Cyclic Variations of 3-Quinolinecarboxamides and Effects on Antiherpetic Activity

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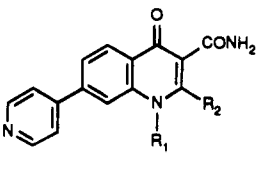
Supported by the antiherpetic properties of 3-quinolinecarboxamides and the importance of the planar intramolecular H-bonded β-keto amide pharmacophore, a series of novel conformationally rigid analogues that contain a heterocyclic bridge between the 3- and 4-positions of the quinoline ring have been evaluated. Two isoxazolo-fused derivatives **17** and **23** displayed good in vitro antiherpetic potency that was similar to that of **1**, the 3-quinolinecarboxamide that served as the comparison structure for this study. The pyrazolo, pyrrolo, and pyrimido derivatives showed considerably less or no activity. In vitro activity did not translate to in vivo efficacy. For **17**, the lack of in vivo activity is likely a consequence of insufficient plasma drug levels (both C_{max} and duration) in mice relative to the MIC versus HSV-2.

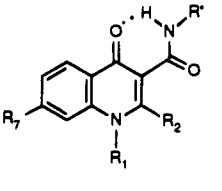
Introduction

We recently reported the in vitro plaque reduction and in vivo antiherpetic activities resident in a series of novel 3-quinolinecarboxamides.[1] The lead compound for this study was the 1-ethyl derivative **1** which was 5-fold less potent in vitro than acyclovir (**5**), a clinically useful antiherpetic agent. Extensive SAR studies revealed that the intramolecular H-bonded structure **4** was a prerequisite for activity. This conclusion was based on several observations, including the lack of in vitro activity of the 2-methyl analogue **2** due to destabilization of the active conformation **4** by $A_{1,3}$ strain between the 2-methyl and carbonyl groups. The 1-(4-FC_6H_4) derivative **3** emerged from that study at the most potent analogue in the series. Compared to acyclovir, **3** was 5-fold more potent in vitro and had similar oral efficacy and potency in a multiple-dose mouse model of infection.

We now report our results summarizing the syntheses and antiherpetic properties of novel cyclic variants of **1** where the intramolecular H-bonded β-keto amide spanning the 3- and 4-positions of the quinoline ring was replaced by isoxazolo, pyrazolo, pyrrolo, and pyrimido ring fusions. By replacing the hydrogen bond in **1** (or **4**) with a covalent attachment, a lower free energy of binding to the, as yet, undefined molecular target would result through favorable entropic considerations (i.e., highly organized and preformed active conformation). This rationale assumes that the acyclic and cyclic derivatives share the same molecular target, have similar spatial and electronic properties, and have similar cell permeability attributes (i.e., cellular assay used for evaluation). Numerous reports describing the benzodiazapine receptor binding properties of similar heterocyclic derivatives, including **6** (CGS 8216), have appeared.[2]



1: R_1 = CH_2CH_3, R_2 = H
2: R_1 = CH_2CH_3, R_2 = CH_3
3: R_1 = 4-FC_6H_4, R_2 = H



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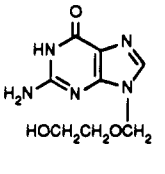

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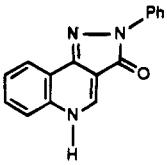

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Chemistry

As shown in Schemes 1–3, target compounds were made from the known ethyl 3-quinolinecarboxylate **7**[3] by conversion first to the more reactive thione derivative **8**. There was no reaction of **8** with phenylhydrazine at elevated temperatures; however, this nucleophile reacted very rapidly with the highly activated quaternary salt **9** (made by treating **8** with CH_3I/THF) to give **20**.

In the synthesis of pyrrolo derivative **21** (Scheme 2), the putative intermediate, **12**, underwent rapid decarboxylation in the mildly acidic medium of the hydrogenolysis. Proton NMR data indicate that both methanesulfonate salts **11** and **21** exist primarily in the enol form. These conclusions are based on the lack of methine (for **11**) and methylene (for **21**) hydrogen resonances and the appearance of one new aromatic hydrogen resonance at δ 7.21 in the spectrum of **21**. The methyl-substituted isoxazole derivative, **23**, was made in straightforward fashion (Scheme 4) from the known isatoic anhydride **15**.[4]

Results and Discussion

HSV-2 Plaque Reduction Activity. The HSV-2 plaque reduction activity in Vero cells of the target

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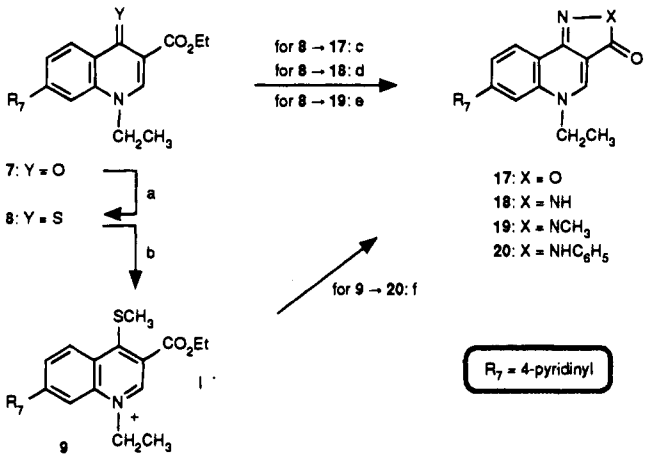
Scheme 1[a]



[a] (a) P_4S_{10}, pyr; (b) CH_3I, THF; (c) NH_2OH·HCl, NaOAc, EtOH; (d) NH_2NH_2·H_2O, DMF; (e) NH_2NHCH_3, DMF; (f) $NH_2NHC_6H_5$, NaOAc, EtOH.

compounds **17**–**23** compared to 3-quinolinecarboxamides **1**, **2**, and **3** as well as acyclovir (**5**) is shown in Table 1 (see footnote for definition of terms and variance of the assay). Relative to **1**, plaque reduction potency was sustained for isoxazolo analogue **17** and was 2-fold greater for the methylisoxazolo derivative **23**. Except for the pyrazolo-fused derivative **18**, which was 4-fold less potent than **1** or **17**, the other target compounds (**19**–**22**) were inactive.

Clearly, the isoxazolo ring fusion represents an effective isosteric replacement for the β-keto amide of **1**. The fact that the methyl-substituted isoxazolo **23** has similar potency to **1** and **17** considerably strengthens our earlier conclusion concerning the role of the 2-methyl group in the acyclic series (i.e., **2**); namely, the inactivity of **2** is a result of raising the free energy of the active conformation due to $A_{1,3}$ strain rather than the molecular target simply not being able to accommodate a methyl group at the 2-position due to steric crowding.

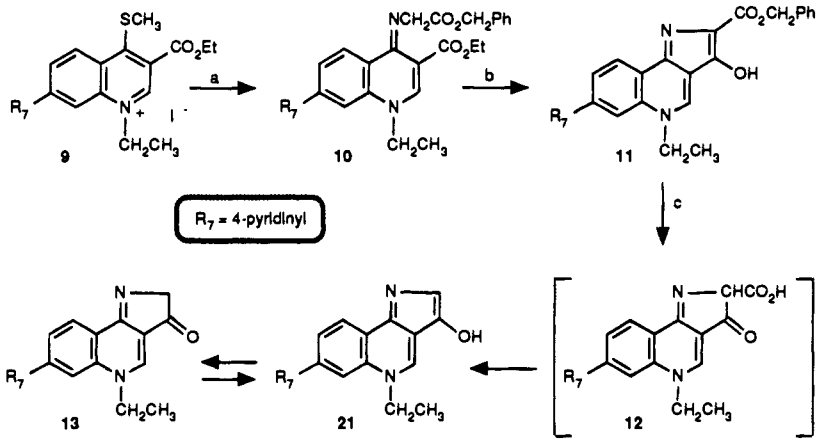
We have no explanation for the diminished activity of derivatives **18**–**20** and **22**. Since pyrrolo analogue **21** (as the methanesulfonate salt) exists primarily in the enol form (**13** ⇌ **21** in Scheme 2), the lack of plaque reduction activity for this derivative may be due to the absence of the carbonyl group resident in all active compounds.

Biological Properties of Target Compounds in Mice. A mouse HSV-2 infection model where drug was administered by the oral route was used to assess the in vivo activity of those targets (**17**, **18**, and **23**) that had HSV-2 plaque reduction activity at or below the MTD (see footnote in Table 1 for definition of terms). All three target compounds were inactive.

In our earlier study, we found that in vitro activity did not correlate nor necessarily translate to in vivo efficacy.[1] Additionally, we also found that the high level of in vivo activity found for **3** could be explained by the high and unexpectedly prolonged plasma drug levels (greater than the MIC) evident in mice following oral administration. We utilized a similar series of experiments to rationalize the divergent in vivo activity profile observed for **1** and **17**, a pair of structurally close derivatives that have nearly identical in vitro potency.

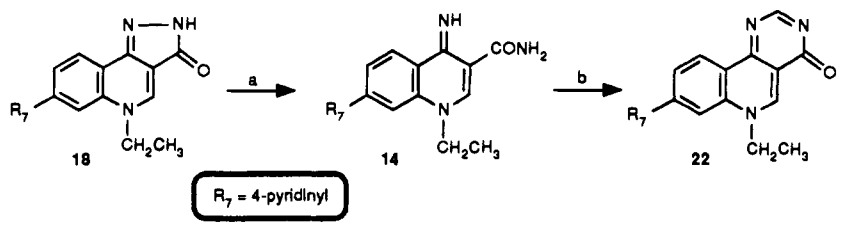
Serum concentrations of **1** and **17** were measured in mice following single oral administration of drug at 25 and 200 mg/kg, respectively (see the Experimental Section). As shown in Figure 1, absorption for both compounds was rapid as evidenced by a mean serum concentration of 7.8 μg/mL for **1** and 5.8 μg/mL for **17** at 25 and 30 min, respectively. Thereafter, serum concentrations of **1** remained above the MIC value of

Scheme 2[a]



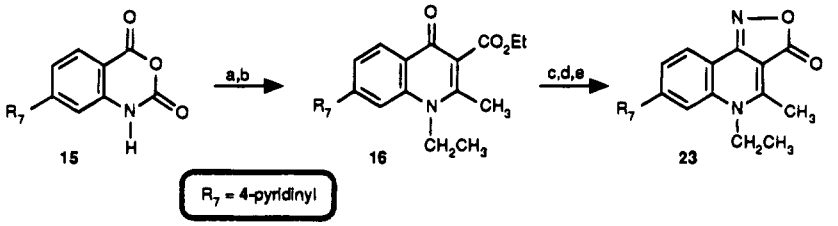
[a] (a) $NH_2CH_2CO_2CH_2Ph$·TsOH, pyr; (b) Et_3N, $CHCl_3$; (c) CH_3SO_3H, H_2/Pd/C, H_2O.

Scheme 3[a]



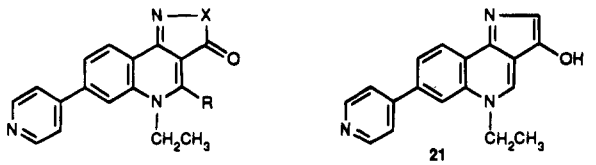
[a] (a) Ra (Ni), EtOH, H_2O, CH_3SO_3H; (b) $(CH_3)_3COCH[N(CH_3)_2]_2$, DMF.

Scheme 4[a]



[a] (a) NaH, CH_3CH_2Br, DMF; (b) NaH, $CH_3COCH_2CO_2Et$, DMF; (c) P_4S_{10}, pyr; (d) CH_3I, THF; (e) NH_2OH·HCl, pyr.

Table 1. Antiherpetic Properties of 5-Ethyl-7-(4-pyridinyl)isoxazolo[4,3-*c*]quinolin-3(5*H*)-one (**17**) and Related Derivatives



			antiherpetic activity			
			in vitro, μg/mL[a]		in vivo, mg/kg po[b]	
compd	X	R	MIC	MTL	MED	MTD
17	O	H	6.3	>100	NA[c]	>400
18	NH	H	27	50	NA	>200
19	NCH_3	H	NA	3.1	*d*	
20	NC_6H_5	H	NA	>100	*d*	
21			NA	100	*d*	
22	−CH=N−	H	NA	>100	*d*	
23	O	CH_3	2.9	>100	NA	>50
1			6.5	12.5	50	50
2			NA	50	*d*	
3			0.24	6.25	25	50
acyclovir (**5**)			1.3	>200	12	>200

[a] HSV-2 plaque reduction assay in Vero cells (see ref 1); the lowest concentration of test compound which inhibited plaque formation by 50% was recorded as the minimum inhibitory concentration (MIC). For active compounds, the MICs shown represent the mean of at least two experiments and had a standard deviation of less than ±50%. The maximum tolerated level (MTL) was the highest concentration of compound that showed no cellular toxicity by visualization of stained cells. [b] HSV-2 infection model in mice where test agent was administered by the oral route (see ref 1). All animal care and use procedures were conducted in accord with the *Guide for the Care and Use of Laboratory Animals* (NIH Pub. No. 86−23, 1985) and were approved by an Institutional Animal Care and Use Committee; the minimum effective dose (MED) was defined as the lowest dose of compound that resulted in survival times that were statistically different from placebo based on known statistical methods. For active compounds, the MED is based on at least two experiments. The maximum tolerated dose (MTD) was defined as the highest dose of compound that did not show any overt signs of toxicity. [c] NA = no activity at the MTL or MTD. [d] Not determined.

6.5 μg/mL for 8 h. In mice medicated with **17**, serum concentrations dropped precipitously with levels well below the MIC of 6.3 μg/mL after 1 h. The fact that **1** has prolonged and high (greater than the MIC) serum levels in mice, whereas relatively low and short-lived serum levels are seen with **17** at a much higher dose, is entirely consistent with the high murine activity found in **1** and the lack thereof for **17**.

Conclusions

In summary, we have demonstrated that in a series of antiherpetic 3-quinolinecarboxamides, an isoxazolo ring fusion is an effective isosteric replacement for the β-keto amide pharmacophore. These data along with information gained by substituting the carbon flanking the isoxazole ring with a methyl group to give **23** provided additional evidence that the active conformation of the acyclic carboxamide **1** is the planar intramolecular H-bonded β-keto amide. For two compounds in this study, **1** and **17**, a correlation between serum plasma levels and in vivo oral antiherpetic efficacy was observed.

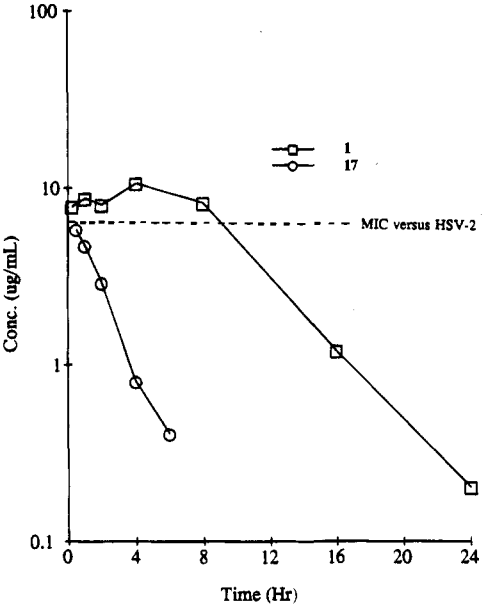


Figure 1. Serum concentrations in mice following oral administration of **1** (25 mg/kg) and **17** (200 mg/kg).

Experimental Section

General. Melting points were determined on a Thomas-Hoover melting point apparatus in open capillaries and are uncorrected. Proton NMR (GE QE-300) spectra, chemical ionization mass spectra (Hewlett-Packard 5980A mass spectrometer), and infrared spectra (Nicolet 10DX FT-IR spectrophotometer) were consistent with the assigned structures. ^1H NMR multiplicity data are denoted by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). Coupling constants are in hertz. Carbon, hydrogen, and nitrogen elemental analyses were performed by Galbraith Laboratories, Knoxville, TN, and were within ±0.4% of theoretical values. Reactions were generally perfored under a N_2 atmosphere.

Ethyl 1-Ethyl-1,4-dihydro-7-(4-pyridinyl)-4-thioxo-3-quinolinecarboxylate (8). Phosphorus pentasulfide (P_4S_{10}, 18.0 g, 40.5 mmol) was added to a mixture of **7**[3] (20.0 g, 62.1 mmol) and 150 mL of pyridine at 25 °C. The resulting mixture was heated at reflux for 2 h, cooled to 100 °C, and poured into 1.5 L of H_2O. A solid was collected, and the filtrate was washed with $CHCl_3$. The solid and $CHCl_3$ extracts were combined, dried (Na_2SO_4), and concentrated to give a brown solid which was recrystallized from CH_3CN to give 16.1 g (77%) of **8**: mp 185 °C, ^1H NMR ($CDCl_3$) δ 9.16 (d, J = 9.0 Hz, 1H), 8.78 (s, 2H), 8.03 (s, 1H), 7.69−7.57 (m, 4H), 4.48−4.43 (m, 4H), 1.65 (t, J = 7.2 Hz, 3H), 1.45 (t, J = 7.1 Hz, 3H). Anal. ($C_{19}H_{18}N_2O_2S$) C, H, N.

5-Ethyl-7-(4-pyridinyl)isoxazolo[4,3-*c*]quinolin-3(5*H*)-one Hydrochloride (17). A mixture of **8** (5.0 g, 14.8 mmol), hydroxylamine hydrochloride (5.0 g, 72.0 mmol), sodium acetate (6.0 g, 73.2 mmol), and EtOH (100 mL) was heated at reflux for 2 h and cooled in ice. The solid that separated was collected, suspended in 300 mL of MeOH, and treated with ethanolic HCl to give a solution. Upon chilling, a solid separated which was collected and dried to give 3.1 g (64%) of **17**: mp 319–320 °C dec; ^{1}H NMR (DMSO-d_6) δ 8.90 (s, 1H), 8.78 (d, J = 5.8 Hz, 2H), 8.25 (s, 1H), 8.21 (d, J = 7.7 Hz, 1H), 8.04 (d, J = 8.3 Hz, 1H), 7.97 (d, J = 5.2 Hz, 2H), 4.60 (q, J = 7.0 Hz, 2H), 1.45 (t, J = 7.0 Hz, 3H). Anal. ($C_{17}H_{13}N_3O_2$·HCl) C, H, N.

5-Ethyl-2,5-dihydro-7-(4-pyridinyl)-3*H*-pyrazolo[4,3-*c*]quinolin-3-one Hydrochloride (18). A mixture of **8** (12.5 g, 36.9 mmol), hydrazine hydrate (4.1 g, 78.8 mmol), and 75 mL of DMF was heated at 100 °C for 3 h, during which time a dark solution formed followed by precipitation of a solid. After cooling to 25 °C, the solid was collected and washed with DMF and ether to give 10.3 g (96%) of the neutral form of **18**: mp >330 °C; ^{1}H NMR (DMSO-d_6) δ 11.48 (s, 1H) 8.74–8.79 (m, 3H), 8.28 (d, J = 8.3 Hz, 1H), 8.11 (s, 1H), 7.95 (d, J = 8.3 Hz, 1H), 7.90 (d, J = 4.6 Hz, 2H), 4.59 (q, J = 7.1 Hz, 2H), 1.45 (t, J = 7.1 Hz, 3H). A portion of this material was dissolved in warm 6 N HCl and cooled to give a hydrochloride salt: mp 352–354 °C. Anal. ($C_{17}H_{14}N_4O$·HCl·H_2O) C, H, N.

5-Ethyl-2,5-dihydro-2-methyl-7-(4-pyridinyl)-3*H*-pyrazolo[4,3-*c*]quinolin-3-one Dihydrochloride (19). Using a procedure similar to that used to prepare **18**, 10.0 g (29.6 mmol) of **8** was treated with 5.0 mL of methyl hydrazine in 40 mL DMF to give 10.2 g (90%) of **19**: mp 320–323 °C; ^{1}H NMR (DMSO-d_6) δ 9.04 (d, J = 6.8 Hz, 2H), 8.91 (s, 1H), 8.57 (d, J = 6.8 Hz, 2H), 8.36 (d, J = 8.3 Hz, 2H), 8.16 (d, J = 8.3 Hz, 1H), 4.66 (q, J = 7.0 Hz, 2H), 3.53 (s, 3H), 1.46 (t, J = 7.0 Hz, 3H). Anal. ($C_{18}H_{16}N_4O$·2HCl·0.5H_2O) C, H, N.

Ethyl 1-Ethyl-7-(4-pyridinyl)-4-(methylthio)-3-quinoliniumcarboxylate Iodide (9). Methyl iodide (28.5 g, 200 mmol) was added to a slurry of 50.0 g (148 mmol) of finely ground **8** in 1.5 L of THF. After the mixture was stirred at 25 °C for 4 h, TLC indicated the presence of **8** along with desired product, and another 28.5 g of CH_3I was added. Stirring was continued for another 14 h at 25 °C, and an additional portion (22.8 g) of CH_3I was added. After 3 h, a solid was collected and washed with THF to give 68.2 g (96%) of **9**. An aliquot was recrystallized from CH_3CN to a material with mp 225 °C dec: ^{1}H NMR (DMSO-d_6) δ 9.01 (d, J = 6.6 Hz, 2H) 8.79 (s, 1H), 8.47–8.42 (m, 3H), 8.25 (s, 1H), 8.02 (d, J = 8.4 Hz, 1H), 4.59 (q, J = 7.0 Hz, 2H), 4.26 (q, J = 7.1 Hz, 2H), 2.51 (s, 3H), 1.44 (t, J = 7.0 Hz, 3H), 1.32 (t, J = 7.1 Hz, 3H). Anal. ($C_{20}H_{21}IN_2O_2S$) H, N; C: calcd, 50.01; found: 49.36.

5-Ethyl-2,5-dihydro-2-phenyl-7-(4-pyridinyl)-3*H*-pyrazolo[4,3-*c*]quinolin-3-one (20). Phenylhydrazine (3.3 g, 30.5 mmol) was added to a suspension of **9** (5.0 g, 10.4 mmol) in EtOH (100 mL) at 60 °C. Sodium acetate (5.0 g, 61.0 mmol) was added to this mixture, which was then stirred at reflux for 2 h. The mixture was diluted with water, and a solid was collected and recrystallized from DMF to give 1.8 g (47%) of **20**: mp 323–324 °C; ^{1}H NMR ($CDCl_3$) δ 8.79 (d, J = 6.0 Hz, 2H), 8.60 (d, J = 8.2 Hz, 1H), 8.37 (s, 1H), 8.26 (d, J = 8.6 Hz, 2H), 7.74–7.82 (m, 2H), 7.59 (d, J = 6.0 Hz, 2H), 7.48 (m, 2H), 7.28–7.21 (m, 2H), 4.43 (q, J = 7.2 Hz, 2H), 1.67 (t, J = 7.2 Hz, 3H). Anal. ($C_{23}H_{18}N_4O$) C, H, N.

Ethyl-1-Ethyl-1,4-dihydro-4-[[2-oxo-2-(phenylmethoxy)ethyl]imino]-7-(4-pyridinyl)-3-quinolinecarboxylate (10). A mixture of **9** (25.0 g, 52.1 mmol), benzyl glycinate (as the *p*-toluenesulfonic acid salt, 26.3 g, 78.0 mmol), and 300 mL of pyridine was stirred at 25 °C for 18 h during which time a solid separated. The solid was partitioned between $CHCl_3$ and water/K_2CO_3, and the organic layer was dried (Na_2SO_4) and concentrated. The residual oil was dissolved in 200 mL of EtOAc, and 12.6 g (52%) of crystalline **10** was collected: mp 153 °C dec; ^{1}H NMR ($CDCl_3$) δ 8.70 (dd, J = 4.6, 1.2 Hz, 2H), 8.59 (d, J = 8.3 Hz, 1H), 7.92 (s, 1H), 7.28–7.55 (m, 9H), 5.23 (s, 2H), 4.27 (q, J = 7.1 Hz, 2H), 4.20 (s, 2H), 4.11 (q, J = 7.2 Hz, 2H), 1.48 (t, J = 7.1 Hz, 3H), 1.33 (t, J = 7.2 Hz, 3H). Anal. ($C_{28}H_{27}N_3O_4$) C, H, N.

Phenylmethyl 5-Ethyl-3-hydroxy-7-(4-pyridinyl)-5*H*-pyrrolo[3,2-*c*]quinoline-2-carboxylate Dimethanesulfonate (11). A solution of **10** (15.5 g, 33.0 mmol), Et_3N (6.7 g, 66.2 mmol), and 250 mL $CHCl_3$ was stirred at reflux for 7 d, during which time a solid separated. The solid was collected and washed with $CHCl_3$ to give 12.7 g (91%) of the neutral form of **11**. A portion was converted to the dimethanesulfonate salt by treatment with excess methanesulfonic acid in EtOH to give **11**: mp 251 °C dec; ^{1}H NMR (DMSO-d_6) δ 9.88 (s, 1H), 9.15 (d, J = 8.6 Hz, 2H), 9.00 (d, J = 6.4 Hz, 1H), 8.79 (s, 1H), 8.51 (d, J = 6.1 Hz, 2H), 8.49 (d, J = 7.9 Hz, 1H), 7.32–7.50 (m, 5H), 5.44 (s, 2H), 5.09 (q, J = 7.1 Hz, 2H), 2.32 (s, 6H), 1.60 (t, J = 7.1 Hz, 3H). Anal. ($C_{26}H_{21}N_3O_3$·2CH_3SO_3H) C, H, N.

5-Ethyl-7-(4-pyridinyl-5*H*-pyrrolo[3,2-*c*]quinolin-3-ol Dimethanesulfonate (21). A mixture of the neutral form of **11** (12.6 g, 29.8 mmol), methanesulfonic acid (6.3 g, 65.4 mmol), 200 mL H_2O, and 0.40 g of 10% Pd/C was subjected to H_2 (ca. 46 psi) in a Paar pressure vessel for 17 h. During this period two additional portions of catalyst were added, and the vessel was evacuated and recharged with H_2 approximately every 3 hours. The mixture was filtered, and the filtrate was concentrated to give an oil which was dissolved in 300 mL of boiling 95% EtOH. Upon cooling, a solid precipitated and was collected to give 12.5 g (87%) of **21**: mp 269 °C dec; ^{1}H NMR (CD_3OD) δ 9.62 (s, 1H), 8.98 (m, 2H), 8.82 (m, 1H), 8.78 (d, J = 9.0 Hz, 1H), 8.59 (m, 2H), 8.42 (d, J = 9.0 Hz, 1H), 7.21 (s, 1H), 5.15 (q, J = 7.3 Hz, 2H), 2.70 (s, 6H), 1.75 (t, J = 7.2 Hz, 3H). Anal. ($C_{18}H_{15}N_3O$·2CH_3SO_3H) C, H, N.

1-Ethyl-1,4-dihydro-4-imino-7-(4-pyridinyl)-3-quinolinecarboxamide Dihydrochloride (14). A mixture of 8.6 g (29.6 mmol) of the neutral form of **18**, 5.7 g (59.3 mmol) of methanesulfonic acid, 4.0 g of Raney nickel, 300 mL of EtOH, and 200 mL of water was stirred at reflux for 14 h. The mixture was filtered, and the filtrate was concentrated to remove most of the EtOH. The aqueous residue was basified with excess K_2CO_3, and a gummy solid was collected. This material was heated at 100 °C for 10 min in 100 mL of 0.7 N HCl to hydrolyze nickel complexes of the product. The resulting solution was filtered and diluted with 50 mL of EtOH. Upon cooling, a white crystalline solid separated and was collected to give 7.0 g (65%) of **14**: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 10.49 (bs, 1H), 10.13 (bs, 1H), 9.50 (s, 1H), 9.01 (d, J = 4.9 Hz, 2H), 8.97 (d, J = 8.9 Hz, 1H), 8.64 (s, 1H), 8.55 (s, 1H), 8.45 (d, J = 4.9 Hz, 2H), 8.34 (d, J = 8.7 Hz, 1H), 7.94 (bs, 1H), 4.82 (q, J = 7.1 Hz, 2H), 1.55 (t, J = 7.1 Hz, 3H). Anal. ($C_{17}H_{16}N_4O$·2HCl) C, H, N.

6-Ethyl-8-(4-pyridinyl)pyrimido[5,4-*c*]quinolin-4(6*H*)-one Dimethanesulfonate (22). A mixture of 10.0 g (34.2 mmol) of the neutral form of **14**, 7.8 g (44.8 mmol) of $(CH_3)_3$-$COCH[N(CH_3)_2]_2$, and 100 mL of DMF was stirred at 25 °C for 3 d, during which time a solid precipitated. This solid was collected and dissolved in 50 mL of water containing excess methanesulfonic acid, and the resulting solution was filtered and diluted with 150 mL of acetone. A solid separated upon standing which was collected to give 12.0 g (74%) of **22**: mp 292 °C dec; ^{1}H NMR (DMSO-d_6) δ 10.17 (s, 1H), 9.22 (d, J = 8.6 Hz, 1H), 8.99 (m, 3H), 8.91 (s, 1H), 8.60 (d, J = 8.7 Hz, 1H) 8.34 (d, J = 5.9 Hz, 2H), 5.32 (q, J = 7.1 Hz, 2H), 2.36 (s, 6H), 1.66 (t, J = 7.1 Hz, 3H). Anal. ($C_{18}H_{14}N_4O$·2CH_3-SO_3H·0.5H_2O) C, H, N.

Ethyl 1-Ethyl-1,4-dihydro-2-methyl-4-oxo-7-(4-pyridinyl)-3-quinolinecarboxylate (16). To 300 mL of DMF was added sodium hydride (50% in mineral oil, 4.37 g, 95.0 mmol, washed with hexane) and **15**[4] (20.7 g, 86.2 mmol). After the mixture was stirred for 30 min, ethyl bromide (10.34 g, 95.4 mmol) was added, and stirring was continued for 14 h at 25 °C. Sodium hydride (50% in mineral oil, 8.74 g, 190 mmol, washed with hexane) was added to the reaction mixture followed by the addition of ethyl acetoacetate (24.7 g, 190 mmol). The reaction mixture was stirred at 25 °C for 30 min and at 100 °C for 7 h and concentrated. The residue was treated with 600 mL of 10% aqueous acetic acid. A gummy solid separated and was extracted into $CHCl_3$. The extracts

were dried ($MgSO_4$) and concentrated to give an oily residue that was purified using flash silica gel chromatography (2.5% isopropylamine in $CHCl_3$ as eluent) to give 8.4 g (29%) of **16**. A portion of this material was recrystallized from ethyl acetate to give analytically pure **16**: mp 161–163 °C; ^{1}H NMR (DMSO-d_6) δ 8.74 (d, J = 4.5 Hz, 2H), 8.30 (d, J = 8.3 Hz, 1H), 8.11 (s, 1H), 7.91 (d, J = 4.5 Hz, 2H), 7.83 (d, J = 8.3 Hz, 1H), 4.50 (q, J = 6.9 Hz, 2H), 4.29 (q, J = 7.1 Hz, 2H), 2.52 (s, 3H), 1.39 (t, J = 6.9 Hz, 3H), 1.30 (t, J = 7.1 Hz, 3H). Anal. ($C_{20}H_{20}N_2O_3$) C, H, N.

5-Ethyl-4-methyl-7-(4-pyridinyl)isoxazolo[4,3-*c*]quinolin-3(5*H*)-one Methanesulfonate (23). Compound **16** (4.0 g, 11.9 mmol) was converted to ethyl 1-ethyl-2-methyl-7-(4-pyridnyl)-4-(methylthio)-3-quinoliniumcarboxylate iodide (4.4 g) using procedures similar to those used to convert **7** to **9**. This crude product was stirred with NH_2OH·HCl (0.91 g, 13.2 mmol) and 75 mL of pyridine at 25 °C for 14 h. The resulting mixture was concentrated, and the residue was triturated with aqueous K_2CO_3 to yield 3.9 g of crude product. This material was purified using flash silica gel chromatography (5% isopropylamine in $CHCl_3$ as eluent) to give 1.9 g of material that was converted to **23** (1.6 g, 33% from **16**) by treatment with 2 mL of methanesulfonic acid in 250 mL of MeOH: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 9.01 (d, J = 6.5 Hz, 2H), 8.44 (d, J = 6.6 Hz, 2H), 8.27 (d, J = 8.3 Hz, 2H), 8.12 (d, J = 8.3 Hz, 1H), 4.58 (q, J = 7.0 Hz, 2H), 2.93 (s, 3H), 2.34 (s, 3H), 1.41 (t, J = 7.0 Hz, 3H). Anal. ($C_{18}H_{15}N_3O_2$·CH_3SO_3H·0.25H_2O) C, H, N.

Serum Concentrations of 1 and 17 in Mice. Female Swiss–Webster mice were dosed intragastrically with **1** at 25 mg/kg or **17** at 200 mg/kg using a protocol identical to that previously reported[1] except for the following: serum from mice treated with **17** was extracted with chloroform and HPLC was performed on a C18 column using 60% MeOH:40% NH_4OAc-(0.2%). The serum from mice treated with **1** was treated with MeOH to precipitate protein, and HPLC was performed on a MCH-5N column using 50% MeOH:50% 0.2 M NH_4OAc at pH 3.5. Data for mice dosed with **17** were obtained on triplicate samples using a single pool from 10 mice/time point. In mice dosed with **1**, there was only one analysis for each time point which came from a pool from 10 mice.

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