Identification of 3,5-Dihydro-2-aryl-1*H*-pyrazolo[3,4-*c*]quinoline-1,4(2*H*)-diones as Novel High-Affinity Glycine Site *N*-Methyl-D-aspartate Antagonists

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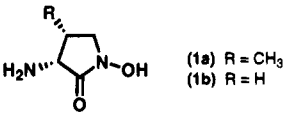
Almost all of the exisiting known antagonists at the glycine site of the N-methyl-D-aspartate (NMDA) receptor have a low propensity for crossing the blood–brain barrier. It has been suggested that in many cases this may be due to the presence of a carboxylic acid which is a common feature of most of the potent full antagonists at this receptor. In this study, 2-aryl-1*H*-pyrazolo[3,4-*c*]quinoline-1,4(2*H*)-diones were found to have high-affinity binding at the glycine receptor. In particular, structure–activity studies identified 7-chloro-3,5-dihydro-2-(4-methoxyphenyl)-1*H*-pyrazolo[3,4-*c*]quinoline-1,4(2*H*)-dione as the most potent of a series of analogues with an IC_{50} of 3.3 nM. The measured pK_a values in this class of compounds (typically 4.0) indicate they are of equivalent acidity to carboxylic acids. Functional antagonism was demonstrated by inhibition of NMDA-evoked responses in rat cortical slices. Anticonvulsant activity in DBA/2 mice was achieved after dosing by direct injection into the cerebral ventricles, but no activity was seen after systemic administration, suggesting low brain penetration with this class of antagonists.

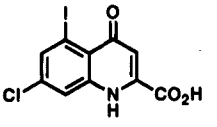
Introduction

Antagonists of the *N*-methyl-D-aspartate (NMDA) receptor have attracted considerable attention in recent years as potential therapeutic agents for the treatment of a variety of neurological and neurodegenerative disorders. Many groups working in this area have focused on the neuroprotective activity of NMDA antagonists which may be clinically useful in reducing brain damage in victims of stroke. The NMDA receptor controls activity of a ligand-gated ion channel which, for activation, requires simultaneous binding of the endogenous ligand (L-glutamate) and glycine, which acts as a coagonist. Several different approaches have been taken to inhibit activity of this receptor, and chemical entities have been described which achieve this by (a) competitively antagonizing binding of L-glutamate;[1] (b) noncompetitively inhibiting the receptor by blocking the open ion channel;[2] (c) antagonism of the coagonist glycine;[3] and (d) modulating binding of the endogenous ligands by action at allosteric sites on the receptor protein.[4] Compounds from each of these categories have now entered clinical trials[5] for the treatment of stroke, and the results of these studies are awaited with interest.

Our interest in glycine site NMDA antagonists has been prompted by the observation that the low-efficacy partial agonist L-687,414 (**1a**) is neuroprotective[6] *in vivo* but does not cause the adverse side effects seen with other types of NMDA receptor antagonists such as the channel blocker dizocilpine (MK801). This partial agonist has relatively weak (IC_{50} 1.4 μM) affinity at the glycine binding site, and although compounds with high receptor affinity have been described, such as 5-iodo-7-chlorokynurenic acid (**2**),[7] L-689,560 (**3**),[8] and MDL 29,-951 (**4**),[9] the central nervous system (CNS) activity of these highly polar carboxylic acids after systemic dosing is extremely weak because of their limited blood–brain barrier penetration. A recent report[10] from our laboratories described a novel series of 3-substituted 4-hydroxyquinolin-2(1*H*)-ones (e.g., **5**) as selective glycine antagonists which possess potent centrally mediated *in vivo* activity after oral administration. As a continuation of this work, we sought to find other glycine site ligands with high receptor affinity but which do not contain a carboxylic acid residue. In this paper we describe the identification of a novel class of high-affinity pyrazoloquinolone glycine antagonists and discuss the results of *in vivo* anticonvulsant experiments.



(1a) R = CH_3
(1b) R = H



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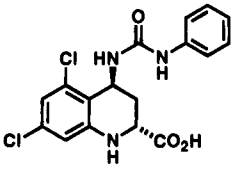

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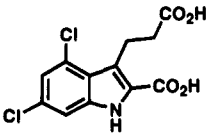

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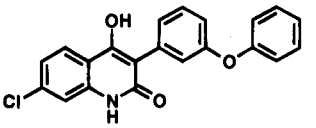

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Biology

Affinities of the compounds in this study for the glycine site on the NMDA receptor were determined by displacement of binding of the glycine site antagonist [^{3}H]-**3** to rat cortical membranes.[11] IC_{50} values were

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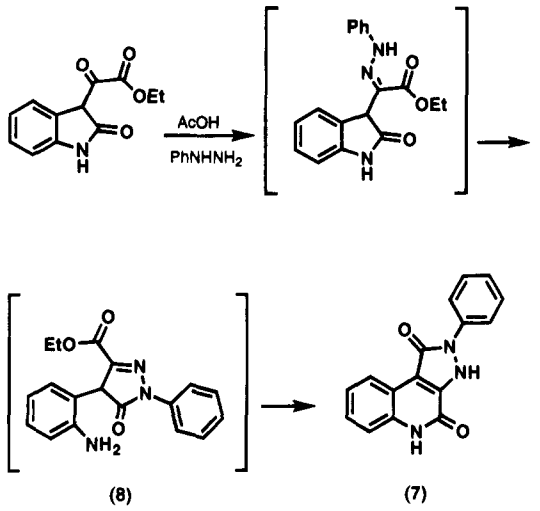
Scheme 1

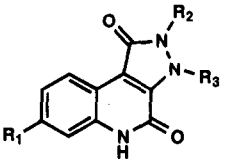


measured from five-point inhibition curves and are the geometric means of at least three independent experiments. Where the IC_{50} is quoted as >100 μM, the test compound inhibited [^{3}H]-**3** binding by less than 50% at 100 μM. Functional antagonism was measured[12] by the ability of test compounds to cause a rightward shift in the dose–response curve for NMDA-induced depolarizations in rat cortical slices. Selectivity for NMDA over AMPA receptors was assessed in similar cortical slice experiments using AMPA to produce depolarizations. Exposure of male and female DBA/2 mice (22-23 days old) to a 120 dB, 1.4 kHz bell induced wild running progressing to tonic extension of the limbs within 7–10 s of the onset of the sound. Groups of eight mice were injected with vehicle or test compound either intraperitoneally (ip) or directly into the lateral cerebral ventricles (icv) under isofurane anesthesia, 15 (icv) or 30 (ip) min before test. The vehicles used were 10% PEG300–water (adjusted to pH 9 with NaOH) for ip dosing and water (pH 11) for icv dosing. Animals failing to show full tonic seizure after 30 s sound exposure were considered protected. The dose protecting 50% of the mice (ED_{50}) was calculated by probit analysis.

Chemistry

As part of our research into antagonists at the glycine site of the NMDA receptor, we had occasion to carry out a reaction first reported in 1924 by Wislicenus and Bubeck[13] in which the condensation of ethyl oxindole-3-glyoxylate with phenyl hydrazine is described. In the original work a single product was obtained which was tentatively assigned the structure of the pyridazinoindole **6**. When we repeated this reaction, a single compound was obtained as the only isolated product in 77% yield. The structure of the compound could not be unambigously assigned solely on the basis of spectral data, but slow crystallization from MeOH gave very fine needles which were suitable for X-ray crystallographic analysis. From this study, the structure of the product was shown to be the pyrazoloquinolone **7** produced (see Scheme 1) by initial formation of a hydrazone followed by opening of the oxindole to give the substituted aniline **8**. Final cyclization of the aniline by closure onto the ester gave the tricyclic quinolone **7**. This compound is, in fact, known in the literature[14] with two previous references in which it was prepared by reaction of

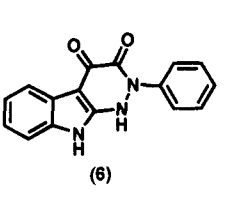
Table 1. Glycine Receptor Binding



Compound	R_1	R_2	R_3	IC_{50} (nM)[a]
7	H	-Ph	H	135
11	H		H	613
12	H		H	162
13	H		H	138
14	H		H	87
15	Cl	-Ph	H	16
16	Cl		H	3.3
17	H	-CH_2Ph	H	562
18	H	-CH_3	H	2020
10	H	-Ph	CH_3	>100,000

[a] Inhibition of [^{3}H]-**3** binding to the glycine site on the NMDA receptor given as the mean of at least three independent determinations. The maximum standard error was always less than 40% of the geometric mean.

phenylhydrazine with 3-mercapto-4-carboxyquinolin-2(1*H*)-one. No biological activity was described in these earlier reports. The *O*-ethyl derivative **9** has also been previously made[15] by a similar type of rearrangement using the preformed phenylhydrazone of ethyl 2-ethoxyindole-3-glyoxylate. We have routinely used the reaction described above with substituted phenylhydrazines to make various analogues with chlorine or methoxy groups on the pyrazolo-substituted phenyl ring. Ethyl 6-chlorooxindole-3-glyoxylate was prepared by a literature method[16] and converted in the same way to the 7-chloro substituted analogues (see Table 1). Alkylation of **7** using NaH/MeI gave the *N*-methyl derivative **10** in low yield. Products of alkylation on the quinolone nitrogen and dimethylated derivatives were also observed; assignment of the position of methylation in **10** was made by comparison of ^{1}H NMR chemical shifts of analogous N- and O-methylated pyrazoloquinolones.[17]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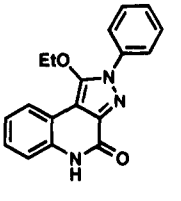
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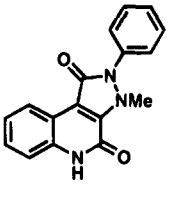
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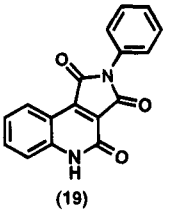
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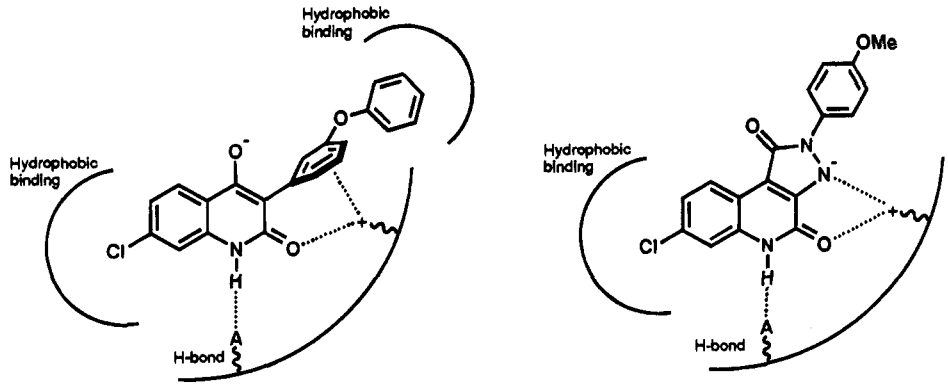

Figure 1. Model for binding of 3-arylquinolinediones and pyrazoloquinolones to the glycine receptor, represented by compounds **5** and **16.** The anions are drawn localized on oxygen and nitrogen for illustrative purposes only.

Results and Discussion

The NMDA receptor has, in recent years, attracted considerable attention as a potential target for therapeutic intervention in the prevention of ischemic brain damage suffered after a stroke. It has also been suggested that antagonists at the glycine site on the NMDA receptor may be useful in the treatment of schizophrenia since compounds such as (+)-HA-966 (**1b**) selectively modulate mesolimbic dopaminergic activity by blocking locomotor activity induced by infusion of amphetamine into the rat nucleus accumbens while having no effect on stereotypy caused by infusion of amphetamine into the striatum.[18] The most challenging problem in the medicinal chemistry of glycine site NMDA antagonists, and indeed with competitive glutamate antagonists, has been to identify compounds which have high-affinity binding but are also able to access receptors in the brain by crossing the blood–brain barrier. Many existing glycine antagonists contain a carboxylic acid, in common with the natural ligand for the receptor, and require this functionality to achieve high affinity. Notable exceptions to this are **1b** and its β-methyl derivative (**1a**) which are considerably less acidic with a pK_a of 8.6 for the hydroxamic acid. **1a**,**b** freely penetrate the blood–brain barrier but have relatively weak activity in binding to the glycine receptor (IC_{50}s 12.5 and 1.4 μM, respectively). Recent work in our laboratories has identified quinolinediones with very high affinity glycine antagonist activity which are also potent inhibitors of audiogenic seizures in DBA/2 mice. The pK_a of these compounds is around 5.5, and so they are essentially fully ionized at physiological pH. Despite these physical properties, the brain penetration of these compounds is excellent as demonstrated by their *in vivo* CNS activity.

We have attempted to find other non-carboxylate antagonists of the glycine receptor and were encouraged to find the pyrazoloquinolone **7** with moderate affinity (135 nM). Structure–activity relationship (SAR) studies were initially directed toward optimizing substitution on the phenyl ring, but introducing chlorine at any of the available positions had little effect on affinity. The *ortho*-substituted derivative **11** was about 4-fold lower in activity than the unsubstituted parent, but there was no effect with the same group at either the *meta* or *para* positions (**12** and **13**). Introduction of a methoxy substituent at the *para* position gave almost a 2-fold increase in affinity (**14**), but it seemed from this limited group of compounds that activity is not very sensitive to the environment around this ring. From a comparison of **7** with the quinolinedione antagonists, and other glycine site ligands, it seemed obvious that activity in this new class of antagonists should be enhanced by addition of a chlorine substituent at C7 of the quinolone ring system. This turned out to be the case, with the parent lead compound **7** improved 8-fold in affinity (**15**) and the *p*-methoxy substituted analogue **16** significantly more active with an IC_{50} of 3.3 nM.

In vitro functional antagonist potency was determined from blockade of NMDA induced depolarizations on rat cortical slices[12]. The test compounds were superfused over the slices for 120 min before their ability to affect increasing concentrations of agonists was determined. During the period of equilibration, the test compounds had no apparent direct action on the recorded base line. In this assay **15** acted as a potent functional NMDA antagonist with a K_b of 29.9 ± 3.9 nM. This compound was also shown to be selective for NMDA receptors since there was no inhibition of AMPA activity in the cortical slice assay at concentrations up to 300 nM. The measured pK_a values of these compounds (**15** is typical with pK_a 4.0) show that they, in common with the quinolinediones, will be fully deprotonated at physiological pH. When the acidity of **7** was removed by methylation (**10**) of the pyrazolone ring, *in vitro* affinity was abolished. In addition, the structurally related succinimidylquinolone **19**, which has been reported as a ligand for the benzodiazepine binding site on the GABA receptor, had no affinity at the glycine receptor. Although the modifications in these molecules (**10** and **19**) affect properties in addition to the acidity of **7** (steric effects, lipophilicity), it seems likely that, in common with most glycine antagonists, the ability to present a negative charge is a key factor in the high-binding affinity of these compounds.

A hypothetical model of binding (Figure 1) has been proposed[19] for 3-arylquinolinedione glycine antagonists which involves binding of the delocalized anion to a positive charge on the receptor through the quinolone oxygen with additional stabilization provided by a Π-cation interaction with the phenyl ring at C3. With the pyrazoloquinolones, a similar type of binding may occur through the quinolone oxygen with a further interaction through the pyrazolone ring nitrogen. In this case, however, there is no opportunity to access the additional lipophilic binding pocket exploited by the quinolinediones where the 3-phenoxyphenyl substituted derivative has 100-fold greater affinity than its corresponding 3-phenyl analogue. In compound **16** the phenyl ring is coplanar with the core quinolone ring

system whereas in **5** the terminal phenyl ring lies out of the plane of the quinolone and is able to sweep out a large region of conformational space which does not include the volume occupied by the phenyl ring in **16**. The benzyl substituted pyrazoloquinolone **17** is able to place the aromatic ring out of the plane of the quinolone and access a greater volume of space but clearly, from its slightly reduced binding affinity compared to **7**, does not benefit from any additional interaction with the receptor. While the pendant aromatic ring in these compounds cannot bind in the same way as the quinolinediones, the 15-fold loss in activity (compared to **7**) when it is replaced by methyl (**18**) indicates it does have an influence in their high-affinity binding although its role is not well defined by the currently available structure–activity dataset.

The pyrazoloquinolones were tested *in vivo* for their ability to prevent audiogenic seizures in DBA/2 mice. None of the compounds in this study were effective at doses up to 50 mg/kg after systemic dosing. However, compound **15** injected icv was a potent anticonvulsant giving protection against seizures with an ED_{50} of 0.8 nmol.[20] This indicates the lack of activity after systemic dosing is due to negligible penetration of the compound into the brain. The explanation for the relative inactivity of these compounds compared to the quinolinediones is unlikely to be due to the greater acidity of the pyrazoloquinolones since both classes of compounds are sufficiently acidic to be fully ionized at physiological pH (pK_a for the quinolinediones is typically 5.5). The measured log *P* values of the compounds in this study indicate they are considerably more hydrophilic (**15**, log $P_{oct,pH7.4} = -0.39$) than the quinolinediones (**5**, log $P_{oct,pH7.4} = 2.9$), and this would be predicted to make CNS penetration more difficult, although previous studies have shown no obvious dependence of anticonvulsant activity on log P_{oct} in several classes of glycine antagonists.

In conclusion, we have identified a novel class of high-affinity glycine site NMDA antagonists which adds to the diversity of structures that bind to this receptor. The lack of *in vivo* activity seen with these compounds after systemic dosing is in common with many other glycine antagonists and remains the most difficult problem in establishing therapeutic agents with this mechanism of action.

Experimental Section

Melting points were determined with a Büchi capillary melting point apparatus and are uncorrected. NMR spectra were recorded at 360 or 250 MHz on a Bruker AM360 or Bruker AM250 instrument. Mass spectral data was generated using a VG Quattro spectrometer. Organic solvents were evaporated on a Büchi rotary evaporator at reduced pressure. Column chromatography was carried out on silica gel (Merck Art. 7734). Petroleum ether used in chromatography had bp 60–80 °C. log *P* measurements were made using a standard shake-flask procedure.[21] pK_a determinations were performed using the Sirius PCA-101 titrator (Sirius Analytical Instruments Ltd., East Sussex, England) equipped with a Ross type combination glass electrode calibrated for mixed solvent titrations. The mixed solvent approach was employed because of the limited aqueous solubility of the compounds across the pH range. A cosolvent of 1,4-dioxane/water (60/40, v/v, ionic strength) adjusted with 0.15 M potassium chloride was used. Three separate titrations were performed for each compound with different water/cosolvent ratios to obtain pK_a values in the presence of cosolvent (p_sK_a values). Aqueous pK_a values were calculated by extrapolation to 0% cosolvent using the Yasuda–Shedlovsky relationship:[22,23] a linear plot of $p_sK_a + \log [H_2O]$ versus $1/\epsilon$, where ϵ is the dielectric constant of the water cosolvent mixture. A Hewlet-Packard 1090L instrument was used for HPLC analyses. Elemental analyses were determined by Butterworth Laboratories Ltd., Teddington, England.

3,5-Dihydro-2-phenyl-1*H*-pyrazolo[3,4-*c*]quinoline-1,4-(2*H*)-dione (7). Ethyl oxindole-3-glyoxylate (220 mg, 0.94 mmol) was heated under reflux with phenylhydrazine (0.1 mL, 1.1 mmol) in AcOH (2 mL) for 10 min. The reaction mixture was cooled and the resulting precipitate filtered and crystallized from MeOH to give the title compound (200 mg, 77%): mp >260 °C slow dec; ^{1}H NMR (250 MHz, DMSO-d_6) δ 11.86 (1H, br s), 8.25 (1H, br d), 7.86 (2H, d, J = 8 Hz), 7.56 (2H, t, J = 8 Hz), 7.22–7.42 (4H, m); MS (ES$^+$) 278 [M + H]$^+$. Anal. ($C_{16}H_{11}N_3O_2$) C, H, N.

The following examples were prepared by the method described above using the appropriately substituted hydrazine with ethyl oxindole-3-glyoxylate or ethyl 6-chlorooxindole-3-glyoxylate.

3,5-Dihydro-2-(2-chlorophenyl)-1*H*-pyrazolo[3,4-*c*]quinoline-1,4(2*H*)-dione (11): mp >310 °C; ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.16 (1H, br s), 7.52–7.77 (4H, m), 7.29–7.41 (3H, m); MS (ES$^+$) 312/314 [M + H]$^+$. Anal. ($C_{16}H_{10}ClN_3O_2{\cdot}0.25H_2O$) C, H, N.

3,5-Dihydro-2-(3-chlorophenyl)-1*H*-pyrazolo[3,4-*c*]quinoline-1,4(2*H*)-dione (12): mp 305 °C dec; ^{1}H NMR (360 MHz, DMSO-d_6) δ 8.37 (1H, d, J = 8 Hz), 8.02 (1H, s), 7.86 (1H, d, J = 8 Hz), 7.58 (1H, t, J = 8 Hz), 7.20–7.44 (4H, m); MS (ES$^+$) 312/314 [M + H]$^+$. Anal. ($C_{16}H_{10}ClN_3O_2$) C, H, N.

3,5-Dihydro-2-(4-chlorophenyl)-1*H*-pyrazolo[3,4-*c*]quinoline-1,4(2*H*)-dione (13): mp >320 °C; ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.25 (1H, d, J = 11 Hz), 7.91 (2H, d, J = 12.6 Hz), 7.62 (2H, d, J = 12.6 Hz), 7.20–7.38 (3H, m); MS (ES$^+$) 312/314 [M + H]$^+$. Anal. ($C_{16}H_{10}ClN_3O_2$) C, H, N.

3,5-Dihydro-2-(4-methoxyphenyl)-1*H*-pyrazolo[3,4-*c*]-quinoline-1,4(2*H*)-dione (14): mp 285 °C; ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.21 (1H, br s), 7.73 (2H, d, J = 9 Hz), 7.31–7.35 (2H, m), 7.12–7.25 (1H, m), 7.11 (2H, d, J = 9 Hz), 3.82 (3H, s); MS (ES$^+$) 308 [M + H]$^+$. Anal. ($C_{17}H_{13}N_3O_3$) C, H, N.

7-Chloro-3,5-dihydro-2-phenyl-1*H*-pyrazolo[3,4-*c*]quinoline-1,4(2*H*)-dione (15): mp >300 °C; ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.20 (1H, d, J = 8 Hz), 7.84 (2H, d, J = 8 Hz), 7.56 (2H, t, J = 8 Hz), 7.37–7.43 (2H, m), 7.30 (1H, dd, J = 3 and 12.2 Hz); MS (ES$^+$, dissolved in the presence of Et_3N) 413/415 [M + Et_3N]. Anal. ($C_{16}H_{10}ClN_3O_2{\cdot}0.33CH_3CO_2H$) C, H, N.

7-Chloro-3,5-dihydro-2-(4-methoxyphenyl)-1*H*-pyrazolo-[3,4-*c*]quinoline-1,4(2*H*)-dione (16): mp >300 °C; ^{1}H NMR (250 MHz, DMSO-d_6) δ 11.46 (1H, br s), 7.85 (1H, d, J = 8.4 Hz), 7.40 (2H, d, J = 9.0 Hz), 7.07 (1H, d, J = 2 Hz), 6.98 (1H, dd, J = 2 and 8.4 Hz), 6.81 (2H, d, J = 9.0 Hz), 3.52 (3H, s); MS (ES$^+$) 342/344 [M + H]$^+$. Anal. ($C_{17}H_{12}ClN_3O_3{\cdot}H_2O$) C, H, N.

3,5-Dihydro-2-benzyl-1*H*-pyrazolo[3,4-*c*]quinoline-1,4-(2*H*)-dione (17): mp >300 °C; ^{1}H NMR (360 MHz, DMSO-d_6) δ 11.55 (1H, br s), 8.11 (1H, br s), 7.26–7.37 (7H, m), 7.19 (1H, t, J = 6.7 Hz), 5.30 (2H, s); MS (ES$^+$) 292 [M + H]$^+$. Anal. ($C_{17}H_{11}N_3O_2{\cdot}2H_2O$) C, H, N.

3,5-Dihydro-2-methyl-1*H*-pyrazolo[3,4-*c*]quinoline-1,4-(2*H*)-dione (18): mp >320 °C; ^{1}H NMR (250 MHz, DMSO-d_6) δ 11.47 (1H, br s), 8.08 (1H, d, J = 7.5 Hz), 7.15–7.32 (3H, m), 3.71 (3H, s); MS (ES$^+$) 216 [M + H]$^+$. Anal. ($C_{11}H_9N_3O_2{\cdot}0.25H_2O$) C, H, N.

3,5-Dihydro-2-phenyl-3-methyl-1*H*-pyrazolo[3,4-*c*]quinoline-1,4(2*H*)-dione (10). Compound **7** (244 mg, 0.88 mmol) in DMF (2 mL) was stirred with NaH (45 mg of a 60% dispersion in oil, 1.12 mmol) until evolution of gas ceased. MeI (130 mg, 0.9 mmol) was added, and the reaction mixture was stirred for 2 h and then diluted with H_2O and extracted with Et_2O. The ethereal solution was dried over Na_2SO_4, filtered, and concentrated, and then the residue was purified by chromatography on silica gel eluting with EtOAc–petroleum ether (1:1) to give the title compound as a white solid (13 mg, 5%): mp 273 °C; ^{1}H NMR (360 MHz, DMSO-d_6) δ 12.28 (1H, s), 8.40 (1H, d, J = 7.5 Hz), 7.54–7.62 (4H, m), 7.42–7.48 (3H,

m), 7.28–7.33 (1H, m), 3.52 (3H, s); MS (ES^+) 292 $[M + H]^+$. Anal. ($C_{17}H_{13}N_3O_2$) C, H, N.

X-ray Crystallography of 7: $C_{16}H_{11}N_3O_2 \cdot H_2O$, M_r = 295.300, monoclinic, $C2/c$, a = 29.259(5), b = 4.5868(9), and c = 21.942(5) Å, β = 102.72(1)°, V = 2872(2) Å^3, Z = 8, D_x = 1.366 g cm^{-3}, monochromatized radiation λ(Cu Kα) = 1.541838 Å, μ = 0.76 mm^{-1}, $F(000)$ = 1232, T = 294 K. Data were collected on a Rigaku AFC5 diffractometer to a θ limit of 70° which yielded 3100 measured (3034 unique) reflections. There were 1291 unique, observed reflections (with $I \geq 3\sigma(I)$ as the criterion for being observed) out of the total measured. The structure was solved by direct methods (SHELXS-86)[24] and refined using full-matrix least-squares on F (SDP-PLUS).[25] The final model included the organic moiety and a disordered water molecule and was refined using 209 parameters and the observed data. All non-hydrogen atoms were refined with anisotropic thermal displacements; hydrogen atoms were included at their idealized positions.The final agreement statistics are R = 0.080, R_w = 0.112, S = 4.19 with $(\Delta/\sigma)_{max}$ = 0.02. The least-squares weights were defined using $1/\sigma^2(F)$. The maximum peak height in a final difference Fourier map was 0.25(4) eÅ^{-3} and is associated with one of the disordered water molecule positions in the lattice. The atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre. The coordinates can be obtained on request from the Director, Cambridge Crystallographic DataCentre, 12 Union Rd., Cambridge CB2 1EZ, U.K.

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Supplementary Material Available: Details of the X-ray crystal structure determination for compound **7** including interatomic distances and angles and positional and thermal parameters (4 pages). Ordering information is given on any current masthead page.

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