood–brain barrier.

Table 1. SAR of 4-hydroxyquinolin-2(1*H*)-ones at the NMDA receptor glycine site

Compd no.	W	X	Y	Z	Ar	[^{3}H]DCKA IC_{50} (μM)
A	C–H	C–H	C–Cl	C–H	*m*-$PhOC_6H_4$	0.023 ± 0.001
13a	N	C–H	C–H	C–H	C_6H_5	> 100
13b	N	C–H	C–Cl	C–H	C_6H_5	3.3 ± 0.7
13c	N	C–H	C–Cl	C–H	*p*-ClC_6H_4	13 ± 3
13d	N	C–H	C–Cl	C–H	*m*-ClC_6H_4	3.0 ± 0.1
13e	N	C–H	C–Cl	C–H	*o*-ClC_6H_4	24 ± 6
13f	N	C–H	C–Cl	C–H	*m*-$MeOC_6H_4$	2.9 ± 0.2
13g	N	C–H	C–Cl	C–H	*m*-MeC_6H_4	1.8 ± 0.3
13h	N	C–H	C–Cl	C–H	*m*-$NO_2C_6H_4$	7.4 ± 1.6
13i	N	C–H	C–Cl	C–H	*m*-$PhOC_6H_4$	0.11 ± 0.02
13j	N	C–H	C–Cl	C–H	3-thienyl	4.3 ± 0.6
13k	N	C–H	C–Cl	C–H	2-thienyl	9.8 ± 1.9
13l	C-H	N	C–H	C–H	*m*-$PhOC_6H_4$	20 ± 5
13m	C-H	C–H	N	C–H	*m*-$PhOC_6H_4$	5.4 ± 1.3
13n	C-H	C–H	C–H	N	*m*-$PhOC_6H_4$	31 ± 7
13o	C-H	C–H	C–H	N	C_6H_5	> 100
13p	C–H	C–Cl	C–H	N	*m*-$PhOC_6H_4$	19 ± 3
13q	C–H	C–Cl	C–H	N	C_6H_5	> 100
28	N	C–H	C–Cl	C–H	NO_2	29 ± 4
29	C–H	C–H	C–Cl	C–H	NO_2	1.5 ± 0.1[a]
E	C–Cl	C–H	C–Cl	C–H	NO_2	0.22 ± 0.05[a]

[a]Ref 7.

Table 2. Functional antagonism of rat NMDA receptors expressed in *Xenopus* oocytes[a]

Compd	NMDA (glycine) K_b (μM)	*n*
A	0.0035 (0.0032–0.0037)	3
13b	0.49 (0.46–0.52)	4
13c	0.46 (0.42–0.50)	3
13f	0.60 (0.57–0.64)	4
13i	0.011 (0.010–0.012)	3
13l	5.1 (4.6–5.6)	3
13p	5.8 (5.3–6.3)	4

[a]Inhibition of NMDA receptors was measured in oocytes expressing the rat subunit combination NR1A/2C. K_b values for glycine binding sites were determined from inhibition of currents elicited by 1 μM glycine and 100 μM glutamate. Values are given to two significant figures and numbers in parentheses are 95% confidence intervals adjusted to the linear scale. Current ranges and mean responses for the NMDA evoked currents were (in nA): 315–800 (570±31, $n=24$).

Conclusion

The 5-aza-3-aryl-7-chloro-4-hydroxyquinolin-2(1*H*)-ones represent a novel class of potent, systemically active NMDA receptor glycine site antagonists. The corresponding 6-, 7- and 8-aza analogues appear to be significantly less active. The most potent compound synthesized in this series, 5-aza-7-chloro-4-hydroxy-3-(3-phenoxyphenyl)quinolin-2(1*H*)-one (**13i**), has an IC_{50} of 110 nM in [^{3}H]DCKA binding, a K_b of 11 nM for functional antagonism of cloned NMDA receptors expressed in oocytes and an ED_{50} of 2.7 mg/kg IP in a mouse MES assay.

Experimental

Melting points were determined in open capillary tubes on a Mel-Temp apparatus and are uncorrected. The ^{1}H NMR spectra were recorded at 300 MHz in $CDCl_3$ unless otherwise stated. Chemical shifts are reported in ppm (δ), and *J* coupling constants are reported in Hz. Elemental analyses were performed by Desert Analytics, Tucson, AZ, USA. Mass spectra (MS) were obtained with a VG 12-250 or VG ZAB-2FHF mass spectrometer. Reagent grade solvents were used without further purification unless otherwise specified. Reverse phase HPLC analyses were monitored at 254 nM on a 4.6×250 mM microsorb-MV C18 column, using as solvents 0.1% trifluoroacetic acid in water (A) and 0.1% trifluoroacetic acid in acetonitrile (B). The linear gradient was 20% B in A to 95% B in A with a flow rate of 1 mL/min.

Typical procedure for synthesis of 3-aryl-5-aza-4-hydroxyquinoxaline-2-ones

Ethyl 3-amino-5-chloropicolinate (7). A mixture of 3-amino-5-chloropicolinic acid (**2**, 2.30 g, 9.36 mmol) in 100 mL of absolute EtOH and 10 mL of concentrated H_2SO_4 (96%, d = 1.84) was refluxed for 12 h. The resulting solution was cooled to rt and poured over ice, and neutralized to pH 5 (pH paper) with solid $NaHCO_3$. The resulting mixture was extracted with EtOAc (3×30 mL). The organic layer was dried over Na_2SO_4 and rotevaporated to dryness, giving 1.64 g (87%) of the title compound as an orange solid: mp 149–151 °C (lit.[13] 148–150 °C); ^{1}H NMR δ 1.44 (t, $J=6.9$ Hz, 3H), 4.45 (q, $J=6.9$ Hz, 3H), 5.84 (brs, 2 H), 7.05 (d, $J=1.5$ Hz, 1H), 7.99 (d, $J=1.8$ Hz, 1H).

Ethyl 3-(*m*-phenoxyphenyl)acetylamido-5-chloropicolinate (12i). To a solution of *m*-phenoxyphenylacetic acid (3.0 g, 13 mmol) in 50 mL of dichloromethane was added 3.3 g (26 mmol) of oxalyl chloride. The resulting solution was allowed to stir at room temperature for 3 h. The solvent was evaporated to give the acid chloride as an oil (3.20 g, 100%), which was used in the next step without purification. To a solution of ethyl 3-amino-5-chloropicolinate (**7**) (0.353 g, 1.76 mmol) in 10 mL of 1,2-dichloroethane and 0.51 mL of triethylamine was added *m*-phenoxyphenylacetyl chloride (3.20 g, 13 mmol). The resulting solution was allowed to reflux for 12 h. After cooling to room temperature, the solvent was evaporated in vacuo to dryness. Water (15 mL) was added to the residue and then it was extracted with ethyl acetate (3×15 mL). The combined extracts were washed with brine and dried over sodium sulfate. The solvent was evaporated in vacuo and the product was obtained by flash chromatography (15% EtOAc in hexane), giving 3.68 g (90%) of the product as a white solid: mp 95–97 °C; ^{1}H NMR δ 1.43 (t, $J=7.2$ Hz, 3H), 3.76 (s, 2H), 4.42 (q, $J=7.2$ Hz, 2H), 6.85 (m, 5H), 7.33 (m, 4H), 8.35 (d, $J=1.2$ Hz, 1H), 9.23(d, $J=1.2$ Hz, 1H), 11.08 (s, 1H).

5-Aza-7-chloro-4-hydroxy-3-(*m*-phenoxyphenyl)quinoline-2-one (13i). To a solution of KHDMS in toluene (42 mL, 21 mmol) in 40 mL of THF was added dropwise a solution of ethyl 3-(*m*-phenoxyphenyl)acetylamido-5-chloropicolinate (**12i**) (2.87 g, 7 mmol) in 40 mL of THF at −78 °C under N_2. The resulting mixture was allowed to warm to room temperature and then stirred for additional 12 h. Water (100 mL) was added to the reaction mixture, which was then extracted with ethyl acetate (30 mL). The aqueous phase was acidified with 4 N HCl to pH = 2. The white solid was obtained by filtration and purified by recrystallization from DMSO-H_2O, giving 2.20 g (86%) of the product as a pale yellow solid: mp 248–250 °C; ^{1}H NMR (DMSO-d_6) δ 6.89 (m, 1H), 7.02 (m, 2H), 7.11 (m, 2H), 7.21 (d, $J=7.2$ Hz, 1H), 7.35 (s, 3H), 7.68 (d, $J=1.5$ Hz, 1H), 8.46 (d, $J=1.5$ Hz, 1H), 11.00 (brs, 1H), 11.67 (s, 1H). EI-MS *m*/*e* 364 (M^+, 40), 77 (100). HR-MS calcd for $C_{20}H_{13}ClN_2O_3$: 364.0603. Found: 346.0609. Anal. calcd for $C_{20}H_{13}ClN_2O_3$: C, 65.85; H, 3.59; N, 7.65. Found: C, 65.51; H, 3.59; N, 7.65.

Ethyl 3-phenylacetylamido-5-chloropicolinate (12b). Mp 120–122 °C. ^{1}H NMR δ 1.43 (t, $J=7.5$ Hz, 3H), 3.79 (s, 2H), 4.45 (q, $J=7.5$ Hz, 2H), 7.37 (m, 5H), 8.32 (d, $J=2.1$ Hz, 1H), 9.21 (d, $J=2.1$ Hz, 1H), 11.08 (s, 1H).

5-Aza-7-chloro-4-hydroxy-3-phenylquinoline-2-one (13b). Mp 328–329 °C (dec.) ^{1}H NMR (DMSO-d_6) δ 7.28–7.44 (m, 5H), 7.71 (d, $J=2.1$ Hz, 1H), 8.48 (d, $J=2.1$ Hz, 1H), 10.88 (s, 1H), 11.69 (s, 1H). Anal. calcd for $C_{14}H_9ClN_2O_2.0.2H_2O$: C, 60.86; H, 3.43; N, 10.14. Found: C, 61.11; H, 3.18; N, 9.82.

Ethyl 3-(*p*-chloro)phenylacetylamido-5-chloropicolinate (12c). Mp 140–142 °C. ^{1}H NMR δ 1.44 (t, J = 6.9 Hz, 3H), 3.75 (s, 2H), 4.47 (q, J = 6.9 Hz, 2H), 7.25–7.38 (m, 4H), 8.34 (d, J = 2.1 Hz, 1H), 9.18 (d, J = 2.1 Hz, 1H), 11.13 (s, 1H).

5-Aza-7-chloro-4-hydroxy-3-(*p*-chloro)phenylquinoline-2-one (13c). Mp 342–344 °C. ^{1}H NMR (DMSO-d_6) δ 7.41 (d, J = 8.1 Hz, 2H), 7.47 (d, J = 8.1 Hz, 2H), 7.72 (d, J = 2.4 Hz, 1H), 8.49 (d, J = 2.4 Hz, 1H), 11.10 (brs, 1H), 11.74 (brs, 1H). Anal. calcd for $C_{14}H_8Cl_2N_2O_2.0.4H_2O$: C, 53.49; H, 2.82; N, 8.91. Found: C, 53.26; H, 2.55; N, 8.84.

Ethyl 3-(*m*-chloro)phenylacetylamido-5-chloropicolinate (12d). Mp 123–124 °C. ^{1}H NMR δ 1.42 (t, J = 7.2 Hz, 3H), 3.81 (s, 2H), 4.42 (q, J = 7.2 Hz, 2H), 6.85 (m, 3H), 7.30 (s, 1H), 8.34 (s, 1H), 9.21 (d, J = 1.2 Hz, 1H), 11.08 (s, 1H).

5-Aza-7-chloro-4-hydroxy-3-(*m*-chloro)phenylquinoline-2-one (13d). Mp 324–326 °C. ^{1}H NMR (DMSO-d_6) δ 7.400 (m, 4H), 7.692 (d, J = 1.5 Hz, 1H), 8.484 (d, J = 1.2 Hz, 1H), 8.463 (s, 1H), 11.158 (brs, 1H), 11.738 (s, 1H). Anal. calcd for $C_{14}H_8Cl_2N_2O_2$: C, 54.75; H, 2.63; N, 9.12. Found: C, 54.52; H, 2.42; N, 9.00. (HPLC purity >98%).

Ethyl 3-(*o*-chloro)phenylacetylamido-5-chloropicolinate (12e). Mp 163–165 °C. ^{1}H NMR δ 1.40 (t, J = 6.9 Hz, 3H), 3.95 (s, 2H), 4.42 (q, J = 6.9 Hz, 2H), 7.19–7.30 (m, 3H), 7.39 (d, J = 10.5 Hz, 1H), 8.34 (s, 1H), 9.23 (s, 1H), 11.09 (s, 1H).

5-Aza-7-chloro-4-hydroxy-3-(*o*-chloro)phenylquinoline-2-one (13e). Mp 248–250 °C. ^{1}H NMR (DMSO-d_6) δ 7.29 (m, 1H), 7.34–7.37 (m, 2H), 7.49 (m,1H), 7.73 (d, J = 2.1 Hz, 1H), 8.50 (d, J = 2.1 Hz, 1H), 11.14 (brs, 1H), 11.72 (s, 1H). Anal. calcd for $C_{14}H_8Cl_2N_2O_2$: C, 54.75; H, 2.63; N, 9.12. Found: C, 54.82; H, 2.52; N, 8.83.

Ethyl 3-(*m*-methoxy)phenylacetylamido-5-chloropicolinate (12f). Mp 108–110 °C. ^{1}H NMR δ 1.42 (t, J = 7.2 Hz, 3H), 3.80 (s, 3H), 3.81 (s, 2H), 4.42 (q, J = 7.2 Hz, 2H), 6.85 (m, 3H), 7.30(s, 1H), 8.34 (d, J = 1.2 Hz, 1H), 9.21 (d, J = 1.2 Hz, 1H), 11.08 (s, 1H).

5-Aza-7-chloro-4-hydroxy-3-(*m*-methoxy)phenylquinoline-2-one (13f). Mp 256–258 °C. ^{1}H NMR (DMSO-d_6) δ 3.74 (s, 3H), 6.80–7.00 (m, 3H), 7.28 (m, 1H), 7.71 (d, J = 2.1 Hz, 1H), 8.48 (d, J = 2.1 Hz, 1H), 10.90 (brs, 1H), 11.68 (s, 1 H). Anal. calcd for $C_{15}H_{11}ClN_2O_3$: C, 59.52; H, 3.66; N, 9.25. Found: C, 59.34; H, 3.57; N, 8.98.

Ethyl 3-(*m*-Methyl)phenylacetylamido-5-chloropicolinate (12g). Mp 94–96 °C. ^{1}H NMR δ 1.41 (t, J = 6.9 Hz, 3H), 2.36 (s, 3H), 3.73 (s, 2H), 4.43 (q, J = 6.9 Hz, 2H), 7.15–7.28 (m, 4H), 8.31 (m,1H), 9.21 (m, 1H), 11.06 (s, 1H).

5-Aza-7-chloro-4-hydroxy-3-(*m*-methyl)phenylquinoline-2-one (13g). Mp 300–302 °C. ^{1}H NMR (DMSO-d_6) δ 2.31 (s, 3H), 7.09 (d, J = 6.9 Hz, 1H), 7.18–7.28 (m, 3H), 7.70 (d, J = 2.1 Hz, 1H), 8.48 (d, J = 2.1 Hz, 1H), 10.82 (brs, 1H), 11.68 (s, 1H). Anal. calcd for $C_{15}H_{11}ClN_2O_2$: C, 62.84; H, 3.87; N, 9.77. Found: C, 62.63; H, 3.61; N, 9.61.

Ethyl 3-(3-thienyl)phenylacetylamido-5-chloropicolinate (12j). Mp 116–118 °C. ^{1}H NMR δ 1.43 (t, J = 6.6 Hz, 3H), 3.83 (s, 2H), 4.44 (q, J = 6.6 Hz, 2H), 7.09 (s, 1H), 7.27 (s, 1H), 7.37 (s, 1H), 8.34 (s, 1H), 9.22 (s, 1H), 11.07 (s, 1H).

5-Aza-7-chloro-4-hydroxy-3-(3-thienyl)phenylquinoline-2-one (13j). Mp 310–312 °C. ^{1}H NMR (DMSO-d_6) δ 7.48 (m, 1H), 7.68 (m, 2H), 7.98 (d, J = 2.1 Hz, 1H), 8.48 (d, J = 2.1 Hz, 1H), 11.17 (brs, 1H), 11.71 (brs, 1H). Anal. calcd for $C_{12}H_7ClN_2O_2S.0.15H_2O$: C, 51.22; H, 2.61; N, 9.95. Found: C, 51.10; H, 2.46; N, 9.72.

Ethyl 3-(2-thienyl)phenylacetylamido-5-chloropicolinate (12k). Mp 141–143 °C. ^{1}H NMR δ 1.43 (t, J = 6.9 Hz, 3H), 4.01 (s, 2H), 4.43 (q, J = 6.9 Hz, 2H), 7.04–7.07 (m, 2H), 7.30 (m, 1H), 8.35 (d, J = 1.8 Hz, 1H), 9.22 (d, J = 1.8 Hz, 1 H), 11.15 (s, 1H).

5-Aza-7-chloro-4-hydroxy-3-(2-thienyl)phenylquinoline-2-one (13k). Mp 318–320 °C (dec.). ^{1}H NMR (DMSO-d_6) δ 7.13 (m, 1H), 7.53 (d, J = 3.9 Hz, 1H), 7.73 (s, 1H), 8.22 (s, 1H), 8.53 (s, 1H), 11.89 (brs, 2H). Anal. calcd for $C_{12}H_7ClN_2O_2S$: C, 51.71; H, 2.53; N, 10.05. Found: C, 51.83; H, 2.53; N, 9.88.

Methyl 3-aminopicolinate (6). To a solution of 3-aminopicolinic acid (**1**, 0.58 g, 4.24 mmol) in 45 mL of absolute MeOH was added an ethereal solution of diazomethane (0.30 M, 30 mL). The solution was stirred for an additional 30 min and the solvent was removed in vacuo. The crude residue was chromatographed on a column of silica using $CHC1_3$–MeOH as eluent, giving 0.33 g (52%) of the title product as a yellow solid. Mp 139–146 °C; ^{1}H NMR δ 3.98 (t, J = 7.2 Hz, 3 H), 5.73 (d, J = 1.8 Hz, 2H), 7.05 (dd, J_1 = 1.2 Hz, J_2 = 8.4 Hz, 1H), 7.22 (dd, J_1 = 4.2 Hz, J_2 = 8.4 Hz, 1H), 8.07 (dd, J_1 = 1.2 Hz, J_2 = 4.2 Hz, 1H); IR (KBr): 3455, 3295, 3160, 1689, 1617, 1408, 1335, 1244, 1115.

Methyl 3-amido-(*N*-phenylacetyl)picolinate (12a). Mp 64–69 °C. ^{1}H NMR δ 3.78 (s, 2H), 3.97 (s, 3H), 7.38 (m, 6H), 8.39 (dd, J_1 = 0.6 Hz, J_2 = 3.9 Hz, 1H), 9.10 (dd, J_1 = 8.4 Hz, J_2 = 8.3 Hz, 1H), 10.96 (s, 1H); EI–MS (*m*/*e*): 270 (M^+, 35), 211 (35), 179 (85), 153 (40), 147 (65), 119 (40), 94, (40), 91 (100).

5-Aza-4-hydroxy-3-phenylquinotin-2(1*H*)-one (13a). Mp 297–299 °C. ^{1}H NMR (DMSO-d_6) δ 7.29 (d, J = 7.2 Hz, 1H), 7.37 (t, J = 7.2 Hz, 2H), 7.45 (d, J = 7.5 Hz, 2H), 7.60 (dd, J_1 = 4.2 Hz, J_2 = 8.1 Hz, 1H), 7.69 (d, J = 8.1 Hz, 1H), 8.49 (d, J = 4.2 Hz, 1H), 11.62 (s, 1H). IR (KBr): 3427, 3161, 2923, 2854, 1655, 1477, 1402, 1123, 693; EI-MS (*m*/*z*): 238 (M^+, 100), 210 (10), 181 (15), 93 (20), 89 (15), 78 (15), 63 (15), 51 (10), 39 (25).

Ethyl 4-aminonicotinate (8). Mp 109–111 °C (lit.[14] 109–111 °C). ^{1}H NMR δ 1.39 (t, J = 7.0 Hz, 3H), 4.35 (q, J = 7.0 Hz, 2H), 6.27 (bm, 2H), 6.53 (d, J = 3.9 Hz, 1H),

8.19 (d, $J=2.1$ Hz, 1H), 8.92 (dd, $J_1=3.3$ Hz, $J_2=4.5$ Hz, 1H).

Ethyl 4-amido-(*N*-[3-phenoxylphenylacetyl)nicotinate (12l). Mp 92–93.5 °C. ^{1}H NMR δ 1.41 (t, $J=7.2$ Hz, 3H), 3.77 (s, 2H), 4.37 (q, $J=7.2$ Hz, 2H), 7.20 (m, 9H), 8.58 (d, $J=5.4$ Hz, 1H), 8.66 (d, $J=5.4$ Hz, 1H), 9.14 (s, 1H), 11.24 (bs, 1H); IR (KBr): 3448, 3243, 3141, 2991, 1702, 1586, 1511, 1450, 1416, 1300, 1259, 1225, 1116, 802, 707, 543; HPLC 100%; EI-MS (*m*/*z*): 377 (20), 376 (M+, 80), 210 (65), 193 (40), 184 (25), 183 (35), 168 (10), 167 (65), 165 (70), 147 (100), 89 (30), 77 (20), 51 (10); HR-MS calcd for $C_{22}H_{20}N_2O_4$: 376.1423. Found: 376.1430.

6-Aza-4-hydroxy-3-(3′-phenoxy)phenylquinolin-2(1*H*)-one (13l). Mp 165–167 °C. ^{1}H NMR (DMSO-d_6) δ 7.20 (m, 10 H), 8.47 (dd, $J_1=1.2$ Hz, $J_2=3.6$ Hz, 1H), 9.04 (s, 1H), 11.81 (s, 1H); IR (KBr): 3434, 3134, 1654, 1491, 1402, 1239, 1130, 700; HPLC 100%; LR-MS (*m*/*z*): 331 (30), 330 (M$^+$, 100), 329 (100). 236 (20), 121 (40), 93 (40), 77 (50), 66 (20), 51 (40), 39 (25); HR-MS calcd for $C_{20}H_{14}N_2O_3$: 330.1004. Found: 330.1002.

Ethyl 3-aminoisonicotinate (9). Mp 63–64 °C (lit.[15] 65 °C). ^{1}H NMR δ 1.40 (t, $J=7.0$ Hz, 3H), 4.37 (q, $J=7.0$ Hz, 2H), 5.77 (d, $J=1.2$ Hz, 2H), 7.64 (d, $J=5.1$ Hz, 1H), 7.91 (d, $J=5.1$ Hz, 1H), 8.25 (s, 1H).

Ethyl 3-amido-(*N*-[3-phenoxylphenylacetyl)isonicotinate (12m). Oil; ^{1}H NMR δ 1.40 (t, $J=7.2$ Hz, 3H), 3.77 (s, 2H), 4.35 (q, $J=7.2$ Hz, 2H), 7.10 (m, 9H), 7.77 (d, $J=5.1$ Hz, 1H), 8.41 (d, $J=5.1$ Hz, 1H), 10.01 (s, 1H), 10.65 (brs, 1H); IR (KBr): 3291, 3066, 2991, 1709, 1593, 1518, 1491, 1416, 1368, 1293, 1259, 1225, 1191, 1116, 1020, 973, 796, 768, 700; HPLC: 98.3%; EI–MS (*m*/*z*): 377 (15), 376 (M$^+$, 70), 211 (15), 210 (100), 193 (25), 184 (20), 183 (45), 166 (25), 165 (15), 147 (40), 89 (20); HR-MS calcd for $C_{22}H_{20}N_2O_4$: 376.1423. Found: 376.1430.

7-Aza-4-hydroxy-3-(3′-phenoxy)phenylquinolin-2(1*H*)-one (13m). Mp 277–280 °C (dec.). ^{1}H NMR (DMSO-d_6) δ 7.20 (m, 9H), 7.81 (d, $J=5.4$ Hz, 1H), 8.32 (d, $J=5.4$ Hz, 1H), 8.62 (s, 1H), 10.65 (d, $J=2.4$ Hz, 1H), 11.74 (s, 1H); IR (KBr): 3427, 3148, 2936, 2861, 1661, 1614, 1498, 1402, 1307, 1252, 1225, 1198, 1136, 857, 700, 564; HPLC: 100%; LR-MS (*m*/*z*): 332 (20), 331 (90), 330 (M$^+$, 100), 121 (15), 93 (15), 77 (10); HR-MS calcd for $C_{20}H_{14}N_2O_3$: 330.1004. Found: 330.1018. Anal. calcd for $C_{20}H_{14}N_2O_3.H_2O$: C 69.0%, H 4.6%, N 8.0%. Found: C 69.3%, H 4.3%, N 7.7%.

Ethyl 2-aminonicotinate (10). Mp 90.5–92.5 °C (lit.[16] 94–95 °C). ^{1}H NMR δ 1.38 (t, $J=7.2$ Hz, 3H), 4.34 (q, $J=7.2$ Hz, 2H), 6.40 (bm, 2H), 6.62 (dd, $J_1=4.9$ Hz, $J_2=7.8$ Hz, 1H), 8.14 (dd, $J_1=1.0$ Hz, $J_2=8.4$ Hz, 1H), 8.21 (dd, $J_1=1.2$ Hz, $J_2=3.8$ Hz, 1H).

Ethyl 2-amido-[*N*-(3-phenoxy)phenylacetyl]nicotinate (12n). Mp 90–91 °C. ^{1}H NMR δ 1.39 (t, $J=6.8$ Hz, 3H), 3.83 (s, 2H), 4.35 (q, $J=6.8$ Hz, 2H), 7.10 (m, 9H), 8.29 (dd, $J_1=1.5$ Hz, $J_2=7.8$ Hz, 1H), 8.59 (dd, $J_1=1.5$ Hz, $J_2=4.8$ Hz, 1H), 10.81 (s, 1H); IR (KBr): 3441, 3168, 1723, 1668, 1660, 1549, 1490, 1436, 1409, 1307, 1246, 1211, 1143, 1027, 980, 775, 700; HPLC 100%; EI-MS (*m*/*z*): 377 (10), 376 (M$^+$, 45), 211 (15), 210 (100), 183 (10), 167 (15), 166 (25), 147 (35), 121 (10), 94 (35), 89 (30), 77 (15), 51 (10), 39 (10); HR-MS calcd for $C_{22}H_{20}N_2O_4$: 376.1423. Found: 376.1419.

8-Aza-4-hydroxy-3-(3′-phenoxy)phenylquinolin-2(1*H*)-one (13n). Mp 246–248 °C (dec.). ^{1}H NMR (DMSO-d_6) δ 7.09 (m, 6H), 7.23 (dd, $J_1=2.4$ Hz, $J_2=7.8$ Hz, 1H), 7.38 (m, 3H), 8.30 (d, $J=7.8$ Hz, 1H), 8.50 (d, $J=2.4$ Hz, 1H), 10.52 (bm, 1H), 11.81 (s, 1H); IR (KBr) 3434, 3127, 1648, 1607, 1498, 1409, 1341, 1246, 1150, 946, 789, 761, 700, 571, 543, 475; HPLC 100%; EI-MS (*m*/*z*): 332 (15), 331 (75), 330 (M$^+$, 100), 121 (10), 93 (10), 77 (1 0), 51 (10), 39 (10); HR-MS calcd for $C_{20}H_{14}N_2O_3$: 330.1004. Found: 330.1015.

Ethyl 2-amido-(*N*-phenylacetyl)nicotinate (12o). Oil; ^{1}H NMR δ 1.37 (t, $J=6.9$ Hz, 3H), 3.91 (s, 2H), 4.33 (q, $J=6.9$ Hz, 2H), 7.04 (dd, $J_1=4.2$ Hz, $J_2=7.5$ Hz, 1H), 7.39 (m, 5H), 8.27 (dd, $J_1=0.9$ Hz, $J_2=7.6$ Hz, 1H), 8.60 (d, $J=3.9$ Hz, 1H), 10.76 (s, 1H).

8-Aza-4-hydroxy-3-phenylquinolin-2(1*H*)-one (13o). Mp 340–342 °C. ^{1}H NMR (DMSO-d_6) δ 7.17 (dd, $J_1=4.8$ Hz, $J_2=7.8$ Hz, 1H), 7.25 (m, 1H), 7.34 (t, $J=7.2$ Hz, 2H), 7.42 (dd, $J_1=0.9$ Hz, $J_2=7.2$ Hz, 2H), 8.29 (dd, $J_1=1.2$ Hz, $J_2=7.8$ Hz, 1H), 8.45 (dd, $J_1=1.2$ Hz, $J_2=7.8$ Hz, 1H), 11.64 (s, 1H); HPLC 99.2%; LR-MS (*m*/*z*): 239 (M+, 100), 238 (90), 181 (20), 121 (40), 118 (20), 93 (40), 91 (55), 89 (15), 77 (25), 69 (20), 63 (25), 57 (35), 55 (40), 51 (30), 43 (55), 41 (55); HR-MS calcd for $C_{14}H_{10}N_2O_2$: 238.0742. Found: 238.0737.

Ethyl 2-amido-[*N*-(3-phenoxy)phenylacetyl]-5-chloronicotinate (12p). Mp 103–104 °C. ^{1}H NMR δ 1.39 (t, $J=7.2$ Hz, 3H), 3.88 (s, 2H), 4.35 (q, $J=7.2$ Hz, 2H), 7.10 (m, 9H), 8.24 (d, $J=2.4$ Hz, 1H), 8.52 (d, $J=2.4$ Hz, 1H), 10.70 (d, $J=2.4$ Hz, 1H); IR (KBr) 3434, 3155, 2925, 1722, 1659, 1401, 1252, 1205, 1102, 775, 700; HPLC 99%; LR-MS (*m*/*z*): 41 0 (M$^+$, 40), 211 (35), 210 (100), 200 (35), 183 (30), 181 (40), 128 (15), 89 (50), 77 (20), 51 (15); HR-MS calcd for $C_{22}H_{19}ClN_2O_4$: 410.1033. Found: 410.1026.

8-Aza-6-chloro-4-hydroxy-3-(3′-phenoxy)phenylquinolin-2(1 *H*)-one (13p). Mp 237–240 °C (dec.). ^{1}H NMR (DMSO-d_6) δ 7.10 (m, 9H), 8.33 (d, $J=2.0$ Hz, 1H), 8.54 (d, $J=2.0$ Hz, 1H), 10.70 (m, 1H), 12.04 (s, 1H); IR (KBr): 3434, 3155, 1641, 1409, 1218, 1136, 1096; HPLC: 98.0%; LR-MS (*m*/*z*): 366 (25), 365 (45), 364 (M+, 80), 363 (100), 214 (30), 169 (15), 168 (10), 155 (15), 141 (15), 127 (15), 78 (30), 77 (30), 63 (40), 51 (30), 45 (10), 39 (10); HR-MS calcd for $C_{20}H_{13}CN_2O_3$: 364.0615. Found: 364.0606.

Ethyl 2-amido-(*N*-phenylacetyl)-5-chloronicotinate (12q). Mp 113–114 °C. ^{1}H NMR δ 1.38 (t, $J=7.2$ Hz, 3H), 3.90 (s, 2H), 4.34 (q, $J=7.2$ Hz, 2 H), 7.38 (m, 5H), 8.22 (d, $J=2.4$ Hz, 1H), 8.54 (d, $J=2.4$ Hz, 1H), 10.64 (s,

1H); IR (KBr): 3427, 3196, 1743, 1640, 1600, 1409, 1272, 1218, 1136, 1096, 796; HPLC: 100%; EI-MS (*m*/*z*): 318 (M+, 25), 202 (20), 201 (10), 200 (60), 183 (15), 181 (40), 154 (10), 128 (10), 118 (100), 91 (80), 65 (25), 39 (10); HR-MS calcd for $C_{16}H_{15}ClN_2O_3$: 318.0771. Found: 318.0772.

8-Aza-6-chloro-4-hydroxy-3-phenylquinolin-2(1*H*)-one (13q). Mp 324–326 °C (dec.). ^{1}H NMR (DMSO-d_6) δ 7.36 (m, 5H), 8.35 (d, J = 2.1 Hz, 1H), 8.55 (d, J = 2.1 Hz, 1H), 10.64 (bm, 1H), 12.05 (s, 1H); IR (KBr): 3434, 3134, 1661, 1559, 1402, 1341, 1232, 1143, 673, 564; HPLC: 100%; LR-MS (*m*/*z*): 274 (30), 273 (45), 272 (M$^+$, 100), 271 (90), 215 (10), 157 (10), 155 (35), 127 (20), 89 (15), 77 (10), 63 (10), 51 (10), 39 (10); EI-MS calcd for $C_{14}H_9ClN_2O_2$: 272.0352. Found: 272.0349.

Quinolinimide (15). The procedure of Crum and Fuchsman[9] was modified. A mixture of quinolinic acid (**14**, 16.8 g, 101 mmol) and acetic anhydride (22 mL, d = 1.08, 230 mmol) was heated at 120 °C to distill off 19 mL of distillate. To the cooled residue (100 °C) was added acetamide (13.4 g, 227 mmol) over 5 min. The stirred mixture was heated at reflux for 2.5 h. The mixture was then cooled to rt, filtered, and the filter cake was washed with water (2×25 mL) to obtain a brown solid. The solid was recrystallized in hot ethanol (95%, 50 mL) to give 8.4 g (56%) of the title compound as a brown solid: mp 230–231 °C (lit.[9] 230–233 °C); ^{1}H NMR (DMSO-d_6) δ 7.76 (dd, J_1 = 2.4 Hz, J_2 = 5.1 Hz, 1H), 8.24 (dd, J_1 = 0.9 Hz, J_2 = 7.2 Hz, 1H), 8.96 (dd, J_1 = 0.9 Hz, J_2 = 7.5 Hz, 1H), 11.66 (brs, 1H). IR (KBr): 3484, 3189, 3100, 3083, 1735, 1704, 1086, 736.

3-Aminopicolinic acid (1). The method of Oakes et al.[10] was modified. To a solution of quinolinimide (**15**, 8.0 g, 54 mmol) in an ice-cold 10% NaOH solution (160 mL) was added an ice-cold aqueous sodium hypobromite solution (prepared by adding bromine (3.0 mL, 9.3 g, 58 mmol) to 56 mL of an ice-cold 15% NaOH solution) over 10 min. The brown solution was stirred at rt for 1 h and at 85 °C for 1 h. The solution was then cooled to rt and the pH was adjusted to 5 (pH paper) using sulfuric acid (50%, 50 mL). The solution was stirred at 4 °C for 63 h. The resulting mixture was filtered and the mother liquor was treated with copper (II) acetate-monohydrate (3.2 g, 16 mmol) in 64 mL of hot water containing 1.60 mL of glacial acetic acid. The resulting mixture was cooled to rt and filtered, and the filter cake was washed with water (2×25 mL). The precipitate was resuspended in water (64 mL) and saturated with hydrogen sulfide. The mixture was filtered through a fritted filter to remove the copper sulfide. The filtrate was rotevaporated to dryness, leaving an orange solid. The orange solid was recrystallized from water to give 0.82 g (10%) of the title compound as light brown crystals: mp 207.5–208 °C (lit.[10] 210 °C); ^{1}H NMR (DMSO-d_6) δ 7.26 (d, J = 8.4 Hz, 1H), 7.33 (dd, J_1 = 4.2 Hz, J_2 = 8.4 Hz, 1H), 7.82 (d, J = 3.9 Hz, 1H); IR (KBr) 386, 3200, 3133, 1644, 1565, 1532, 1398, 1286, 806.

2-Amino-5-chloro-3-nitropyridine (17). The procedure of Vaughan et al.[11] was modified. Sulfuric acid (97%, 300 mL) was placed in a 500 mL three-neck round-bottom flask. The flask was equipped with an internal thermometer, a glass funnel and stopper, and placed in a salt/ice bath. When the internal temperature reached 5 °C, 2-amino-5-chloropyridine (**16**, 77.2 g, 0.60 mol) was added over 1 h with stirring. The suspension was then stirred at rt to dissolve the rest of the solid. The resulting solution was heated to 55 °C. Nitric acid (70%, 40.5 mL, d = 1.41, 0.634 mol) was added dropwise through an addition funnel so as to maintain the internal temperature at 57 ± 3 °C. The reaction solution was poured over ice (1.5 kg), and the resulting mixture was partially neutralized with 40% NaOH (~600 mL). The mixture was filtered to leave a yellow/orange solid. This solid was washed by resuspension in water (600 mL). The mixture was filtered and the resulting solid was dried in the oven 48 h to yield 66.3 g (64%) of the title compound as an orange/yellow solid: mp 191–193 °C (lit.[11] 190–103 °C); ^{1}H NMR δ 6.69 (brs, 2H), 8.33 (d, J = 1.5 Hz, 1H), 8.43 (d, J = 1.5 Hz, 1H).

2-Bromo-5-chloro-3-nitropyridine (18). To HBr (48%, d = 1.49, 214 mL, 1.89 mol) was added 2-amino-5-chloro-3-nitropyridine (**17**, 66.0 g, 0.38 mol) in portions at 0 °C with a mechanical stirring. The mixture was stirred until the internal temperature < 0 °C, and then bromine (65 mL, d = 3.102, 1.3 mol) was added dropwise. The resulting orange mixture was stirred < 0 °C and a solution of $NaNO_2$ (91.3 g, 1.32 mol) in 125 mL of water was added slowly as to maintain the internal temperature < 0 °C. The mixture was stirred for 45 min at < 0 °C, and then a solution of NaOH (139.3 g, 3.48 mol) in 200 mL of water was added to the mixture slowly to maintain the internal temperature < 20 °C. The mixture was stirred at < 20 °C for an additional h, and then filtered. The recovered brown solid was dried at 25 °C under vacuum for 6 h. It was purified by recrystallization from 95% ethanol to give 46.0 g (52%) of the title compound as a yellow solid: mp 73–75 °C (lit.[17] 75 °C); ^{1}H NMR δ 8.15 (d, J = 2.1 Hz, 1H), 8,57 (d, J = 2.1 Hz, 1H).

5-Chloro-3-nitropicolinonitrile (19). The procedure of Berrie et al. was modified.[17] 2-Bromo-5-chloro-3-nitropyridine (**18**, 6.0 g, 25 mmol) was mixed with copper (I) cyanide (4.6 g, 51 mmol) in a 100 mL round-bottom flask fitted with a condenser loosely plugged with cotton. The flask was slowly heated in an oil bath. When the temperature reached ~150 °C (takes 2 h), the reaction mass began to turn black. When the reaction mass turned completely black (or close to it), the pressure was reduced to ~1 mm Hg by vacuum and the oil bath removed after 30 s. The mixture was cooled to rt, and the sublimed solid (on the cotton) and reaction mass were treated with hot acetone (100 mL). The resulting mixture was filtered, and the mother liquor rotaevaporated to dryness to yield the crude title compound as a dark brown solid. This solid was purified by column chromatography using 4:1 hexane-EtOAc (R_f = 0.13) to give 7.30 g (80%) of the title compound as a white solid: mp 95–97 °C (lit.[17] 98 °C); ^{1}H NMR δ 8.62 (d, J = 1.5 Hz, 1H), 8.95 (d, J = 1.5 Hz, 1H).

3-Amino-5-chloropicolinamide (20). The procedure of McCaustland and Cheng was modified.[18] Raney nickel

(10 g of 50% slurry in water) was washed with water (100 mL), 5% AcOH (100 mL), water (100 mL), and 95% EtOH (3×100 mL). The slurry was added to a solution of 5-chloro-3-nitropicolinonitrile (**19**, 3.0 g, 16.3 mmol) in 95% EtOH (100 mL). The mixture was hydrogenated at 45 psi for 2.5 h. The mixture was then filtered over a bed of Celite and washed with 95% EtOH (2×100 mL). The red/brown filtrate was rotaevaporated to dryness, leaving 2.7 g (95%) of the title compound as a light brown solid: mp 162–163 °C (lit.[18] 165–166 °C); ^{1}H NMR δ 5.42 (bs, 1H), 6.05 (bs, 2H), 7.00 (d, J = 1.2 Hz, 1H), 7.71 (bs, 1H), 7.79 (d, J = 1.2 Hz, 1H).

3-Amino-5-chloropicolinic acid hydrochloride (2). Concentrated HCl (38%, 32 mL, d = 1.20, 400 mmol) was added to 3-amino-5-chloropicolinamide (**20**, 2.3 g, 13 mmol). The mixture was stirred and heated to reflux. The resulting solution was refluxed (100 °C) for 17 h. The resulting mixture was cooled to rt, then placed in the cold room for 3 h. The mixture was filtered, leaving 2.5 g (85%) of the title compound as its hydrochloride salt: mp 235–236 °C; ^{1}H NMR (DMSO-d_6) δ 6.68 (brs, 2H), 7.33 (d, J = 1.8 Hz, 1H), 7.80 (d, J = 2.1, 1H). calcd for $C_6H_6Cl_2N_2O_2$: C, 34.47; H, 2.89; N, 13.40. Found: C, 34.19; H, 2.75; N, 13.40.

5-Chloro-2-cyano-3-methoxypyridine (21a). To a solution of 5-chloro-3-nitropicolinonitrile (**19**) (183.5 mg, 1.0 mmol) in 5 mL of methanol was added 1.2 mL of 10% sodium hydroxide aqueous solution at 0 °C. The resulting solution was allowed to stir at rt for 12 h. Methanol was evaporated in vacuo to give a suspension, which was neutralized with 4 N HCl to pH = 6. A yellow solid was collected by filtration and dried in vacuo to give 110 mg (65%) of the product: mp 93–95 °C; ^{1}H NMR δ 4.00 (s, 3 H), 7.36 (s, 1H), 8.25 (s, 1H). Anal. calcd for $C_7H_5ClN_2O$: C, 49.87; H, 2.99; N, 16.62. Found: C, 49.91; H, 3.02; N, 16.5.

5-Chloro-2-cyano-3-ethoxypyridine (21b). To a solution of 5-chloro-3-nitropicolinonitrile (**19**) (134 mg, 0.73 mmol) in 5 mL of ethanol was added 0.8 mL of 10% sodium hydroxide aqueous solution at 0 °C. The resulting solution was allowed to stir at rt for 12 h. Methanol was evaporated in vacuo to give a suspension, which was neutralized with 4 N HCl to pH = 6. A yellow solid was collected by filtration and dried in vacuo to give 75 mg (55%) of the product: mp 53–55 °C; ^{1}H NMR δ 1.53 (t, J = 6.9 Hz, 3H), 4.20 (q, J = 6.9 Hz, 2H), 7.33 (s, 1H), 8.23 (s, 1H). Anal. calcd for $C_8H_7ClN_2O$: C, 52.62; H, 3.86; N, 15.34. Found: C, 52.47; H, 3.80; N, 15.35.

4-Nitro-3-picoline-l-oxide (23). The method of Taylor and Crovetti was utilized.[19] To a cooled (0–5 °C) mixture of concentrated HNO_3 (70%, 137.5 mL, d = 1.40, 2.139 mol) and concentrated H_2SO_4 (96%, 175 mL, d = 1.84, 3.15 mol) was slowly added so as to maintain internal temperature <10 °C 3-picoline-l-oxide (**22**, 50.0 g, 0.458 mol). The resulting solution was heated slowly to 100–105 °C and held there for 2.5 h. The orange solution was cooled to rt, and then poured over ice (500 g). The green/yellow solution was partially neutralized with Na_2CO_3 (210 g). The mixture was placed in the cold room overnight and then filtered. The filter cake was washed with water (20 mL) and dried at 45 °C for 6 h, giving 32.4 g (46%) of the title compound as yellow flakes: mp 134–136 °C (lit.[19] 136–137 °C); ^{1}H NMR δ 2.62 (s, 3H), 8.02 (d, J = 6.9 Hz, 1H), 8.10 (s, 1H), 8.13 (s, 1H).

4-Nitronicotinic acid *N*-oxide (24). The procedure of Taylor and Crovetti[20] was used. To concentrated H_2SO_4 (96%, 61 mL, d = 1.84, 1.1 mol) in an ice-bath was added 4-nitro-3-picoline-*N*-oxide (**24**, 9.0 g, 58 mmol) slowly so as to maintain the internal temperature at 20–25 °C. To the resulting orange solution was added $Na_2Cr_2O_7 \cdot 2H_2O$ (17.4 g, 58.4 mmol) over 45 min while maintaining the internal temperature at 30–40 °C. The dark green mixture was stirred at 30 °C for 2.5 h and then poured onto ice (125 g). The mixture was diluted with water (total volume: 215 mL) and placed in the cold room overnight. The mixture was filtered and dried in vacuo to give 2.8 (26%) of the title compound as a light green solid: mp 171 °C (dec.) (lit.[20] 170–172 °C); ^{1}H NMR (DMSO-d_6) δ 8.13 (d, J = 6.9 Hz, 1H), 8.47 (d, J = 5.1 Hz, 1H), 8.64 (s, 1H).

4-Aminonicotinic acid (3). The method of Herz and Murty was modified.[21] 4-Nitronicotinic acid *N*-oxide (**24**, 0.8 g, 4 mmol) was added to water (20 mL). The slurry was stirred while concentrated NH_4OH (29%, d = 0.9, 0.6 mL, 4 mmol) was added dropwise. The resulting suspension was diluted with water (total volume, 40 mL) and then 10% Pd/C (0.2 g) was added. The resulting mixture was hydrogenated at 50 psi for 2 h. The mixture was filtered over a bed of Celite and washed with 0.3% NH_4OH (20 mL). The filtrate was concentrated to 10 mL and neutralized with 4 N HCl (four drops). The mixture was filtered leaving 0.2 g (40%) of the title compound as a gray solid: mp 327.5–329.5 °C (lit.[21] 330 °C); ^{1}H NMR (DMSO-d_6) δ 6.71 (d, J = 6.0 Hz, 1H), 8.54 (s, 1H).

3-Aminoisonicotinic acid (4). The procedure of Chen and Deady was modified.[15] 3,4-Pyridinedicarboximide (**25**, 4.95 g, 30 mmol) was added in portions to an ice-cold solution of sodium hypobromite, prepared by adding Br_2 (1.75 mL, d = 3.102, 34.0 mmol) to 100 mL of an ice-cold 10% NaOH solution. More 10% NaOH (60 mL) was added and the resulting yellow solution was heated over 25 min to 85 °C. The stirred solution was maintained at 85 °C for 10 min and then cooled to rt. The solution was cooled in an ice bath and acidified to pH 3 with 50% H_2SO_4 (20 mL). The mixture was suction filtered, leaving an orange/brown solid. This solid was dried at 45 °C for 1.5 h to leave 3.7 g (79%) of the title compound as an orange solid: mp 283–285 °C (lit.[15] 280 °C); ^{1}H NMR (DMSO-d_6) δ 7.45 (d, J = 7.2 Hz, 1H), 7.71 (d, J = 5.4 Hz, 1H), 8.18 (s, 1H).

Ethyl 2-amino-5-chloronicotinate (11). To a solution of ethyl 2-aminonicotinate (**10**, 163 mg, 0.99 mmol) in 1 mL of concentrated HCl (35%) was added 0.10 mL of H_2O_2 (30%, d = 1.110, 0.98 mmol). The resulting solution was heated at 55–60 °C for 2 h. The solution was cooled to rt, diluted with water (10 mL) and basified to pH 8 (pH

paper) with solid $NaHCO_3$. The mixture was filtered, washed with water (2×10 mL) and dried at 45 °C for 2 h to leave 0.186 g (94%) of the title compound as a white solid. Mp 119.5–121.5 °C; ^{1}H NMR δ 1.39 (t, J = 7.05 Hz, 3H), 4.35 (q, J = 7.05 Hz, 2H), 6.44 (bm, 2H), 8.11 (d, J = 2.40 Hz, 1H), 8.16 (d, J = 2.4 Hz, 1H); IR (KBr): 3448, 3441, 3284, 3161, 1709, 1641, 1402, 1307, 1232, 1150, 1109, 796; HPLC: 100%; EI-MS (m/e): 202 (30), 200 (M^+, 100), 172 (15), 156 (20), 155 (30), 154 (60), 130 (15), 129 (20), 128 (50), 127 (45), 126 (15), 100 (15), 92 (10), 73 (20), 65 (15), 64 (15); HR-MS calcd for $C_8H_9ClN_2O_2$: 200.0352. Found: 200.0355.

Ethyl 3-acetylamido-5-chloropicolinate (26). To a solution of ethyl 3-amino-5-chloropicolinate (**7**, 2.0 g, 10 mmol) in 10 mL of 1,4-dioxane was added 4 mL of acetic anhydride. The resulting solution was allowed to stir at 50 °C for 24 h. Solvent was evaporated and water (10 mL) was added to the residue. The mixture was neutralized by saturated sodium bicarbonate solution to pH = 7. A pale yellow solid was collected by filtration and dried in vacuo, giving 2.0 g (83%) of the product: mp 98–100 °C; ^{1}H NMR δ 1.48 (t, J = 7.2 °Hz, 3H), 4.45 (q, J = 7.2 Hz, 3H), 8.35 (d, J = 2.1 Hz, 1H), 9.22 (d, J = 2.1 Hz, 1H), 11.09 (s, 1H).

5 - Aza - 7 - chloro - 4 - hydroxyquinolin - 2(1*H*) - one (27). KHMDS (6.0 mL of 0.5 M soln in toluene) was placed in a 25 mL round-bottom flask under nitrogen. The solution was cooled to −78 °C (acetone/dry ice). After 15 min, a solution of ethyl 3-amido-(*N*-acetyl)-5-chloropicolinate (**26**, 243.0 mg, 1.00 mmol) in dry THF (4.0 mL) was added via syringe. The resulting mixture was stirred under nitrogen and allowed to warm to rt. The mixture was stirred at rt overnight. The reaction mixture was quenched with water (10 mL) and washed with EtOAc (2×5 mL). The aqueous layer was acidified to pH < 2 by the addition of 4 N HCl. The resulting mixture was filtered and the filter cake washed with water. The solid was dried in vacuo to leave 152.8 mg (78%) of the title compound as a light brown solid: mp 294–296 °C; ^{1}H NMR (DMSO-d_6) δ 5.86 (s, 1H), 7.66 (s, 1H), 8.42 (s, 1H), 11.35 (s, 1H); IR (KBr): 3434, 3134, 2929, 2854, 1682, 1470, 1396, 1239, 1218, 1164, 802; HPLC: 98.2%; EI-MS (*m*/*z*): 198 (35), 196 (M^+, 100), 170 (20), 168 (60), 127 (20), 113 (15), 69 (25), 64 (15); HR-MS calcd for $C_8H_5ClN_2O_2$: 196.0040. Found: 196.0045.

5-Aza-7-chloro-4-hydroxy-3-nitroquinolin-2(1*H*)-one (28). To a suspension of 5-aza-7-chloro-4-hydroxyquinolin-2(1*H*)-one (**25**, 107.0 mg, 0.54 mmol) in 2 mL of glacial acetic acid was added 0.2 mL of HNO_3 (70%, d = 1.40, 3 mmol). The orange mixture was heated at 100 °C for 1 h, then cooled to rt. The mixture was quenched with water (3 mL) and the mixture was filtered, giving yellow crystals. The crystals were dried (2 h at 45 °C) to give 45.6 mg (46%) of the title compound as yellow crystals: mp 252–254 °C; ^{1}H NMR (DMSO-d_6) δ 7.74 (s, 1H), 8.58 (s, 1H), 12.01 (d, J = 1.5 Hz, 1H); IR (KBr) 3441, 3141, 3004, 2930, 2861, 1675, 1546, 1470, 1402, 1293, 1239, 1157, 11 02, 932, 904, 809, 625, 468; HPLC: 100%; EI–MS (*m*/*e*): 243 (25), 241 (M^+, 80), 225 (30), 213 (35), 212 (15), 211 (100), 197 (15), 196 (15), 183 (15), 181 (30), 157 (15), 155 (50), 154 (20), 153 (25), 139 (15), 128 (15), 127 (20), 126 (15), 125 (15), 114 (15), 112 (50), 103 (15), 91 (25), 76 (30), 73 (20), 65 (15), 64 (45), 52 (25), 38 (25); HR-MS calcd for $C_8H_4ClN_3O_4$: 240.9890. Found: 240.9888.

Binding assay. The [^{3}H]DCKA binding assay was performed as described previously.[8]

Electrophysiology. Agonist concentration–response curves were analyzed as described previously.[8] EC_{50} and slope values for glycine on NR1a/2C receptors were 0.327 μM and 1.81, respectively (n = 4). K_b values were estimated from five point concentration-inhibition data using a generalized form of the Cheng–Prusoff equation.

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