Computer-Aided Molecular Modeling, Synthesis, and Biological Evaluation of 8-(Benzyloxy)-2-phenylpyrazolo[4,3-*c*]quinoline as a Novel Benzodiazepine Receptor Agonist Ligand

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Using computer-aided conformational analysis, based on molecular dynamics simulation, cluster analysis, and Monte Carlo techniques, we have designed and synthesized compounds in which a benzyloxy substituent has been incorporated into a series of pyrazoloquinoline benzodiazepine receptor (BZR) ligands. Earlier studies had shown that the benzyloxy group could act as part of the agonist pharmacophoric determinant in the β-carboline ring system. Furthermore, the agonist β-carboline had been correlated with a binding site orientation and volume fit for an agonist 6-phenylimidazobenzodiazepine carboxylate. The present study was undertaken to determine whether the benzyloxy substituent could be used as an agonist pharmacophoric descriptor for the phenylpyrazolo[4,3-*c*]quinolin-3-one BZR ligands. The results of a determination of GABA shift ratios for the synthetic ligands indicate that 8-(benzyloxy)-2-phenylpyrazolo[4,3-*c*]quinolin-3-one can be predicted to be an agonist at the BZR.

Since the discovery of the benzodiazepine receptor (BZR),[1,2] a wide variety of structurally unique classes of BZR ligands have been identified. Thus, in addition to the benzodiazepines, the β-carbolines and pyrazoloquinolines are two classes of compound possessing intrinsic activity as agonist, antagonists, and inverse agonists, depending on the pattern of substitution within each class.[3–5]

In the β-carbolines series, 6-(benzyloxy)-4-(methoxymethyl)-β-carboline-3-carboxylic acid ethyl ester, ZK93423 (**1**, Figure 1), is the only compound reported to have full agonist activity.[6] It has also been reported[7] that replacement of the benzyloxy group in compound **1**, either by methoxy (**2**, Figure 1) or by hydrogen, results in high-affinity antagonist ligands. In contrast, the otherwise unsubstituted ethyl ester, β-CCE (**3**, Figure 1), has been shown to have full inverse agonist activity. From an examination of these three structures, it can be seen that the 6-benzyloxy group would appear to be an important part of the agonist pharmacophore for β-carbolines.

In an earlier molecular modeling study,[8] the π_1 and ring A binding sites for compound **1** were constrained to fit similar sites for the benzodiazepine agonist Ro 21 8384 (**4**, Figure 2). This resulted in a high degree of overlap between the A and C ring of Ro 21-8384 and the six-membered rings of **1** (see Figure 2). Moreover, the volume fit of the two phenyl rings (the 6-benzyloxy group of the β-carboline and the 6-phenyl group of **4**) was also sufficient to propose a common nonspecific hydrophobic site. Analogs of **1**, in which electronic and steric substituents, known to be allowed for imidazobenzodiazepine agonists, were copied onto the β-carboline. These analogs were shown to act as agonist ligands even though the modifications chosen were those known to deactivate β-carboline inverse agonists.[9]

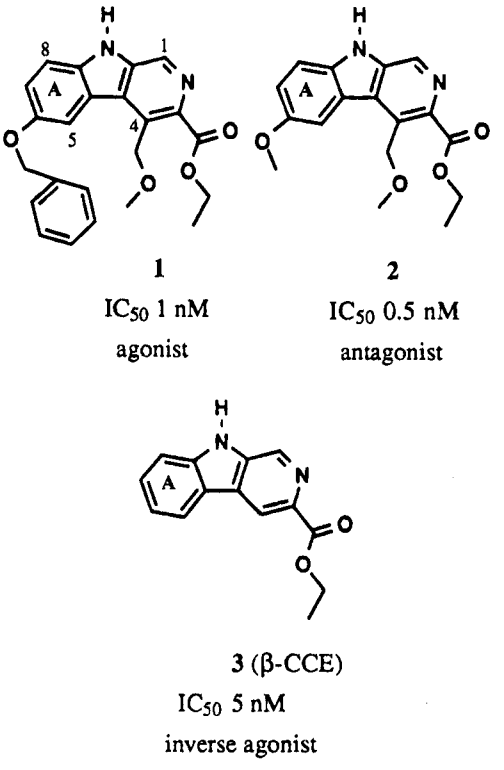

Figure 1. Effect of substituents on intrinsic activity of β-Carboline BZR Ligands.

On the basis of these findings, three-dimensional predictive models for both agonist and antagonist BZR ligands have been proposed.[10] In these models, it was determined that an aromatic or heteroaromatic ring (A) which can undergo π/π stacking within the receptor and a proton-accepting group (labeled π_1) spatially related to the plane of A ring were determined as minimum requirements for agonist ligand binding. A second proton-accepting group (designated π_2) is thought to be

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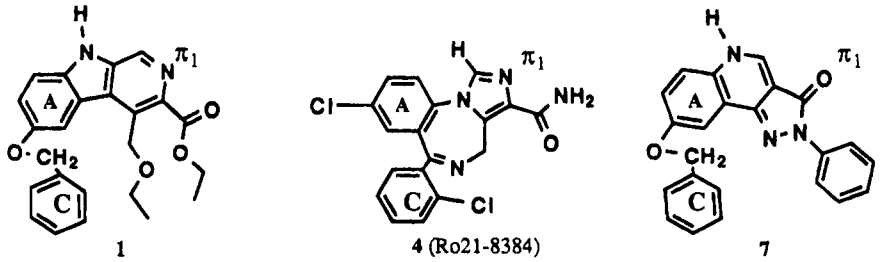
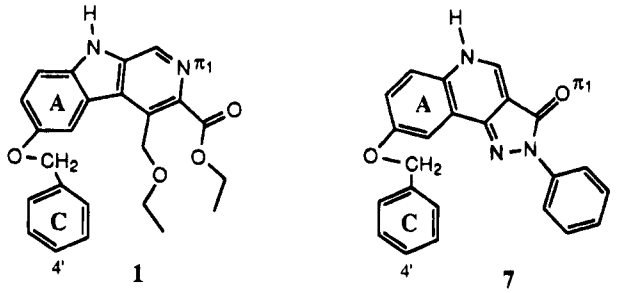


Figure 2. Schematic representation of the relative orientations of superpositioned compounds **1**, **4**, and **7** obtained by computer modeling analysis.

Table 1. Cluster Analysis: Geometric Parameters for Predominant Clusters



compd	mean distance 4'-π_1, Å	mean torsion angle between planes A and C
1	12.10	1.35
7	12.60	1.82
5	10.82	−6.08
6	13.23	6.97
9	8.63	−0.32

directly related to ligand binding in either the antagonist or the inverse agonist receptor conformations. The binding site requirements for compounds **1** and **4** are labeled according to the agonist models.[9]

In the phenylpyrazolo[4,3-*c*]quinolin-3-one series of compounds, the 2-phenyl (CGS8216), 2-(4-methoxyphenyl) (CGS 9895), and 2-(4-chlorophenyl) (CGS 9896) analogs have been reported to be biologically active as inverse agonist, antagonist and partial agonist ligands respectively.[4]

Since the 6-benzyloxy group is an important determinant for the β-carboline molecule to exhibit agonist activity at the BZR, molecular modeling and synthetic studies were carried out to determine whether this pharmacophoric descriptor would have a similar effect on the pyrazoloquinoline series of compounds. That is, whether the addition of a benzyloxy moiety would force these pyrazoloquinolines to adopt an agonist conformational fit at the BZR. Starting with compound **1**, we investigated the conformational behavior of the benzyloxy side chain by means of molecular dynamics (MD option of the SYBYL[11] molecular modeling software package) and a clustering procedure (FAMILY option of SYBYL), since this procedure has been shown to be a valuable tool in pharmacophoric pattern determination.[12] In the predominant cluster, the phenyl ring of the side chain was close to the plane defined by the polycyclic heteroaromatic system (mean distance (Table 1) C-4' to π_1 = 12.1 Å, mean torsional angle to the plane 1.3°). On the basis of these results, compounds **5**–**7**, and **9** were constructed (structures shown in Schemes 1 and 2) and subjected to identical analyses. While the geometric features of the main cluster of **7** exhibited an excellent fit with the parent molecule **1** (mean distance 12.6 Å, mean torsion angle 1.8°), compounds **5**, **6**, and **9** yielded main clusters with different conformational features (Table 1).

Attempts to fit the main cluster conformers of **1** and **7** to the low-energy conformers of the imidazobenzodiazepine **4** were unsuccessful. Therefore, additional low-energy conformers of **1** and **7** were sought using a Monte Carlo like randomized search strategy (RANDOMSEARCH option of SYBYL; see the Experimental Section). Using this procedure, the conformers of **1** and **7** with the lowest potential energy of each search run revealed a great similarity of conformational features, not only with each other, but also with **4**. This permitted a good fit of the crucial moieties (aromatic A ring, dipole region π_1, and the lipophilic region corresponding to the benzyloxy or phenyl group, respectively). A schematic representation of the orientation used for the fit of compounds **1**, **4**, and **7** is depicted in Figure 2. In addition to **7**, compounds **5**, **6**, **8**, and **9** were synthesized to provide a complete evaluation of the substituent effects of the benzyloxy group on activity at the BZR.

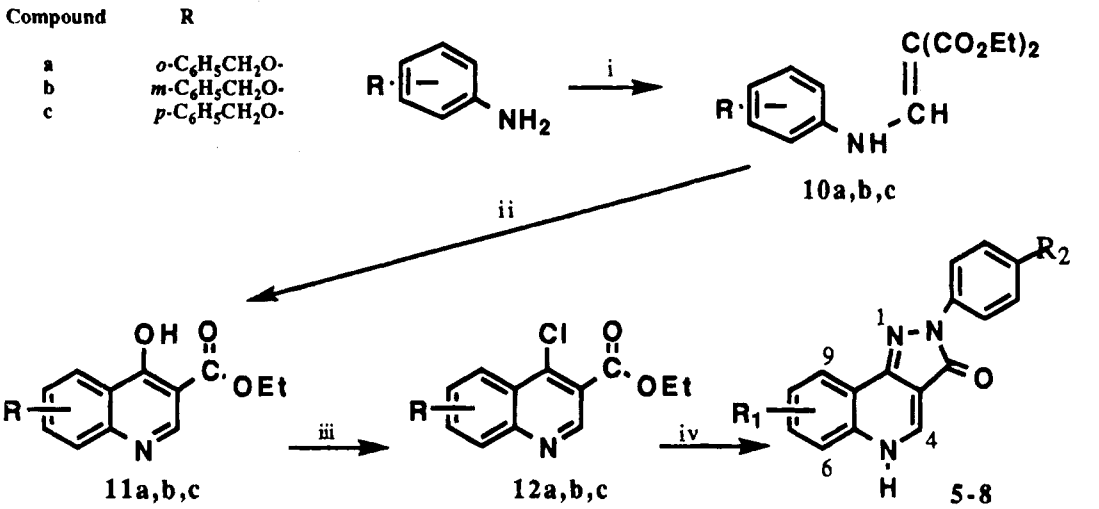
Chemistry

The synthetic pathways for novel compound **5**–**8** were essentially based on literature procedures (Scheme 1).[4,13] (Benzyloxy)anilines were combined with diethyl (ethoxymethylene)malonate to give compounds **10a**–**c**, which were thermally ring closed to give the corresponding 4-hydroxyquinoline-3-carboxylic esters **11a**–**c**. Treatment of the esters with phosphorus oxychloride afforded the 4-chloro derivatives **12a**–**c**, which were condensed with the corresponding phenylhydrazines to give the target compounds.

Due to the fact that cyclization of the *meta*-substituted (anilinomethylene)malonic ester **10b** occurs exclusively at the least hindered position, giving only **11b**, the synthesis of compound **9** required the use of a bromine substituent as a blocking group (Scheme 2). In this instance, it was decided to use 2-bromo-4-methoxyaniline (**13**), rather than the corresponding benzyloxy analog as the starting material, in order to avoid possible steric problems with the subsequent ring closure reaction.

However, even with the methoxy group, attempts at thermal ring closure of the substituted anilinomalonate **14** were unsuccessful, and when **14** was heated as in the previous work, either in Dowtherm or in diphenyl ether, both at reflux, extensive decomposition (tar) took place and less than 5% of the quinoline **15** could be isolated. When polyphosphoric acid (PPA) was used to effect ring closure, it was found that both reaction temperature and workup conditions were critical. At a temperature of 100 °C, only starting material and decomposition products were detected. At a tempera-

Scheme 1



Compound	R_1	R_2
5	6-$C_6H_5CH_2O$-	H
6	7-$C_6H_5CH_2O$-	H
7	8-$C_6H_5CH_2O$-	H
8	8-$C_6H_5CH_2O$-	Cl

i) Diethyl ethoxymethylenemalonate, steam bath; ii) Diphenylether, reflux; iii) $POCl_3$, steam bath; iv) Phenylhydrazine/xylene, reflux

ture of 170 °C, hydrolysis of the ester and decarboxylation occurred, and only the quinoline (**20**) was obtained. By heating at the intermediate temperature of 140 °C, initially, **15** was isolated in 2.5% yield together with 20% of the de-esterified acid **21**. The acid was obtained by extensive extraction of the aqueous workup mixture with methylene chloride. It was reasoned that hydrolysis was taking place in the PPA mixture, and therefore the reaction mixture was refluxed in absolute ethanol prior to workup. Using this procedure to esterify any acid present in the reaction mixture, **15** could be isolated in better than 70% yield. Debromination by hydrogenolysis of **15** with Pd/C was clean (**16**). The pyrazolo ring was added by the known literature procedure to give **17**.[4] Replacement of the methyl group with a benzyl group involved demethylation in aqueous HBr to give the phenol **18** and protecting the nitrogen at the 5-position as the *tert*-butyl carbamate. Benzylation and deprotection to the desired product **9** were completed in a single step, since the *tert*-butyl carbamate was thermally unstable and could be removed simply by reflux. Attempts to O-benzylate **18** directly were not successful, only the N-benzylated product **19** being isolated.

Result and Discussion

The results of the in vitro evaluation of the synthesized benzyloxy-substituted 2-phenylpyrazoloquinoline analogs (**5**–**9**) are listed in Table 2, together with the same data for the methoxyphenyl (**17**) and hydroxyphenyl derivatives (**18**). Literature values for the inverse agonist and agonist ligands from the phenylpyrazoloquinoline series (phenyl [CGS 8216] and *p*-chlorophenyl [CGS 9896] analogs respectively) are tabulated for comparative purposes.

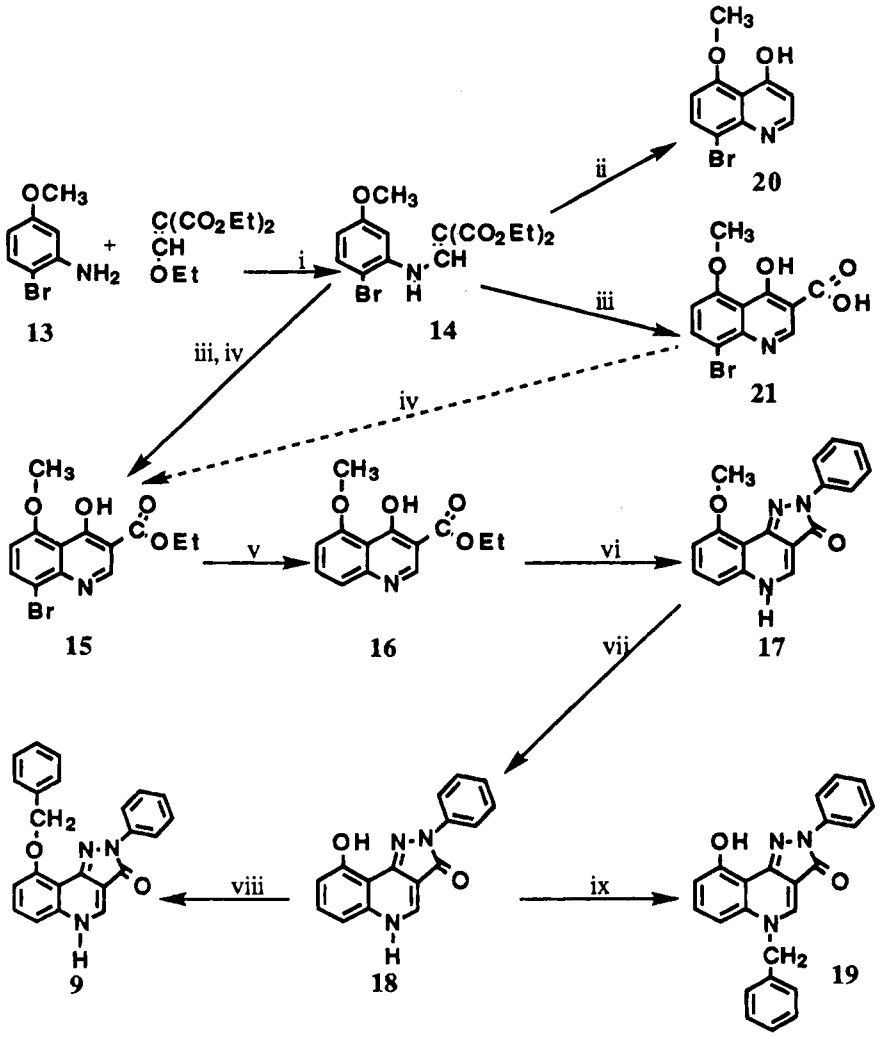
Compound **7** binds with high affinity to BZR, and its GABA shift ratio indicates that, as predicted, it should act as an agonist.[14] The other positional isomers (**5**, **6**, and **9**) of compound **7** exhibit low affinity for BZR, which is in accord with the computer-modeling experiments.

The 9-OCH_3 derivative, **17**, and the 9-OH compound, **18**, are both high-affinity BZR ligands. Based on GABA shift ratio data, they are probably antagonists or partial agonists. The corresponding 7- and 8-substituted methyl ethers are also high-affinity ligands (IC_{50} 2.1 and 0.38 nM, respectively) with antagonist or partial inverse agonist activity (GABA shift ratios, 0.97 and 0.93, respectively).[15] These findings indicate that the fused benzene ring can tolerate small substituents without significant effect of position or type (electron donating or withdrawing[12]) and still exhibit high affinity for the BZR.[15] However, consistent with the predictions of the modeling studies, there is a strict positional requirement for the benzyloxy pharmacophoric descriptor, proposed as a combined lipophilic and steric replacement of the 5-phenyl substituent of agonist 1,4-benzodiazepines.

It is difficult to explain the intrinsic activity of **8** the 2-(*p*-chlorophenyl) derivative of **7**. The affinity of **8** has decreased by more than 1 order of magnitude, and the GABA shift ratio indicates intrinsic activity as either an antagonist or a partial inverse agonist. Thus, the biological profile of this ligand now closely resembles that of the 7-methoxy-2-phenyl analog discussed above. If it is assumed that the *p*-chloro substituent in **8** sterically prohibits the fit of the 7-benzyloxy compound in the agonist conformation of the receptor, then **8** may adopt an antagonist or inverse agonist conformation comparable to that of the 7-methoxy analog, albeit with much lower affinity.

In summary, molecular-modeling techniques indicate three structurally different classes of heterocyclic ring systems can be constrained to fit (volume and electrostatic potentials) a three-dimensional model for agonist ligands at the BZR. The proposed equivalence of a benzyloxy substituent on an agonist β-carboline to the 6-phenyl substituent of an imidazobenzodiazepine[7] has now been extended to include the pyrazoloquinoline series of BZR ligands. Thus, as predicted by molecular-modeling studies, the benzyloxy group can act as an

Scheme 2



i) Steam bath; ii) PPA/170 °C; iii) PPA/140 °C; iv Reflux ethanol; v) H_2/Pd-C;
vi) a. $POCl_3$, steam bath, b. Phenylhydrazine/xylene/120°C; vii) Reflux H_2O/HBr;
viii) a. EtONa/EtOH/di-t-butyl di-carbonate, b. Benzyl bromide, reflux;
ix) EtONa / benzyl bromide.

Table 2. In Vitro Activities of Synthetic 2-Phenylpyrazoloquinoline Derivatives

compd	IC_{50}	GABA shift ratio
5	>1 μM	
6	>1 μM	
7	3.7 nM	1.3
8	52.3 nM	0.94
9	>1 mM	
17	0.68 nM	1.09
18	0.74 nM	1.03
diazepam	20.6 nM	1.82
Ro 15−1788	2.86 nM	1.00
2-phenyl analog (CGS 8216)[a]	0.4 nM	0.94
2-(4-chlorophenyl) analog (CGS 9896)[a]	0.6 nM	1.3

[a] Data from ref 4.

agonist pharmacophoric descriptor and force the ligand into an agonist conformation.

Experimental Section

General. Melting points were determined with Mel-Temp apparatus and are uncorrected unless specified. Infrared spectra were obtained by using the Fourier infrared spectrophotometer, and KBr pellets were used for all crystalline compounds. Mass spectra were recorded on Hewlett-Packard HP5869 gas chromatograph−Finnigan Mat Incos 50 mass spectrometer (70 eV). The ^1H NMR spectra were determined at 200 MHz with a Bruker Model WP 200 or an IBM Model WP-200SY Fourier transform spectrometer. Spectra were recorded in $CDCl_3$ or Me_2SO-d_6, and chemical shifts are expressed in parts per million (ppm) on the δ scale relative to a Me_4Si internal standard. ^1H NMR spectra are reported as follows: (solvent) chemical shift (multiplicity [s = single, d = doublet, t = triplet, q = quartet, qn = quintet, m = multiplet, br = broad], coupling constant in hertz, interpretation). Microanalyses were carried out at Hoffmann-La Roche, Inc., Nutley, NJ.

Unless specified otherwise, commercially available solvents from Fisher Scientific Co. and reagents from Aldrich Chemical Co. were used as received. Purification by column chromatography was accomplished on 230−400 mesh silica gel, Merck, grade 60 (Aldrich). DC-Plastikfolien Kieselgel 60 F254 (Art. 5735) from Alltech Associates, Inc. were used for thin layer chromatography.

Radioligand Binding. The affinities of the compounds for the central benzodiazepine receptor were assessed using a modification of previously described techniques.[16] Sprague−Dawley rats were decapitated, and the brains were rapidly

removed and placed in 320 mM sucrose (0–4 °C) before dissection. After dissection, cerebral cortex was weighed and then homogenized in 50 volumes of 50 mM Tris-citrate buffer (pH 7.4) using a Polytron (Brinkman Instruments) at a setting of 6.5 for 15 s. The homogenate was centrifuged at 20000*g* (0–4 °C) for 20 min, and the pellet was resuspended in 50 volumes of Tris-citrate buffer. This "washing" procedure was repeated five times. After the last wash, the pellet was resuspended in 20 volumes of buffer and stored at −80 °C for no more than 30 days before use.

Prior to assay, the tissue preparation containing BZR was thawed and a 50 μL aliquot (containing ~0.12 mg of protein) added to each assay tube, which also contained 50 μL of the compound to be tested (0.01 nM to 10 μM, final concentration), 50 μL of [^{3}H]Ro 15-1788 (final concentration, 1 nM), and sufficient Tris-citrate buffer to yield a final volume of 500 μL. The "GABA shift" values were determined by adding 50 μL aliquots of NaCl and GABA (final concentrations, 120 mM and 200 μM respectively) to each assay tube. The ratio of the IC_{50} values in the absence and presence of GABA is the GABA shift. Total and nonspecific [^{3}H]Ro 15-1788 binding was determined separately in triplicate, using 10 μM Ro 14-7437 to define nonspecific binding. Assays were terminated after incubation (1 h at 25 °C) by rapid filtration over Whatman GF/B filter strips using a Brandel M-24R filtering manifold. Samples were washed with 2 × 5 mL aliquots of cold buffer. The radioactivity retained by the filters was measured in a Beckman LS 5802 liquid scintillation spectrometer.

Competition curves were fitted to the data using nonlinear regression techniques (Inplot4, GraphPad Software, San Diego CA) and the concentrations of test compound required to inhibit the specific binding of [^{3}H]Ro 15-1788 by 50% (IC_{50}) determined.

Molecular Modeling. The molecular modeling study was performed using the Sybyl program package (either version 5.5 mounted of a VAX 11/780, or version 6.0 mounted on a SGI Indigo). The molecules were built using X-ray coordinates, if available, or from the Sybyl standard fragment library and minimized to the nearest local minimum using the Maximin minimizer option. The molecular dynamics simulations (MD) were run for 10 000 fs at 1000 K in order to surmount conformational barriers and were then continued for 90 000 fs at 300 K. Conformers were saved from the second period every 100 fs. The potential energy was coupled with the temperature bath every 5 fs; the time step of all simulations was 1 fs.

After MD, the distance and torsion values were recorded and were submitted to the FAMILY clustering option (distance grid size 0.5 Å, torsion angle grid size 1°, wrapping allowed). The main cluster containing a statistically representative population was extracted for all compounds investigated, and the mean distance and torsional angle values were calculated.

The Monte Carlo simulation was done using the Random Search option of SYBYL, starting from a fully relaxed, low-energy conformer, derived from the previously determined MD analysis. Conformers within a range of 5 kcal above the starting conformer were saved (steric fit RMS convergence value, 0.5 Å; minimizer energy convergence threshold, 0.0001; maximum number of minimizer iterations, 750).

Diethyl [[2-(Benzyloxy)anilino]methylene]malonate (10a). A mixture of 4.4 g (22 mmol) of 2-(benzyloxy)aniline[13c] and 4.8 g (22 mmol) of diethyl (ethoxymethylene)malonate was heated on a steam bath for 2 h. The mixture was cooled to room temperature, and the residue was recrystallized from hexane to give 8.2 g (90%) of **10a** as fluffy white crystals: mp 82.0–82.5 °C; ^{1}H NMR ($CDCl_3$) δ 11.27 (d, J = 14.2 Hz, 1H), 8.58 (d, J = 14.1 Hz, 1H), 7.53–7.25 (m, 6H), 7.11–6.95 (m, 3H), 5.22 (s,2H), 4.26 (m, 4H), 1.33 (t, J = 7.1 Hz, 6H); IR 3230, 3070, 2900, 2980, 1680, 1648, 1618, 1582, 1435, 1350, 1250, 1100, 1030, 810, 748, 715, 690 cm^{-1}. Anal. ($C_{21}H_{23}NO_5$) C, H, N.

Diethyl [[3-(Benzyloxy)anilino]methylene]malonate (10b). A mixture of 8.4 g (42 mmol) of 3-(benzyloxy)aniline and 9.1 g (42 mmol) of diethyl (ethoxymethylene)malonate was heated on a steam bath for 2 h. The 17.5 g of product **10b** containing approximately 11% EtOH was homogeneous by TLC (silica gel, MeCN eluent, R_f 0.90). A small portion of the oil was purified by short-path distillation. Anal. ($C_{21}H_{23}NO_5$) C, H, N.

Diethyl [[4-(Benzyloxy)anilino]methylene]malonate (10c). A mixture of 7.0 g (35 mmol) of 4-(benzyloxy)aniline and 7.6 g (35 mmol) of diethyl (ethoxymethylene)malonate was heated on a steam bath for 2 h. The reaction mixture was dried in a vacuum oven overnight (50 °C) to give 13 g of solidified product **10c**, which was homogeneous by TLC (silica gel, MeCN/CH_2Cl_2 eluent, R_f 0.90). A small portion was purified by recrystallization from methanol, mp 120-122 °C. Anal. ($C_{21}H_{23}NO_5$) C, H, N.

Ethyl 8-(Benzyloxy)-4-quinolone-3-carboxylate (11a). Diphenyl ether (40 mL) was heated to the boiling poin,t and 3.5 g (9.5 mmol) of **10a** was added portionwise. After the addition, the mixture was refluxed for 30 min. The solution was cooled to room temperature when 50 mL of hexane was added to give a white precipitate, which was collected by filtration and washed with hexane, and recrystallization from DMF to give 2.4 g (78%) of **11a** as white fluffy crystals: mp 213–214 °C; ^{1}H NMR (DMSO-d_6) δ 11.75 (br, D_2O exchangeable, 1H), 8.39 (s, 1H), 7.74–7.29 (m, 8H), 5.37 (s, 2H), 4.22 (q, J = 7.1 Hz, 2H), 1.27 (t, J = 7.1 Hz, 3H); IR 3300–2510, 1710, 1560–1530, 1290, 750 cm^{-1}. Anal. ($C_{19}H_{17}NO_4$) C, H, N.

Ethyl 7-(Benzyloxy)-4-quinolone-3-carboxylate[17] (11b). Diphenyl ether (40 mL) was heated to the boiling point, and 6.5 g (16 mmol) of **10b** was added portionwise. After the addition was complete, the mixture was refluxed for 30 min and then cooled to room temperature. The mixture was diluted with 20 mL of hexane to give 3.8 g (74%) of **11b** as a white precipitate which was collected by filtration and washed with hexane and dried: mp 248–252 °C; ^{1}H NMR (DMSO-d_6) δ 8.58 (s, 1H), 8.07 (d, J = 8.9 Hz, 1H), 7.58–7.21 (m, 5H), 7.11 (s, 1H), 6.97 (d, J = 8.9 Hz, 1H), 5.20 (s, 2H), 4.19 (q, J = 7.1 Hz, 2H), 1.26 (t, J = 7.1 Hz, 3H).

Ethyl 6-(Benzyloxy)-4-quinolone-3-carboxylate[18] (11c). Diphenyl ether (40 mL) was heated to the boiling point, and 1.8 g (4.9 mmol) of **10c** was added portionwise. After the addition was complete, the mixture was refluxed for 30 min and then cooled to room temperature. The mixture was diluted with 20 mL of hexane to give 1.4 g (88%) of **11c** as a white precipitate which was collected by filtration and washed with hexane and dried: mp 278–282 °C (lit. mp 279); ^{1}H NMR (DMSO-d_6) δ 8.50 (s, 1H), 8.07 (d, J = 8.9 Hz, 1H), 7.67–7.00 (m, 8H), 5.21 (s, 2H), 4.21 (q, J = 7.1 Hz, 2H), 1.26 (t, J = 7.1 Hz, 3H).

Ethyl 8-(Benzyloxy)-4-chloroquinoline-3-carboxylate (12a). A mixture of 4.6 g (14 mmol) of **11a** and 3.6 g (24 mmol) of phosphorus oxychloride was heated on a steam bath for 15 min. The mixture was cooled in an ice bath, and 90 mL of dilute aqueous ammonium hydroxide was added. The aqueous layer was extracted with two portions (80 mL) of ether. The combined organic layers were washed with water, dried ($MgSO_4$), and filtered. After evaporation of ether at reduced pressure, the residual oil was recrystallized from hexane to give **12a** as yellow crystals (3.8 g, 78%): mp 114–115 °C; ^{1}H NMR ($CDCl_3$) δ 9.24 (s, 1H), 7.96 (dd, J = 1.0, 8.6 Hz, 1H), 7.57–7.15 (m, 8H), 5.46 (s, 2H), 4.51 (q, J = 7.1 Hz, 2H), 1.46 (t, J = 7.1 Hz, 3H); IR 2970, 2940, 2870, 1738, 1579, 1370, 775, 750 cm^{-1}. Anal. ($C_{19}H_{16}NO_3Cl$) C, H, N.

Ethyl 7-(Benzyloxy)-4-chloroquinoline-3-carboxylate (12b). A mixture of 3.0 g (9.1 mmol) of **11b** and 2.4 g (16 mmol) of phosphorus oxychloride was heated on a steam bath for 15 min. The mixture was cooled in an ice bath and treated with 90 mL of diluted aqueous ammonium hydroxide solution. The aqueous layer was extracted with two portions (80 mL) of ether. The combined organic layers were washed with water, dried ($MgSO_4$), and filtered. After evaporation of ether, 2.3 g (76%) of **12b** was obtained as an oil which was homogeneous by TLC (CH_2Cl_2/MeCN, 1:1). A small portion of the oil was purified by short-path distillation. Anal. ($C_{19}H_{16}NO_3Cl$) C, H, N.

Ethyl 6-(Benzyloxy)-4-chloroquinoline-3-carboxylate (12c). A mixture of 1.0 g (3.1 mmol) of **11c** and 0.81 g (5.3 mmol) of phosphorus oxychloride was heated on a steam bath

for 10 min. The mixture was cooled in an ice bath and treated with dilute aqueous ammonium hydroxide. The aqueous solution was extracted with two portion (50 mL) of ether. The combined organic layers were washed with water, dried ($MgSO_4$), and filtered. After evaporation of ether, 0.81 g (77%) of **12c** was obtained as an oil which was homogeneous by TLC (CH_2Cl_2/MeCN, 1:1). The product was used directly for the next step.

6-(Benzyloxy)-2-phenylpyrazolo[4,3-*c*]quinolin-3-one (5). A mixture of 1.48 g (4.0 mmol) of **12a** and 1.0 g (9.2 mmol) of phenylhydrazine was refluxed in 30 mL of xylene for 30 min, during which time a yellow precipitate was formed. At room temperature the mixture was treated with hexane (20 mL), and the product was collected by filtration to give 1.1 g (75%) of **5** as a yellow amorphous solid which was crystallized by dissolving in ethanolic solution of NaOH (3 M); the ethanol solution was subsequently filtered and neutralized with 1 M HCl. An analytical sample was prepared by recrystallization from a mixture of methanol and methylene chloride: mp 268–269 °C; ^{1}H NMR (DMSO-d_6) δ 11.69 (s, D_2O exchangeable, 1H), 8.39 (s, 1H), 8.25 (d, J = 7.8 Hz, 2H), 7.93 (d, J = 7.4, 1H), 7.58–7.40 (m, 9H), 7.18 (m, 2H), 5.28 (s, 2H); IR 3130, 3030, 1610, 1570, 1270, 1060, 750 cm^{-1}. Anal. ($C_{23}H_{17}N_3O_2$) C, H, N.

7-(Benzyloxy)-2-phenylpyrazolo[4,3-*c*]quinolin-3-one (6). A mixture of 2.2 g (7.0 mmol) of **12b** and 1.6 g (15 mmol) of phenylhydrazine was refluxed in 20 mL of xylene for 30 min, and a yellow precipitate was formed. At room temperature the mixture was treated with 20 mL of hexane, and the product was collected by filtration to give 2.1 g (82%) of **6** as a yellow amorphous powder which was crystallized by dissolving in a NaOH and ethanol solution and neutralizing with 1 M HCl. An analytical sample was prepared by recrystallization from a mixture of methanol and methylene chloride: mp 290.5–291.5 °C; ^{1}H NMR (DMSO-d_6) δ 12.69 (d, J = 6.7 Hz, D_2O exchangeable, 1H), 8.70 (d, J = 6.3 Hz, 1H), 8.19 (m, 3H), 7.54–7.26 (m, 9H), 7.16 (t, J = 7.3 Hz, 1H), 5.25 (s, 2H); IR 3119, 3062, 2968, 2887, 1629,1592, 1527, 1452, 1364, 1248, 1187, 1024, 874, 840, 735, 760, 694 cm^{-1}. Anal. ($C_{23}H_{17}N_3O_2 \cdot 0.5H_2O$) C, H, N.

8-(Benzyloxy)-2-phenylpyrazolo[4,3-*c*]quinolin-3-one (7). A mixture of 0.81 g (2.4 mmol) of **12c** and 0.5 g (5.4 mmol) of phenylhydrazine was refluxed in 20 mL of xylene for 30 min, and a yellow precipitate was formed. At room temperature the mixture was treated with 20 mL of hexane, and the product was collected by filtration to give 0.50 g (57%) of **7** as a yellow amorphous powder which was crystallized by dissolving in a NaOH and ethanol solution and neutralizing with 1 M HCl. An analytical sample was prepared by recrystallization from a mixture of methanol and methylene chloride: mp 299–300 °C; ^{1}H NMR (DMSO-d_6) δ 12.85 (br, D_2O exchangeable, 1H), 8.68 (s, 1H), 8.25 (d, J = 7.6 Hz, 1H), 7.73–7.35 (m, 10H), 7.21 (t, J = 7.3 Hz, 1H), 5.30 (s, 2H); IR 3100, 3060, 2900, 1642, 1600,1548, 1480, 1400, 1350, 1275, 1235, 1050, 840, 790, 760, 690 cm^{-1}. Anal. ($C_{23}H_{17}N_3O_2$) C, H, N.

8-(Benzyloxy)-2-(*p*-chlorophenyl)pyrazolo[4,3-*c*]quinolin-3-one (8). A mixture of 0.80 g (2.5 mmol) of **12c** and 0.60 g (3.4 mmol) of *p*-chlorophenylhydrazine was refluxed in 20 mL of xylene for 30 min during which time a yellow precipitate was formed. At room temperature the mixture was treated with 20 mL of hexane to give 0.47 g (47%) of **8** as a yellow precipitate which was collected by filtration. Recrystallization from a mixture of methylene chloride and methanol gave the pure product as fluffy yellow crystals: mp 294–295 °C dec; ^{1}H NMR (DMSO-d_6) δ 12.90 (br, D_2O exchangeable, 1H), 8.56 (s, 1H), 8.13 (d, J = 8.7 Hz, 2H), 7.74–7.36 (m, 10H), 5.23 (s, 2H); IR 3383, 3065, 3028, 2903, 1619, 1549, 1488, 1460, 1412, 1353, 1313, 1289, 1235, 1196, 1092, 1010, 920, 859, 825, 770, 746, 695 cm^{-1}. Anal. ($C_{23}H_{16}N_3O_2Cl \cdot H_2O$) C, H, N.

9-(Benzyloxy)-2-phenylpyrazolo[4,3-*c*]quinolin-3-one (9). A mixture of 0.72 g (2.6 mmol) of **17**, 0.60 g (2.6 mmol) of 97% di-*tert*-butyl dicarbonate, and 0.19 g (2.8 mmol) of sodium ethoxide was stirred in 20 mL of absolute ethanol at room temperature for 2 h. Another 0.42 g (6.2 mmol) of sodium ethoxide was added, followed by 0.65g (3.8 mmol) of benzyl bromide, and the reaction mixture was stirred overnight. The reaction mixture was added to 20 mL of water, extracted with three 40 mL portions of methylene chloride, neutralized with dilute hydrochloric acid, and extracted with three more portions of 40 mL of methylene chloride. The combined organic layers were dried ($MgSO_4$), filtered, and concentrated under reduced pressure, and the residue was flash chromatographed on 30 g of silica gel (CH_2Cl_2/MeCN, 3:1, eluent). Fractions of 30 mL were collected and analyzed by TLC. Fractions containing the two less polar compounds were combined and evaporated to give 0.25 g of an oil. This residue was again treated with 0.14 g (2.1 mmol) of sodium ethoxide and 0.21 g (1.3 mmol) of benzyl bromide, as described above, and the resulting mixture was refluxed in absolute ethanol (25 mL) for 24 h. During reflux, a yellow precipitate formed. The yellow precipitate was added to 20 mL of water and extracted with three portions of methylene chloride (60 mL). The combined organic layers were dried ($MgSO_4$), filtered, and evaporated under reduced pressure, and the residue was recrystallized from a mixture of methylene chloride and methanol to give 0.17 g (18%) of **9** as yellow needles: R_f 0.15 (CH_2Cl_2/MeCN, 3:1, eluent); mp 294–295 °C; ^{1}H NMR (DMSO-d_6) δ 12.69 (br, D_2O exchangeable, 1H), 8.64 (s, 1H), 8.27 (d, J = 7.8 Hz, 2H), 7.90 (d, J = 7.6 Hz, 2H), 7.64–7.14 (m, 9H), 5.35 (s, 2H); IR 3353, 3068, 2923, 2853, 1642, 1624, 1595, 1571,1548, 1483, 1433, 1379, 1314, 1275, 1136, 1087, 1055, 798, 755, 732, 690 cm^{-1}. Anal. ($C_{23}H_{17}N_3O_2 \cdot H_2O$) C, H, N.

2-Bromo-5-methoxyaniline[19] (13) (Modified literature procedure[17]). A solution of 6.8 g (30 mmol) of 4-bromo-3-nitroanisole[17] in a mixture of tetrahydrofuran (100 mL) and ethanol (150 mL) containing 2.0 mL of concentrated aqueous ammonia was hydrogenated in a Parr bomb at room temperature with 3 g of commercial Raney nickel as the catalyst. After the absorption of hydrogen stopped (4 h), catalyst was removed by filtration over Celite, and solvents were evaporated under reduced pressure to give 5.8 g (98% of **13** as an oil, homogeneous by TLC (silica gel, CH_2Cl_2/hexane, 2:1)): MS m/z 202 (M^+). This oil was used in the following step without further purification.

Diethyl [(2-Bromo-5-methoxyanilino)methylene]malonate (14). A mixture of 5.8 g (29 mmol) of **13** and 6.5 g (30 mmol) of diethyl (ethoxymethylene)malonate was heated on a steam bath for 2 h. The reaction mixture was then recrystallized from 30 mL of methanol to give 9.1 g (83%) of **14** as yellow fine needles: mp 98–99 °C; ^{1}H NMR (DMSO-d_6) δ 11.09 (d, J = 10.4 Hz, D_2O exchangeable, 1H), 8.50 (d, J = 10.4 Hz, 1H), 7.58 (d, J = 8.8 Hz, 1H), 7.18 (d, J = 2.8 Hz, 1H), 6.73 (dd, J = 8.9, 2.8, 1H), 4.19 (m, 4H), 3.95 (s, 3H), 1.26 (m, 6H); IR 3136, 2984, 2904, 1681, 1648, 1618, 1609, 1587, 1426, 1368, 1346, 1271, 1226, 1096, 1021, 965, 869, 817, 803 cm^{-1}. Anal. ($C_{15}H_{18}NO_5Br$) C, H, N.

Ethyl 8-Bromo-5-methoxy-4-oxoquinolin-3-carboxylate (15). Compound **14** (1.0 g, 3.1 mmol) was added slowly and with stirring to 12 g of commercial polyphosphoric acid at 140 °C, and then the mixture was stirred for 30 min. After the mixture cooled to room temperature, absolute ethanol (20 mL) was added and the resulting mixture was refluxed for 45 min. The mixture was neutralized with aqueous sodium bicarbonate at 25 °C, extracted with four portions (40 mL) of methylene chloride, which were combined, dried ($MgSO_4$), and concentrated to give 0.61 g (71%) of crude **15**. A small portion was recrystallized from methanol to give **15** as light yellow plates: mp 184–185 °C; ^{1}H NMR (DMSO-d_6) δ 11.11 (s, D_2O exchangeable, 1H), 8.29 (s, 1H), 7.89 (d, J = 8.8 Hz, 1H), 6.87 (d, J = 8.8 Hz, 1H), 4.20 (q, J = 7.0 Hz, 2H), 3.83 (s, 3H), 1.76 (t, J = 7.0 Hz, 3H); IR 3490, 3110, 3070 , 2983, 2838, 1703, 1629, 1597, 1529, 1460, 1408, 1362, 1197, 1182, 1098, 1023, 974, 852, 800, 766, 711, 636, 608 cm^{-1}. Anal. ($C_{13}H_{12}NO_4Br$) C, H, N.

Ethyl 5-Methoxy-4-oxoquinolin-3-carboxylate (16). A mixture of 3.5 g (12.6 mmol) of **15** and 1.75 g (12.6 mmol) of sodium acetate in 120 mL of glacial acetic acid was hydrogenated in a Parr bomb at 2.5 atm pressure over a 10% Pd/C catalyst (0.85 g) for 8 h. The catalyst was removed by filtration, and the reaction mixture was concentrated under reduced pressure. The resulting oil was dissolved in 200 mL of methylene chloride. The solution was washed with aqueous

sodium bicarbonate solution (15%, 100 mL) and then with water, dried ($MgSO_4$), and filtered. Evaporation of solvent gave 1.9 g (72%) of **16** which was recrystallized from ethyl acetate to give a yellow microcrystalline solid: mp 252–255 °C; ^{1}H NMR (DMSO-d_6) δ 11.95 (br, D_2O exchangeable, 1H), 8.32 (s, 1H), 7.54 (t, J = 8.1 Hz, 1H), 7.07 (d, J = 8.3 Hz, 1H), 6.84 (d, J = 8.2 Hz, 1H), 4.16 (q, J = 7.1 Hz, 2H), 1.25 (t, J = 7.1 Hz, 3H); IR 3163–2724, 1703, 1619, 1587, 1527, 1467, 1377, 1345, 1293, 1184, 1131, 1092, 973, 887, 822, 758, 696, 612 cm^{-1}; HRMS found 247.0485 (M), calcd for $C_{13}H_{13}NO_4$ 247.0481 (M). Anal. ($C_{13}H_{13}NO_4$·0.25H_2O) C, H, N.

9-Methoxy-2-phenylpyrazolo[4,3-*c*]quinolin-3-one (17). A mixture of 0.45 g (1.8 mmol) of **16** and 0.36 g (2.4 mmol) of phosphorus oxychloride was heated on a steam bath for 15 min. The mixture was cooled in an ice bath, treated with 90 mL of dilute aqueous ammonium hydroxide, and extracted with two portions (80 mL) of ether. After evaporation of the ether under reduced pressure, the residue was treated with 0.25 g (2.3 mmol) of phenylhydrazine and then refluxed in xylene (10 mL) for 30 min. Compound **17** was formed as a yellow precipitate which was recrystallized from methanol to give 0.15 g (29%) of yellow needles: mp 304–305 °C; ^{1}H NMR (DMSO-d_6) δ 12.66 (s, D_2O exchangeable, 1H), 8.65 (s, 1H), 8.24 (d, J = 8.2 Hz, 2H), 7.59 (t, J = 8.2, 1H), 7.45 (t, J = 7.8 Hz, 2H), 7.28–7.08 (m, 3H), 4.00 (s, 3H); IR 3059, 2930, 1638, 1598, 1544, 1513, 1483, 1431, 1379, 1315, 1266, 1217, 1171, 1101, 1058, 983, 895, 798, 775, 746, 731, 715, 694 cm^{-1}; HRMS found 291.0949 (M), calcd for $C_{17}H_{13}N_3O_2$ 291.1007 (M). Anal. ($C_{17}H_{13}N_3O_2$·0.25H_2O) C, H, N.

9-Hydroxy-2-phenylpyrazolo[4,3-*c*]quinolin-3-one (18). A solution of 0.60 g (2.1 mmol) of **17** in 25 mL of 48% aqueous hydrogen bromide was refluxed for 48 h and then cooled to room temperature. The reaction mixture was added to 30 mL of water and then neutralized with aqueous sodium bicarbonate. The solution was dark brown and turbid. The mixture was extracted with four portions of 40 mL of methylene chloride, which were combined, washed, dried ($MgSO_4$), filtered, and concentrated. Recrystallization of the residue from methanol gave 0.41 g (72%) of **17** as white needles: mp 314–316 °C; ^{1}H NMR (DMSO-d_6) δ 12.93 (br, D_2O exchangeable, 1H), 9.59 (s, D_2O exchangeable, 1H), 8.76 (s, 1H), 8.17 (d, J = 8.4 Hz, 2H), 7.58–7.16 (m, 5H), 7.00 (d, J = 8.2 Hz, 1H); IR 3581, 3057, 2877, 1620, 1597, 1493, 1460, 1443, 1366, 1308, 1253, 1190, 971, 902, 854, 799, 767, 747, 732, 684 cm^{-1}; HRMS found 277.0843 (M), calcd for $C_{16}H_{11}N_3O_2$ 277.0851 (M). Anal. ($C_{16}H_{11}N_3O_2$·0.25H_2O) C, H, N.

5-Benzyl-9-hydroxy-2-phenylpyrazolo[4,3-*c*]quinolin-3-one (19). A mixture of 0.19 g (0.70 mmol) of **18**, 0.34 mL of 3 M sodium ethoxide in ethanol, and 0.14 g (0.82 mmol) of benzyl bromide was refluxed in 3 mL of absolute ethanol for 1 h. The reaction mixture was cooled to room temperature and neutralized with 10 mL of aqueous sodium bicarbonate solution. The aqueous solution was extracted with three 30 mL portions of methylene chloride, and the combined organic layers were dried ($MgSO_4$), filtered, and evaporated on rotary evaporator. The residue was chromatographed over 10 g of silica gel (CH_2Cl_2/MeCN, 1:1, as eluent) to give, after removal of the solvent, 46 mg (18%) of **19** as a yellow powder: mp 322–327 °C; ^{1}H NMR (DMSO-d_6) δ 9.81 (s, 1H, D_2O exchangeable), 9.20 (s, 1H) 8.19 (d, J = 7.8 Hz, 2H), 7.54–7.20 (m, 10H), 7.04 (d, J = 8.1 Hz, 1H), 5.72 (s, 2H); HRMS found 367.1304 (M), calcd for $C_{17}H_{13}N_3O_2$ 367.1320 (M).

8-Bromo-5-methoxyquinolin-4-one (20). To 10 g of stirred commercial polyphosphoric acid kept at 170 °C was slowly added 0.65 g (1.8 mmol) of **14**, and the mixture was stirred for 30 min. After cooling to room temperature, the reaction mixture was neutralized with aqueous ammonium hydroxide solution while keeping the temperature below 10 °C. The aqueous solution was extracted with three 30 mL portions of methylene chloride, and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated by rotary evaporator to give 0.33 g (60%) of **20** as a yellow powder: mp 183–187 °C; ^{1}H NMR (DMSO-d_6) δ 10.40 (br, D_2O exchangeable, 1H), 7.89 (d, J = 8.9 Hz, 1H), 7.75 (d, J = 7.0 Hz, 1H), 6.78 (d, J = 8.9 Hz, 1H), 6.10 (d, J = 7.0 Hz, 1H), 3.88 (s, 3H); HRMS found 252.9745 (M), calcd for $C_{10}H_8NO_2Br$ 252.9738 (M).

Ethyl 8-Bromo-5-methoxy-4-oxoquinolin-3-carboxylic Acid (21). To 12 g of stirred commercial polyphosphoric acid at 140 °C was slowly added 1.3 g (3.6 mmol) of **14**, and the mixture was stirred for 30 min. The reaction mixture was cooled to room temperature and neutralized with aqueous ammonium hydroxide solution while keeping the temperature below 10 °C. The aqueous solution was extracted with three 30 mL portions of methylene chloride. The combined organic layers were dried ($MgSO_4$), filtered, and concentrated by rotary evaporator to give 0.07 g (2.5%) of **15**. The aqueous layer was re-extracted with 10 more 40 mL portions of methylene chloride. The combined organic layers were dried ($MgSO_4$), filtered, and concentrated to give 0.5 g (20%) of **21** as a yellow powder: mp 259–264 °C; ^{1}H NMR (DMSO-d_6) δ 12.15 (s, D_2O exchangeable, 1H), 8.58 (s, 1H), 8.10 (d, J = 8.9 Hz, 1H), 7.06 (d, J = 8.9 Hz, 1H), 3.91 (s, 3H); HRMS found 296.9619 (M), calcd for $C_{11}H_8NO_4Br$ 296.9636 (M). Anal. ($C_{11}H_8NO_4Br$) C, H, N.

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