Notes

Structure–Activity Relationship Studies at the Benzodiazepine Receptor (BZR): A Comparison of the Substituent Effects of Pyrazoloquinolinone Analogs†

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The synthesis of a series of 2-phenylpyrazolo[4,3-*c*]quinolin-3-one derivatives and their in vitro biological evaluation as ligands for the benzodiazepine receptor are described. The in vitro activities, as determined by an analysis of GABA shift ratios, and binding affinities of these compounds to BZR are compared in terms of the electronic, lipophilic, and steric effect changes of their substituents.

Benzodiazepine receptor (BZR) ligands exert a continuous profile of pharmacological activities,[1,2] ranging from (i) agonists, which enhance γ-aminobutyric acid ($GABA_A$) gated Cl^- current to induce anticonvulsant, hypnotic, muscle relaxant, and anxiolytic effects; (ii) inverse agonists, which are proconvulsants, convulsants, and anxiogenics; and (iii) antagonists, which bind to the BZR but have no pharmacological effects. The detailed tertiary structure of the BZ/GABA receptor supramolecular complex remains obscure even though recent reports suggested that at least three distinct cDNA's must be coexpressed in vitro to obtain a fully functional chloride channel.[3]

Our interest in pyrazolo[4,3-*c*]quinolin-3-one compounds, such as CGS-9896, CGS-9895, and CGS-8216 (**4**, **7**, **15** respectively, Table I), resulted from the report that these compounds bind to BZR with the very high affinity and their different pharmacological profiles are related only to the nature of the substituent in the para position of the 2-phenyl ring.[4] The difference in the pharmacological activities for the pyrazolo[4,3-*c*]quinolin-3-one compounds has been separately discussed in terms of either lipophilic and electronic[5] or steric effects[6] of the substituents. Since the marked difference of these biological profiles could be attributed to any factor or to a combination of them, it does not seem reasonable to discuss some effects without concomitant consideration of the others.

In an attempt to systematically evaluate these substituent effects on receptor affinity and on the activity profile of pyrazolo[4,3-*c*]quinolin-3-ones, a series of pyrazoloquinolinone compounds was synthesized. These compounds were designed on the basis that the relative lipophilicity of both the fused benzene ring and the 2-phenyl ring can be altered by an appropriate substitution. On the other hand, a change of the substituent position on the same aryl ring would not be expected to affect lipophilicity[7] in any substantial manner, although such changes would be expected to alter both the electronic properties and the spatial orientation of the substituents in the molecules.

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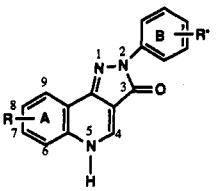
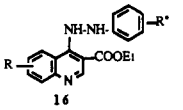
Table I. Pyrazoloquinolinones[a]



no.	R	R′	no.	R	R′
4	H	*p*-Cl	**10**	8-Cl	H
5	H	*m*-Cl	**11**	8-Cl	*p*-Cl
6	H	*o*-Cl	**12**	8-Cl	*o*-Cl
7	H	*p*-OMe	**13**	8-OMe	H
8	H	*m*-OMe	**14**	7-OMe	H
9	H	*o*-OMe	**15**	H	H

[a] In three instances (during the preparation of compounds **6**, **12**, and **14**), the initially formed hydrazines **16** were isolated prior to cyclization of the pyrazole ring. These intermediates were readily characterized by the ^{1}H-NMR spectroscopy. No attempt was made to further purify these hydrazines, and they were cyclized to their respective pyrozoloquinolinones by heating in Dowtherm A.



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Chemistry

All pyrazolo[4,3-*c*]quinolin-3-one compounds were made using slightly modified literature procedures.[4,8] In brief, the substituted anilines were refluxed with diethyl (ethoxymethylene)malonate in Dowtherm A to give 4-hydroxy-3-quinolinecarboxylic acid esters **2** via thermal ring closure. Treatment of **2** with phosphorus oxychloride led to the corresponding chloro derivatives **3**, which were condensed with the appropriately substituted phenylhydrazines in xylene or Dowtherm A to afford **4**–**15** (Scheme I and Table I). The known intermediate compounds **2a**–**d** and **3a**–**d** had melting points consistent with literature values, and their spectra were compatible with the assigned structures.[8] The final compounds were purified by recrystallization from ethanol. The three known compounds (**4**, **7**, and **15**) were also resynthesized as biological standards and had melting points and exhibited spectral properties consistent with literature values.[4]

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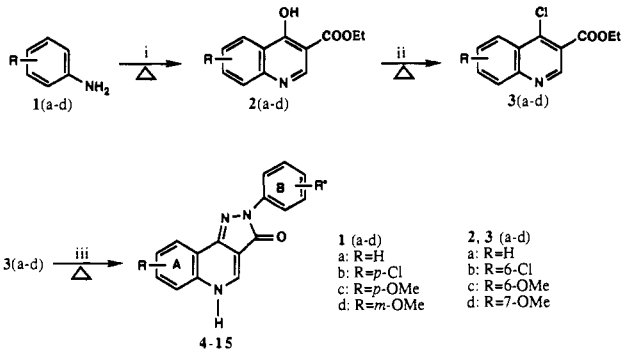
Scheme I. Synthesis of Pyrazoloquinolinone Analogues[a]



[a] Reagents and conditions: (i) diethyl(ethoxymethylene)malonate, Dowtherm A; (ii) $POCl_3$; (iii) phenyls hydrazine, (*o*; *m*-, or *p*-chloro- and -methoxyphenyl)hydrazine, xylene, or Dowtherm A.

Table II. Potencies and GABA Shift Ratio of Pyrazoloquinolinone Analogs Compared with Classical 1,4-Benzodiazepines[a]

compound	IC_{50} (nM)[b]	GABA shift ratio	activities
4(CGS-9896)	0.56 ± 0.04 (0.62)	1.07 (1.32)	partial agonist[c]
5	3.90 ± 0.31	1.7	
6	70.0 ± 2.3	1.08	
7(CGS-9895)	0.38 ± 0.03 (0.06)	0.94 (0.86)	partial agonist/
8	0.58 ± 0.07	1.1	antagonist[c]
9	990 ± 46	ND[e]	
10	0.24 ± 0.05	1.1	
11	4.00 ± 0.15	1.47	
12	8.00 ± 0.23	1.34	
13	0.38 ± 0.03	0.91	
14	2.10 ± 0.3	0.97	
15(CGS-8216)	0.13 ± 0.01 (0.15)	0.99 (0.94)	partial inverse agonist[d]
diazepam	20.6 ± 2.3 (15.1)	1.78 (1.82)	agonist
flumazenil	1.82 ± 0.28 (2.3)	1.00 (1.00)	antagonist

[a] The data in the parentheses were K_D values taken from ref 5, and GABA shift ratio in the parentheses were taken from the same reference, IC_{50} values reported here were determined at a 1 nmol final concentration of radioactive ligand. [b] Results reported as 95% fiducial limits. [c] See ref 17. [d] See ref 18. [e] GABA ratio was not determined because of its low potency.

Results and Discussions

Studies have indicated that the GABA shift ratio (the ratio of IC_{50} without GABA/IC_{50} with GABA) can be related to[9] and has been extensively employed as a predictor of[10] the biological spectrum of BZR ligands, in which GABA modulates the binding of BZR ligands, enhancing that for agonists (ratio > 1.0), not affecting that for antagonists (ratio = 1.0) and diminishing that for inverse agonists (ratio < 1.0).[11] Using the GABA shift ratio of the standard BZR ligands (diazepam and flumazenil) shown in Table II as a basis for comparison, compounds **5** and **6**, isomers of CGS-9896 (**4**), have the same relative lipophilicity as that of **4** and show the character of an agonist-like and a partial agonist/antagonist-like pharmacological profile, respectively. Compound **6**, which has very similar lipophilic and electronic properties as compound **4**, seems to retain the pharmacological profiles of the parent compound. The reduction of binding affinity of **6** is attributed to the steric effects caused by the different substituent position. Compound **5**, on the other hand, possesses different electronic effects and different steric effects from **4**, thus altering both its potency and its pharmacological profile. Since the steric effect (compound **6**) reduces only the binding affinity, the profile change of **5** from partial agonist/antagonist to the

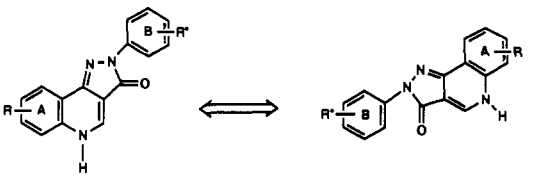


Figure 1.

character of agonist may be ascribed, at least partially, to a difference in the electronic effect of the parasubstituent from the metasubstituent. The same type of combined electronic and steric effect on the potencies and pharmacological profiles is also observed in the methoxy isomers (**7**, **8**, and **9**) which are related to CGS-9895 (**7**). These results imply that the potencies of these ligands are modulated by the substituent position on the 2-phenyl ring, while their pharmacological profiles might be related to the electronic effects of the substituents. Thus, the findings presented here do not agree with those described by Shindo and co-workers,[6] in which the pharmacological activities of substituted 2-thienylpyrazoloquinolinones, the analogs of 2-phenylpyrazoloquinolinones, were predicted based only on the relative distance of the substituents on a thiophene ring measured from the axis along the N–C bond connecting the pyrazolo and thiophene rings. It should be noted here that as the substituents on the 2-phenyl ring are moved from the para position through to the ortho position, steric hindrance can possibly induce rotation about the bond between N2 and the 2-phenyl ring, causing an increasing out of plane twist of the 2-phenyl ring. The lack of binding affinity of compound **9** is believed to be such an example caused by a twist of the 2′-methoxyphenyl ring.

A second series of compounds (**10**, **13**, and **14**) was synthesized in which the B ring was kept unsubstituted, while the A ring was substituted either by Cl or by OCH_3. Substitution at the 8-position was considered to be comparable to the para position of the 2-phenyl ring if the orientation of the molecule is inverted within the receptor pocket (Figure 1). It was reasoned that a pyrazoloquinolinone with the same relative lipophilicity between the A and B rings as that for the partial agonist/antagonist **7** could be synthesized by the removal of the methoxy (π value around −0.02[12]) substituent from the 2-phenyl group of the compound **7** and adding a chlorine atom (π value around 0.70[12]) to the 8-position of the parent compound **15**, i.e. **10**.[13] Thus, **10** would have the opposite relative lipophilicity between the A and B rings compared with **4** and the same relative lipophilicity as for compound **7**. Both potencies and pharmacological profiles of **4** and **10**, as shown by GABA ratios, appear to be the same. This suggests that the binding orientations of the substituted rings within the receptor sites must coincide. In other words, the results seem to infer that one of these two molecules would bind in an inverted configuration within the receptor pocket (Figure 1) in order for the substituents to be superimposed. Similarly, compounds **7** and **13** (methoxy analogs of **4** and **10**) also exhibit the same potencies and the same pharmacological profiles despite the opposite relative lipophilicity between their A and B rings. These findings would seem to imply that ligand orientation within the receptor active sites can be modulated by the relative lipophilicity of the aromatic rings in pyrazoloquinolinones.

The dichloro-substituted compounds **11** and **12** have the "GABA-positive" character indicating an agonist-like

profile and have the same potencies at the BZR. The pharmacological profiles and potencies of 11 and 12 appear to be different from those of their A-ring unsubstituted analogs (4 and 6). The latter show a partial agonist/antagonist profile, and their potencies exhibit a difference of 2 orders of magnitude. As mentioned above, moving the Cl on the freely rotating phenyl ring from 4'- to 2'-position resulted in a remarkable loss of binding affinity, presumably caused by an increase in out of plane rotation. This might well indicate that this aromatic ring is acting as the molecular determinant which has the π-π aromatic stacking interactions with the BZR for the monosubstituted analogs. For the corresponding disubstituted analogs (11 and 12) this loss of affinity was not apparent for the 2'-Cl-substituted analog. This result can be rationalized as being due to the fact that it is now the fused benzene ring that acts as the molecular determinant. Thus, the findings presented here are at variance with those described by Villar et al.[5] in which it is proposed that the substituted 2-phenyl ring of pyrazoloquinolinones acts as the molecular determinant, interacting with BZR to define an agonist profile.

The enol configuration of the vinylogous amide was required by Villar's model in order to explain the agonist activity of CGS-9896 (two imine nitrogen atoms are defined as binding sites). From the NMR (in DMSO-d_6) and FT-IR (KBr) data for the pyrazoloquinolinones described in this paper, no evidence for the existence of enol formation was ever detected. This is consistent with reported data with a series of 2-thienylpyrazoloquinolinones[6] and 2-phenylpyrazoloisoquinolinols.[14] Thus, the physical data for these agonist ligands indicate only the carbonyl form of the amide, which again is at variance with Villar's required model for BZR agonists.

Conclusion

The present studies demonstrate that the affinity of pyrazoloquinolinone ligands to BZR can be correlated with the steric effects due to substituents on the 2-phenyl ring in these compounds. Furthermore, their pharmacological profiles seem to be modulated by the electronic effects of these substituents. The relative lipophilicity appears to define the aromatic ring determinant of pyrazoloquinolinones binding to the BZR. Nonetheless, the correlation between the relative lipophilicity and the aromatic ring determinant is not discernible as described by Villar et al.[5] It is also apparent that the rationalization of the intrinsic activity of these compounds based solely on steric effects[6] of the substituents on the 2-phenyl ring is not a general phenomenon. The consideration of any one substituent effect (i.e., lipophilic, electronic, or steric) without concomitant consideration of the others is inadequate as a predictor of biological activity for the pyrazoloquinolinone ligands. Further work is needed in order to better define a predictive model for the pharmacophores of pyrazoloquinolinone compounds.

Experimental Section

Melting points were determined with the Mel-Temp apparatus and are uncorrected. Infrared spectra were obtained by using the Mattson Polaris FT-IR spectrometer with KBr pellets. ^{1}H NMR spectra were taken either on a Bruker WP-200SY or on a Varian 400 Fourier transform spectrometers using DMSO-d_6 solvent. Microanalyses were performed at Hoffmann-La Roche and were all within ±0.4% of calculated values for carbon, hydrogen, and nitrogen. All chemicals were purchased from the Aldrich Chemical Co.

Radioligand Binding. The affinity of the compounds for the central benzodiazepine receptor was assessed by using a modification of the previously described techniques.[15] Sprague–Dawley rats were decapitated, and the brains were rapidly removed and placed in 320 nmol of sucrose (0–4 °C) before dissection. The cortex was homogenized in 50 volumes of 50 mM Tris-citrate buffer (pH 7.4) using a Polytron (Brinkmann Instruments) at a setting of 6.5 for 15 s. The homogenate was centrifuged at 20000g (0–4 °C) for 20 min, and the pellet was resuspended in an equal volume 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.

Before assay, the tissue preparation was thawed, and a 50-μL aliquot (containing about 0.12 mg of protein) was added to each assay mixture, which also contained 50 μL of [^{3}H]Ro15-1788 (final concentration, 1 nM) and 50 μL of Ro14-7437 for nonspecific binding (final concentration, 10 μM). Sufficient Tris-citrate buffer was used to bring the final assay volume to 0.5 mL. The assay was terminated after incubation (1 h at 0–4 °C) by rapid filtration over Whatman GF/B filter strips using a Brandel M-24R filtering manifold. Samples were washed twice with 5 mL of cold buffer. The filters were then placed in vials and 4 mL of scintillation fluid was added. In order to determine the GABA shift ratio, a similar procedure was carried out in the presence of NaCl (400 mM) and GABA (200 mM).

The radioactivity retained by the filters was measured in a Beckman LS 5802 liquid scintillation spectrometer. The concentration of test compounds which inhibit the specific binding of [^{3}H]Ro15-1788 to BZR in rat cortex by 50% (IC_{50}, nM) were derived from displacement curves with 10 concentrations of each compound, each assayed in triplicate.

General Procedures for the Preparation of Pyrazoloquinolinones. A mixture of compounds **3a–d** and the appropriate analog of phenylhydrazine (approximately 1.2 molar equiv) in xylene or Dowtherm A was heated at the boiling point temperature of xylene or in those cases requiring higher temperature (at about 180 °C) in Dowtherm A. After 1 h, the reaction mixture was cooled to room temperature and filtered. The yellowish solid was washed several times with hexane and then several times with acetone. The solid was then dissolved in the hot ethanol and filtered to remove undissolved impurities, and the compound was crystallized by cooling. In cases when the compound had poor solubility in ethanol, a few drops of 3 N sodium hydroxide solution was added to the ethanol solution, which was then filtered, and the filtrate was carefully neutralized with 1 N HCl (to avoid precipitation) to pH 7 (pH paper). The solution was cooled and the product allowed to crystallize. Filtration afforded the crystalline product which was washed well with distilled water and dried in vacuo.

Preparation of 2a–d and 3a–d. Compounds **2a–d** and **3a–d** were prepared according to the literature procedure.[8] The melting points of these compounds were consistent with the literature values.[8]

2-(3-Chlorophenyl)pyrazolo[4,3-*c*]quinolin-3-one (5). A mixture of 3-carbethoxy-4-chloroquinoline (**3a**) (2.0 g, 9 mmol) and (3-chlorophenyl)hydrazine (2.0 g, 13 mmol) in 60 mL of xylene was heated according to the general procedure. Recrystallization of product from the solvent system as described in the general procedure afforded **5** (1.62 g, 61%) as yellow crystals: mp 325–326 °C (lit.[16] mp 336–337 °C); ^{1}H NMR (DMSO-d_6) δ 12.9 (br, NH, D_2O exchangeable), 8.8 (d, 2 H, J = 6.5 Hz), 8.35 (s, 1 H), 8.2 (t, 2 H, J = 6.7 Hz), 7.7 (m, 2 H), 7.55 (dd, 1 H, J = 2.8, 8.0 Hz), 7.45 (t, 1 H, J = 8.2 Hz); IR (KBr) 3377.1, 3135.1, 2975.9, 1630.7, 1588.2, 769.5 cm^{-1}. Anal. ($C_{16}H_{10}ClN_3O\cdot0.5H_2O$) C, H, N.

2-(2-Chlorophenyl)pyrazolo[4,3-*c*]quinolin-3-one (6). Treatment of **3a** (2.0 g, 9 mmol) with (2-chlorophenyl)-hydrazine (2.0 g, 13 mmol) in 50 mL of xylene solution under reflux for 1.5 h afforded the corresponding uncyclized intermediate **16** formed by nucleophilic attack of hydrazine at the 4-position of quinoline. This structure was confirmed by ^{1}H NMR spectroscopy. The intermediate was then reheated in 60 mL of Dowtherm A at about 180 °C for 30 min. Removal of solvent afforded **6** (1.48 g, 56%) as bright yellow crystals: mp 330–331 °C (lit.[16] mp 336–339 °C); ^{1}H NMR (DMSO-d_6) δ 12.95 (br, NH, D_2O exchangeable), 8.75 (d, 1 H, J = 6.4 Hz), 8.1 (dd, 1 H, J = 2.0, 7.6 Hz), 7.5–7.7 (m, 7 H): IR (KBr) 3441.7, 3060.8, 2840.9, 1615.2, 1455.2, 758.9 cm^{-1}. Anal. ($C_{16}H_{10}ClN_3O$) C, H, N.

2-(3-Methoxyphenyl)pyrazolo[4,3-*c*]quinolin-3-one (8). A solution of **3a** (2.0 g, 9 mmol) and (3-methoxyphenyl)hydrazine (2.0 g, 14 mmol) in 50 mL of xylene was heated for 1 h and after workup afforded 1.49 g (57%) of **8** as yellow crystals: mp 248–249 °C; ^{1}H NMR (DMSO-d_6) δ 13.0 (br, NH, D_2O exchangeable), 8.8 (s, 1 H), 8.25 (d, 1 H, J = 7.6 Hz), 7.5–7.9 (m, 5 H), 7.6 (t, 1 H, J = 8.1 Hz), 6.8 (dd, 1 H, J = 1.5, 8.4 Hz), 3.85 (s, 3 H); IR (KBr) 3487.0, 3104.2, 2957.3, 2864.1, 1650.9, 1600.8, 1453.2, 1234.3, 759.9 cm^{-1}. Anal. ($C_{17}H_{13}N_3O_2$) C, H, N.

2-(2-Methoxyphenyl)pyrazolo[4,3-*c*]quinolin-3-one (9). A mixture of **3a** (2.0 g, 9 mmol) and (2-methoxyphenyl)hydrazine (2.0 g, 14 mmol) in 50 mL of xylene was heated under reflux as described above and after workup afforded 1.3 g (50%) of **9** as yellow needles: mp 305–307 °C; ^{1}H NMR (DMSO-d_6) δ 13.1 (br, NH, D_2O exchangeable), 8.65 (s, 1 H), 8.2 (d, 1 H, J = 7.6 Hz), 7.25–7.75 (m, 5 H), 7.2 (d, 1 H, J = 8.3 Hz), 7.12 (t, 1 H, J = 7.5 Hz), 3.75 (s, 3 H); IR (KBr) 3479.4, 3401.2, 3056.9, 2839.0, 1621.1, 1579.5, 1454.2, 763.76 cm^{-1}. Anal. ($C_{17}H_{13}N_3O_2\cdot0.5H_2O$) C, H, N.

8-Chloro-2-phenylpyrazolo[4,3-*c*]quinolin-3-one (10). A solution of 2.0 g (7 mmol) of 4-chloro-6-chloro-3-carbethoxyquinoline (**3b**) and 1 mL (10 mmol) of phenylhydrazine in 60 mL of xylene was heated for about 30 min. After workup **10** was obtained as yellow crystals (1.07 g, 52%): mp >400 °C dec; ^{1}H NMR (DMSO-d_6) δ 12.95 (br, NH, D_2O exchangeable), 8.9 (s, 1 H), 8.1 (m, 3 H), 7.85 (m, 2 H), 7.5 (t, 2 H, J = 8.8 Hz), 7.2 (t, 1 H, J = 7.3 Hz); IR (KBr) 3466.8, 3184.3, 3054.7, 2939.3, 2839.0, 1625.9, 1450.3, 758.9 cm^{-1}. Anal. ($C_{16}H_{10}ClN_3O$) C, H, N.

8-Chloro-2-(4-chlorophenyl)pyrazolo[4,3-*c*]quinolin-3-one (11). A mixture of **3b** (2.69 g, 10 mmol) and (4-chlorophenyl)hydrazine (1.7 g, 12 mmol) was heated in 60 mL of xylene for 30 min, and **11** precipitated as a yellow solid. Crystallization afforded 1.0 g (30%) of the product as yellow crystals: mp 366–369 °C; ^{1}H NMR (DMSO-d_6) δ 12.85 (br, NH, D_2O exchangeable), 8.75 (d, 1 H, J = 4.9 Hz), 8.25 (d, 2 H, J = 9.0 Hz), 8.15 (d, 1 H, J = 1.9 Hz), 7.75 (m, 2 H), 7.45 (d, 2 H, J = 8.9 Hz); IR (KBr) 3421.5, 3095.6, 2938.4, 1629.7, 1491.8, 823.5 cm^{-1}. Anal. ($C_{16}H_9Cl_2N_3O$) C, H, N.

8-Chloro-2-(2-chlorophenyl)pyrazolo[4,3-*c*]quinolin-3-one (12). A mixture of 2.7 g (10 mmol) of **3b** and 1.7 g (12 mmol) of (2-chlorophenyl)hydrazine was heated in 50 mL of xylene for 1 h. Workup afforded the uncyclized intermediate (confirmed by ^{1}H NMR spectrum) which was reheated in 60 mL of Dowtherm A at a temperature of about 260 °C for 1 h in order to effect cyclization. Workup afforded **12** as yellow crystals (1.98 g, 60%): mp 379–383 °C; ^{1}H NMR (DMSO-d_6) δ 12.9 (br, NH, D_2O exchangeable), 8.8 (s, 1 H), 8.05 (s, 1 H), 7.75 (s, 2 H), 7.65 (m, 1 H), 7.45 (m, 3 H); IR (KBr) 3435.0, 3124.5, 2940.3, 1666.4, 1607.6, 1440.7, 1271.0, 759.9 cm^{-1}. Anal. ($C_{16}H_9Cl_2N_3O$) C, H, N.

8-Methoxy-2-phenylpyrazolo[4,3-*c*]quinolin-3-one (13). The reaction of 2.7 g (10 mmol) of (4-chloro-3-carbethoxy-6-methoxyphenyl)hydrazine (**3c**) with 2 mL (20 mmol) of phenylhydrazine in 50 mL of Dowtherm A at 250 °C for 30 min afforded **13**. Recrystallization of **13** from ethanol afforded yellow crystals (1.4 g, 48%): mp 322–325 °C; ^{1}H NMR (DMSO-d_6) δ 12.9 (br, NH, D_2O exchangeable), 8.7 (d, 1 H, J = 5.3 Hz), 8.27 (d, 2 H, J = 8.0 Hz), 7.7 (d, 1 H, J = 9.1 Hz), 7.6 (s, 1 H), 7.45 (t, 2 H, J = 7.8 Hz), 7.25 (d, 1 H, J = 9.1 Hz), 7.2 (t, 1 H, J = 7.1 Hz), 3.95 (s, 3 H); IR (KBr) 3445.6, 3064.7, 2923.9, 2849.6, 1632.6, 1439.8, 1239.2, 757.8 cm^{-1}. Anal. ($C_{17}H_{13}N_3O_2$) C, H, N.

7-Methoxy-2-phenylpyrazolo[4,3-*c*]quinolin-3-one (14). A mixture of 2.0 g (7 mmol) of 4-chloro-7-methoxy-3-carbethoxyquinoline (**3d**) and 1 mL (10 mmol) of phenylhydrazine was heated under reflux in 50 mL of xylene giving only the uncyclized intermediate. Compound **14** was obtained by reheating the intermediate in 60 mL of Dowtherm A at 240 °C for 30 min. Purification by crystallization afforded 1.6 g (76%) of **14** as yellow crystals: mp 297–299 °C; ^{1}H NMR (DMSO-d_6) δ 12.75 (br, NH, D_2O exchangeable), 8.7 (d, 1 H, J = 6.0 Hz), 8.25 (d, 2 H, J = 8.6 Hz), 8.15 (d, 1 H, J = 9.6 Hz), 7.45 (t, 2 H, J = 7.7 Hz), 7.2 (m, 3 H), 3.8 (s, 3 H); IR (KBr) 3340.5, 3117.7, 2963.4, 1630.7, 1469.7, 1250.8, 1032.8, 865.9, 766.6 cm^{-1}. Anal. ($C_{17}H_{13}N_3O_2$) C, H, N.

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