Heterocyclic Analogues of Quinone Methide: Preparation and Cytotoxicity of 3-Oxo-3*H*-pyrazolo[1,5-*a*]indole Derivatives

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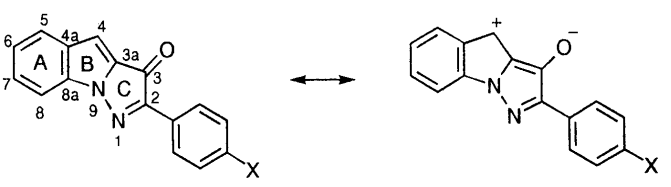
A series of 3-oxo-3*H*-pyrazolo[1,5-*a*]indole derivatives was prepared and characterized as heterocyclic analogues of quinone methide. These compounds showed some cytotoxicity to cancer cells, but were ineffective in an *in vivo* test against murine leukemia L1210.

Key words quinone methide heterocyclic analogue; preparation; UV-VIS spectrum; NMR spectrum; cytotoxicity; 3-oxo-3*H*-pyrazolo[1,5-*a*]indole derivative

Quinone methide is a chemical unit which is derived from either *o*- or *p*-benzoquinone by replacing one carbonyl group with a methylene unit.[1] The importance of this unit in bioreductive alkylating agents is well recognized,[2] and attention has recently been focused on the interaction of this type of intermediate with DNA.[3] We have reported the preparation and reactions of 3*H*-pyrazolo[1,5-*a*]indole derivatives.[4] We also prepared **1a** (X=H), which is of interest because of its electrophilic character as expressed by the resonance form **1′** (Chart 1). The skeletal unit **A** involved in the structure **1** constitutes a heterocyclic analogue of *o*-quinone methide (bold line). Replacement of N-1 with a carbon atom in **A** gives the electronically equivalent 1*H*-pyrrolo[1,2-*a*]indol-1-one **B**,[5] which is an isoelectronic analogue of the quinone methide, fluoren-1-one **C** (Chart 1). The structural unit **B** is a constituent of the widely used anticancer agent, mitomycin C.[6] Here we present the characterization and cytotoxic activity of a series of heterocyclic analogues of quinone methide, **1a—d**, **7** and **9**.

Previously we have reported two pathways for the preparation of **1**.[4] The compounds **1b—d** were prepared from indoline-2-carboxylic acid **2** *via* the hydrazone **3**, by the pathway reported for **1a** (Chart 2).[4b] Slight modification was required for the preparation of **4d**, since the hydrazone **3d** was not available by this method. Thus, the nitro group of **4b** was reduced catalytically and the resulting labile amine **4e** was methylated with a combination of formalin and sodium cyanoborohydride in acetic acid[7] to give the dimethylamino product **4d** in 60% yield. Several other problems arose during the preparation of **1b—d**. 1) Recrystallization of the acid **3c** from ethanol resulted in partial decomposition. An attempt to trap a possible intermediate (azomethine imine) with diethyl acetylenedicarboxylate failed. 2) The phenolic product **5d** was readily susceptible to air oxidation, so basic hydrolysis of **4d** leading to **5d** was always accompanied with formation of **1d**. Oxidation of **5d** was readily carried out with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), but the high susceptibility of **5d** to air oxidation allowed the oxidation of **5d** to **1d** by the weaker oxidant chloranil in good yield. Chloranil, an inexpensive oxidant was not effective in the oxidation of **5a** to **1a**. The preparations of **7** and **9** were carried out by starting with the alcohol **6**[4a] (Chart 2). DDQ oxidation of **6** afforded **7**. Hydrogenation of **7** resulted in the reduction of the quinone-methide unit and saturation of the styrene-type double bond to give the phenolic product **8**. Oxidation of **8** again with DDQ gave the desired product **9**.

IR and NMR data for the selected heterocyclic analogues of quinone methide are summarized in Table 1. The effect of C-2 substituents was not marked in the IR spectra (KBr) and the carbonyl absorption band appeared in the range of 1687—1695 (Δ 8 cm^{-1}). In the ^{13}C-NMR spectra ($CDCl_3$) the effects of C-2 substituents were reflected in the chemical shifts of C-3 and C-4, and those of C-3 were observed in the range of 179.5—182.4 ppm (Δ 2.9 ppm). In this range, the highest chemical shift was observed with **1b**, which has a *p*-nitrophenyl group (X=NO_2), and the lowest one with **1d** having a *p*-dimethylaminophenyl group (X=NMe_2). This difference can be explained in terms of the resonance contribution of **1′** (Chart 1). The nitro group (X=NO_2) of **1b** in the resonance form **1′** withdraws electrons from the electron-rich pyrazole ring C by inductive and resonance effects and increases the participation of the resonance form **1′**. The increase of importance of **1′** in



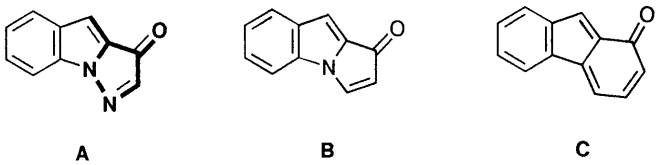


Chart 1

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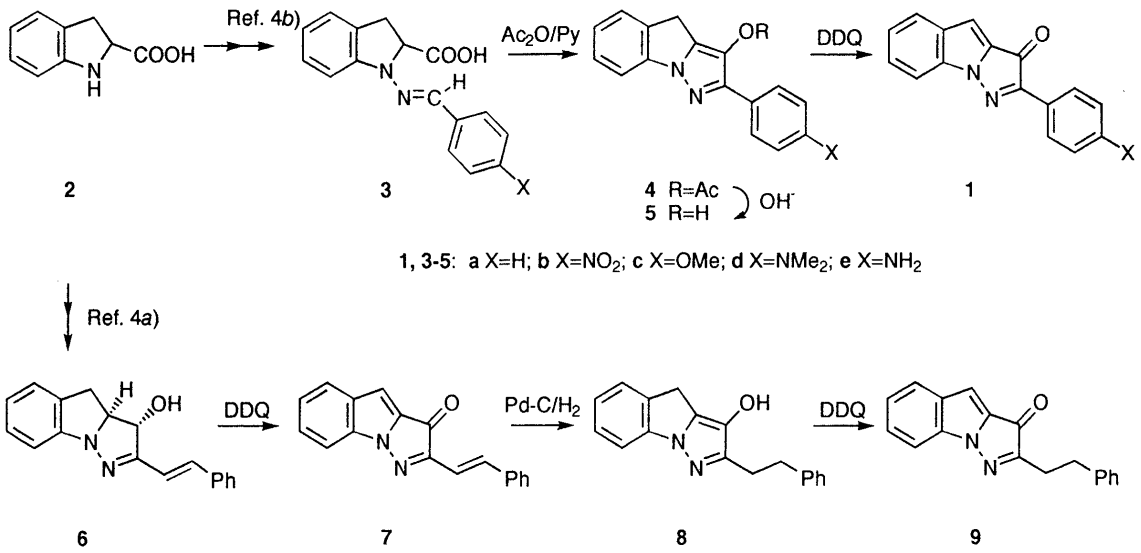

Chart 2

Table 1. IR and NMR Spectral Values of Selected Heterocyclic Analogues of Quinone Methide

Ent.	Compd.	C=O[a]	4-H[b]	C-3[c]	C-4[c]
1	**1a**[d]	1688	7.19	180.7	111.0
2	**1b**	1693	7.27	179.5	113.1
3	**1c**	1688	7.17	181.3	110.3
4	**1d**	1694	7.12	182.4	109.0
5	**7**	1695	7.13	180.7	110.7
6	**9**	1687	7.10	180.7	110.6

a) IR absorption band for C-3 carbonyl group (cm^{-1}). *b*) Chemical shifts (δ in ppm) of these protons in ^{1}H-NMR spectra. *c*) Chemical shifts (δ in ppm) of these carbons in ^{13}C-NMR spectra. *d*) Spectral data for **1a** were taken from the literature.[4a]

Table 2. UV-VIS Data for Selected Heterocyclic Analogues of Quinone Methide[a]

Ent.	Compd.	λ_{max} nm (log ε)	λ_{max} nm (log ε) (+HCl)[b]
1	**1a**[c]	293 (4.22), 392 (4.17), 508 (3.64)	294 (4.20), 392 (4.09), 509 (3.56)
2	**1b**	319 (4.20), 402 (4.16), 515 (3.81)	283 (3.95), 369 (4.23), 511 (3.48)
3	**1c**	250 (4.26), 291 (4.19), 390 (4.18), 523 (3.72)	250 (4.24), 291 (4.18), 391 (4.14), 522 (3.69)
4	**1d**	305 (4.35), 388 (4.22), 568 (3.46)	301 (4.31), 399 (4.03), 510 (3.52)
5	**7**	258 (4.13), 312 (4.48), 325 (4.50), 398 (3.86), 528 (3.83)	257 (4.16), 315 (4.16), 326 (4.18), 392 (3.81), 529 (3.01)

a) Solvent: MeCN. *b*) A drop of aqueous 2 M HCl solution was added to the solution (2 ml). *c*) Spectral data for **1a** were taken from the literature.[4a]

the resonance would cause the chemical shift of C-3 to move to higher magnetic field. On the other hand, the dimethylamino group (X = NMe_2) in **1d** donates electrons to the already electron-rich ring C and destabilizes the resonance form **1′**, thus decreasing the participation of **1′** in the resonance. This argument is supported by the fact that the C-3 signal (δ 128.5 ppm) of the phenolic compound **8** was detected at higher magnetic field than that of the ketonic compound **9** (δ 180.7 ppm). Similar stabilization and destabilization effects on the C ring of **1′** by 2-substituents were also reflected in the chemical shifts of 4-H and C-4. An electron-attracting group (X = NO_2) on the C-2 substituent might stabilize the resonance form **1′**, and increase the positive charge at C-4, thus shifting the 4-H and C-4 signals to lower magnetic field. In fact, 4-H of **1b** (X = NO_2) appeared at lowest magnetic field (δ 7.27) and that of **1d** (X = NMe_2) at the highest (δ 7.12). Similarly the lowest and the highest signals for C-4 were assigned in the range between 109.0 and 113.1 (Δ 4.0 ppm), and could be explained similarly. In a series of compounds **1** the remote substituent effect was detected through eight consecutive carbon–carbon bonds.

UV-VIS data are summarized in Table 2. Introduction of substituents (X) at the C-2 phenyl ring of **1a** caused a bathochromic shift in the absorption band at the visible region. Acid addition resulted in no remarkable shift of the maximum absorption band except **1d**. The hypsochromic shift (−58 nm) in the visible region of **1d** on addition of acid is due to protonation at the dimethylamino group. When the B-band absorptions are compared (**1a** 392, **1b** 402, **1c** 390, **1d** 388, **7** 398 nm), the absorption band of **1b** was at the longest wavelength (402 nm) and that of **1d** at the shortest (388 nm). This difference can again be explained in terms of the participation of the resonance form **1′**, as in the case of the NMR spectra. Stabilization of **1′** increases the contribution of **1′** in the resonance of **1**, and shifts the B-band absorption to the longer wavelength region.

Cytotoxicity of the heterocyclic analogues of quinone methides was examined by using cells of murine leukemia (L1210), uterus cervical (HeLa), lung (PC-1), gastric (KATOIII), colon (LoVo), and breast (MCF-7) cancers and expressed in terms of inhibitory activity (IC_{50}: μg/ml) obtained by the MTT method.[8] The results are summarized in Table 3. The acetate **4d** was tested as a prodrug for **1d**, since its hydrolysis gives **5d**, which is readily oxidized to the quinone methide **1d**. However, **4d** did not behave as expected, showing only weak activity compared with **1d**. Among compounds **1**, the order of cytotoxicity

Table 3. Cytotoxicities of Heterocyclic Analogues of Quinone Methide toward Cancer Cells

Compd.[a]	IC_{50} (μg/ml)[b]					
	L1210	HeLa	PC-1	KATOIII	LoVo	MCF-7
MMC	<0.005	0.011	<0.005	0.319	0.099	0.005
1a	0.20	0.42	0.30	0.17	0.41	0.24
1b	0.55	3.85	3.91	2.47	1.51	>5.0
1c	0.41	0.62	0.75	2.15	2.29	0.57
1d	0.28	1.06	2.24	0.96	0.78	1.08
4d	1.11	6.01	4.65	5.94	5.21	3.83
7	0.78	1.93	3.27	2.15	2.19	2.43
9	0.60	1.76	2.27	1.55	1.45	2.59

a) MMC: mitomycin C. *b*) Activities against cancer cells (L1210, murine leukemia; HeLa, uterus cervical; PC-1, lung; KATOIII, gastric; LoVo, colon; MCF-7, breast) were measured by MTT assay after 3 d of incubation.

Table 4. Antitumor Activities of Heterocyclic Analogues of Quinone Methide against Murine Leukemia L1210

Compd. (i.p.)	Dose[a] (mg/kg)	*MST*[b] (Day)	*ILS*[b] (%)	30-day survivors	Body wt. (g) D_5—D_1[c]
Control		7.2±0.4		0/5	2.0
MMC	5×1	>30.0±0.0	316.7	5/5	0.8
1a	200×1	7.2±0.2	0	0/5	1.4
	100×1	7.6±0.6	5.5	0/5	0.8
1b	200×1	6.8±0.2	−5.5	0/5	2.4
1c	200×1	7.6±0.4	5.5	0/5	1.4
Control		7.2±0.4		0/5	—
1d	100×1	9.0±0.4		0/5	—

a) L1210 cells (1×10^5) were inoculated intraperitoneally (i.p.) into BDF1 male mice (6 weeks). *b*) Survival number was monitored daily and the increase in life span (*ILS*, %) was calculated from [(mean survival time of treated group)/(mean survival time of control group)−1]×100. *c*) Body weight increase (g) at the 5th day from the start. Bar denotes no measurement.

was **1a**>**1d**>**1c**>**1b** and this result suggests that the electronic state of the C-2 aromatic ring has little effect on the cytotoxicity of this series of compounds. Compound **1a** was the most active among the listed compounds, but its activity is still only about one-tenth as strong as that of the reference compound, mitomycin C, except against gastric cancer cells KATOIII. As a selective agent against gastric cancer, the activity of **1a** would need to be improved more than ten-fold. The *in vivo* antitumor activity of compounds **1** was investigated with murine leukemia L1210 and the results are listed in Table 4. The drugs were inoculated intraperitoneally (i.p.) and the maximum doses shown were decided by considering acute toxicity. The effect was evaluated in terms of increase of life span (*ILS*%). The compounds tested were all ineffective compared with the reference compound, mitomycin C. No survivor after 30 d was observed. In conclusion, these new hetero analogues of quinone methide **1** showed cytotoxicity against cancer cell lines, being a little less potent than mitomycin C, but they did not show effective antitumor activity *in vivo*. As the quinone methide structure is chemically quite reactive to nucleophiles, a quinone methide drug may react too readily with other nucleophiles, such as protein, before reaching cancer cells in the body. In this context, the design of a prodrug, which can afford a reactive quinone methide after biological transformation, as in the case of mitomycin C and anthracyclines,[2] may be a useful approach to the development of heterocyclic analogues of the quinone methide **1**. We are exploring this idea.

Experimental[9]

The following compounds were prepared by the method described for **1a**.[4b]

1-(4-Nitrobenzylideneamino)indoline-2-carboxylic Acid (3b) 52%. Yellow-red needles, dec. 188—190 °C (EtOH). MS *m/z*: 311 (M^+, 1.3%), 265 (100), 219 (13), 218 (16), 116 (39). IR (KBr): 3200—2800 (br), 1709 (COOH), 1613, 1336 (NO_2), 1229, 1246, 1222, 1192, 1108, 886, 747 cm^{-1}. ^{1}H-NMR dimethyl sulfoxide (DMSO)-d_6) δ: 3.24 (1H, dd, *J*=16.6, 3.9 Hz, 3-H), 3.69 (1H, dd, *J*=16.6, 11.2 Hz, 3-H), 5.08(1H, dd, *J*=11.2, 3.9 Hz, 2-H), 6.89 (1H, m, 5-H), 7.21 (3H, m, 4, 6, 7-H), 7.48 (1H, s, N=CH), 7.90 (2H, m, 2′, 6′-H), 8.22 (2H, m, 3′, 5′-H). ^{13}C-NMR (DMSO-d_6) δ: 32.5 (C-3), 60.3 (C-2), 108.7 (C-7), 121.3 (C-5), 124.0 (C-3′, 5′), 124.9 (C-4), 126.0 (C-2′, 6′), 126.1 (C-3a), 127.9 (C-6), 130.3 (C=N), 142.5 (C-1′), 145.9 (C-8a), 146.5 (C-4′), 171.1 (COOH).

1-(4-Methoxybenzylideneamino)indoline-2-carboxylic Acid (3c) 44%. MS *m/z*: 296 (M^+, 2.1%), 252 (100), 250 (72), 235 (9), 207 (6), 134 (42), 118 (53), 117 (41), 91 (15). IR (KBr): 3100—2800 (br), 1718 (COOH), 1608, 1482, 1254, 1168, 1023, 883, 828, 750 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 3.16 (1H, dd, *J*=16.6, 4.2 Hz, 3-H), 3.63 (1H, dd, *J*=16.6, 11.2 Hz, 3-H), 3.78 (3H, s, OMe), 4.92 (1H, dd, *J*=11.2, 4.2 Hz, 2-H), 6.78 (1H, td, *J*=7.2, 1.2 Hz, 5-H), 6.96 (2H, m, 3′-, 5′-H), 7.05—7.19 (6H, m, Ar-H), 7.34 (1H, s, N=CH), 7.60 (2H, m, 2′, 6′-H). ^{13}C-NMR (DMSO-d_6) δ: 32.6 (C-3), 55.1 (OMe), 60.5 (C-2), 108.1 (C-7), 114.1 (C-3′, 5′), 119.8 (C-5), 124.5 (C-4), 125.2 (C-3a), 127.0 (C-2′, 6′), 127.7 (C-6), 128.5 (C-1′), 133.2 (C=N), 147.7 (C-7a), 159.2 (C-4′), 172.0 (COOH).

3-Acetoxy-2-(4-nitrophenyl)-4*H*-pyrazolo[1,5-*a*]indole (4b) 88%. Yellow needles, mp 208 °C (acetone). UV (MeOH) λ_{max} nm (log ε): 261 (4.15), 344 (4.32); (MeOH+NaOH): 307 (4.21), 430 (4.14). MS *m/z*: 335 (M^+, 2%), 293 (15), 145 (4), 144 (3), 117 (12), 116 (6), 70 (12), 61 (12), 43 (100). IR (KBr): 1752 (C=O), 1599, 1341 (NO_2), 1260, 1240, 855, 756, 701 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.41 (3H, s, $COCH_3$), 4.05 (2H, s, 4-H_2), 7.25 (1H, td, *J*=7.4, 1.1 Hz, 6-H), 7.43 (1H, t, *J*=7.7 Hz, 7-H), 7.47 (1H, d, *J*=7.4 Hz, 5-H), 7.70 (1H, d, *J*=7.7 Hz, 8-H), 8.14 (2H, m, 2′ 6′-H), 8.29 (2H, m, 3′, 5′-H). ^{13}C-NMR ($CDCl_3$) δ: 20.9 ($\underline{C}OCH_3$), 30.8 (C-4), 110.8 (C-8), 123.9 (C-3′, 5′), 125.3 (C-6), 125.9 (C-5), 127.0 (C-2′, 6′), 128.2 (C-7), 128.9 (C-3), 133.3 (C-4a), 135.7 (C-3a), 138.8 (C-1′), 140.1 (C-8a), 142.3 (C-2), 146.9 (C-4′), 166.9 (C=O). *Anal.* Calcd for $C_{18}H_{13}N_3O_4$ (M.W. 335.32): C, 64.48; H, 3.91; N, 12.53. Found: C, 64.64; H, 3.82; N, 12.43.

3-Acetoxy-2-(4-methoxyphenyl)-4*H*-pyrazolo[1,5-*a*]indole (4c) 52%. mp 134.0—134.5 °C (EtOAc–pentane). UV (MeOH) λ_{max} nm (log ε): 297 (4.50); (MeOH+NaOH): 267 (4.21), 342 (4.39). MS *m/z*: 320 (M^+, 72.9), 278 (100), 263 (4.7), 144 (8.2), 134 (3.6), 117 (19.8), 116 (18.9). IR (KBr): 1750, 1626, 1610, 1248, 1221, 1179, 1031, 743 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.35 (3H, s, $COCH_3$), 3.85 (3H, s, OCH_3), 3.99 (2H, s, 4-H_2), 6.98(2H, m, 3′,5′-H), 7.18 (1H, td, *J*=7.6, 0.7 Hz, 6-H), 7.39 (1H, t, *J*=7.7 Hz, 7-H), 7.43 (1H, d, *J*=7.6 Hz, 5-H), 7.66 (1H, d, *J*=7.7 Hz, 8-H), 7.86 (2H, m, 2′,6′-H). ^{13}C-NMR ($CDCl_3$) δ: 20.9 ($\underline{C}OCH_3$), 30.6 (C-4), 55.3 (OCH_3), 110.3 (C-8), 114.0 (C-3′, 5′), 124.4 (C-6), 125.0 (C-1′), 125.7 (C-5), 127.8 (C-3), 128.0 (C-7), 128.2 (C-2′, 6′), 133.0 (C-4a), 135.3 (C-3a), 140.6 (C-8a), 145.1 (C-2), 159.5 (C-4′), 167.4 (C=O). *Anal.* Calcd for $C_{19}H_{16}N_2O_3$ (M.W. 320.35): C, 71.24; H, 5.04; N, 8.74. Found: C, 71.02; H, 5.05; N, 8.63.

3-Acetoxy-2-(4-dimethylaminophenyl)-4*H*-pyrazolo[1,5-*a*]indole (4d) A solution of **4b** in ethyl acetate was catalytically reduced over 5% Pd–C under a hydrogen atmosphere at room temperature for 3 d. Filtration of the solution through a Celite pad and evaporation of the filtrate gave the amine **4e** in quantitative yield.

3-Acetoxy-2-(4-aminophenyl)-4*H*-pyrazolo[1,5-*a*]indole (4e) ^{1}H-NMR ($CDCl_3$) δ: 2.34 (3H, s, $COCH_3$), 3.27 (2H, br, NH_2), 3.98 (2H, s, 4-H_2), 6.74 (2H, m, 3′, 5′-H), 7.16 (1H, td, *J*=7.4, 1.1 Hz, 6-H), 7.38 (1H, t, *J*=7.8 Hz, 7-H), 7.42 (1H, d, *J*=7.4 Hz, 5-H), 7.65 (1H, d, *J*=7.8 Hz, 8-H), 7.73 (2H, m, 2′, 6′-H). ^{13}C-NMR ($CDCl_3$) δ: 20.9 ($\underline{C}OCH_3$), 30.5 (C-4), 110.2 (C-8), 115.1 (C-3′, 5′), 122.7 (C-1′), 124.2 (C-6), 125.6 (C-5), 127.6 (C-3), 127.9 (C-7), 128.1 (C-2′, 6′), 133.0 (C-4a), 135.2 (C-3a), 140.7 (C-8a), 145.6 (C-2), 146.4 (C-4′), 167.5 (C=O).

The labile amine **4e** (0.97 g, 3.2 mmol) thus obtained was stirred for 1 h with aqueous 37% formaldehyde (2.42 ml, 30 mmol) and $NaBH_3CN$

(0.56 g, 9 mmol) in acetonitrile (30 ml), and reaction was initiated by the gradual addition of acetic acid. When the solution remained acid, it was kept overnight. Aqueous sodium carbonate and ether were introduced, and the organic layer was collected. The aqueous layer was extracted with ether. The combined extracts gave, after usual work-up, the crude product (1.0 g), which was purified by flash column chromatography (silica gel 45 g, EtOAc–PE, 2:8) to give **4d** (0.53 g, 60%), mp 137.5—138.0 °C (hexane–EtOAc, 1:1). UV-VIS (MeOH) λ_{max} nm (log ε): 314 (4.54); (MeOH + NaOH): 280 (4.11), 353 (4.54). MS *m/z*: 333 (M^+, 96.9%), 291 (100), 147 (23.0), 146 (17.1), 145 (31.2), 144 (36.0), 121 (10.7), 117 (44.6). IR (KBr): 1761, 1616, 1216, 1185, 895, 823, 752 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.35(3H, s, $COCH_3$), 3.00 (6H, s, NMe_2), 3.98 (2H, s, 4-H_2), 6.79 (2H, m, 3′, 5′-H), 7.16 (1H, td, J=7.4, 1.1 Hz, 6-H), 7.38 (1H, t, J=7.7 Hz, 7-H), 7.42 (1H, d, J=7.4 Hz, 5-H), 7.66 (1H, d, J=7.7 Hz, 8-H), 7.82 (2H, m, 2′, 6′-H). ^{13}C-NMR ($CDCl_3$) δ: 20.9 ($CO\underline{C}H_3$), 30.5 (C-4), 40.4 (NMe_2), 110.2 (C-8), 112.3 (C-3′, 5′), 120.4 (C-1′), 124.1 (C-6), 125.6 (C-5), 127.6 (C-3), 127.8 (C-2′, 6′), 127.9 (C-7), 133.0 (C-4a), 135.2 (C-3a), 140.7 (C-8a), 145.8 (C-2), 153.2 (C-4′), 167.5 (C=O). *Anal.* Calcd for $C_{20}H_{19}N_3O_2$ (M.W. 333.39): C, 72.05; H, 5.74; N, 12.60. Found: C, 72.11; H, 5.70; N, 12.63.

3-Hydroxy-2-(4-nitrophenyl)-4*H*-pyrazolo[1,5-*a*]indole (5b) 93%. Yellow rods, dec. 215—220 °C (acetone). UV-VIS (MeOH) λ_{max} nm (log ε): 279 (4.21), 371 (4.34); (MeOH + NaOH): 309 (4.22), 425 (4.14), 439 (4.17). MS *m/z*: 293 (M^+, 21%), 291 (42), 261 (7), 143 (100), 115 (66), 88 (7). IR (KBr): 3574, 3120 (br), 1626, 1251, 857, 748, 702 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 4.00 (2H, s, 4-H_2), 7.26 (1H, td, J=7.5, 0.9 Hz, 6-H), 7.45 (1H, t, J=7.5 Hz, 7-H), 7.61 (2H, m, 5, 8-H), 8.30 (4H, s, 2′, 3′, 5′, 6′-H), 9.88 (1H, s, OH). ^{13}C-NMR (DMSO-d_6) δ: 27.9 (C-4), 109.7 (C-8), 123.8 (C-3′, 5′), 124.7 (C-6), 125.7 (C-2′, 6′), 126.5 (C-5), 127.9 (C-7), 129.9 (C-3), 133.8 (C-4a), 136.2 (C-3a), 139.5 (C-1′), 139.7 (C-8a), 140.1 (C-2), 145.4 (C-4′). *Anal.* Calcd for $C_{16}H_{11}N_3O_3$ (M.W. 293.29): C, 65.53; H, 3.78; N, 14.33. Found: C, 65.64; H, 3.74; N, 14.33.

3-Hydroxy-2-(4-methoxyphenyl)-4*H*-pyrazolo[1,5-*a*]indole (5c) 95%. Pale red crystals, mp 197.5—200.5 °C (EtOH). UV (MeOH) λ_{max} nm (log ε): 318 (4.49). MS *m/z*: 278 (M^+, 100), 277 (14.4), 276 (67.1), 263 (6.4), 143 (55.2), 133 (8.9), 117 (26.9), 115 (35.4). IR (KBr): 3058 (br, OH), 1623, 1289, 1246, 1176, 1033, 833, 748 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 3.79 (3H, s, OMe), 3.94 (2H, s, 4-H), 6.99 (2H, m, 3′, 5′-H), 7.18 (1H, td, J=7.5, 0.8 Hz, 6-H), 7.39 (1H, t, J=7.7 Hz, 7-H), 7.53 (2H, m, 5, 8-H), 7.98 (2H, m, 2′, 6′-H), 9.23 (1H, s, OH). ^{13}C-NMR (DMSO-d_6) δ: 27.7 (C-4), 55.0 (OMe), 109.0 (C-8), 113.7 (C-3′, 5′), 123.5 (C-6), 126.1 (C-1′), 126.3 (C-5), 126.9 (C-2′, 6′), 127.8 (C-7), 129.6 (C-3), 133.3 (C-4a), 134.1 (C-3a), 140.0 (C-8a), 142.4 (C-2), 158.2 (C-4′). *Anal.* Calcd for $C_{17}H_{14}N_2O_2$ (M.W. 278.31): C, 73.37; H, 5.07; N, 10.06. Found: C, 73.45; H, 4.99; N, 10.15.

2-(4-Dimethylaminophenyl)-3-hydroxy-4*H*-pyrazolo[1,5-*a*]indole (5d) 98%. Yellow crystals, dec. 215.0—218.0 °C (EtOAc). IR: 2980 (br), 1616, 1497, 1477, 1360, 1307, 1246, 1198, 817, 742 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.93 (6H, s, NMe_2), 3.91 (2H, s, 4-H), 6.77 (2H, m, 3′, 5′-H), 7.15 (1H, td, J=7.3, 1.3 Hz, 6-H), 7.38 (1H, td, J=7.5, 1.0 Hz, 7-H), 7.48 (1H, d, J=7.5 Hz, 8-H), 7.53 (1H, d, J=7.3 Hz, 5-H), 7.88 (2H, m, 2′, 6′-H), 9.08 (1H, s, OH). ^{13}C-NMR (DMSO-d_6) δ: 27.7 (C-4), 40.0 (NMe_2), 108.8 (C-8), 112.0 (C-3′, 5′), 121.6 (C-1′), 123.2 (C-6), 126.2 (C-5), 126.6 (C-2′, 6′), 127.8 (C-7), 129.4 (C-3), 133.2 (C-4a), 133.9 (C-3a), 140.1 (C-8a), 143.3 (C-2), 149.3 (C-4′).

2-(4-Nitrophenyl)-3-oxo-3*H*-pyrazolo[1,5-*a*]indole (1b) 77%. Dark reddish brown needles, dec. >170 °C (obscure). MS *m/z*: 291 (M^+, 73%), 143 (100), 115 (66), 88 (8). IR (KBr): 1693 (C=O), 1343 (NO_2), 1299, 1168, 863, 806, 752 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 7.16 (1H, td, J=8.1, 1.7 Hz, 6-H), 7.27 (1H, d, J=0.7 Hz, 4-H), 7.51 (2H, m, 7, 8-H), 7.64 (1H, dt, J=8.1, 0.9 Hz, 5-H), 8.30 (2H, m, 3′, 5′-H), 8.44 (2H, m, 2′, 6′-H). ^{13}C-NMR ($CDCl_3$) δ: 110.2 (C-8), 113.1 (C-4), 123.3 (C-6), 123.9 (C-3′, 5′), 126.1 (C-5), 128.0 (C-2′, 6′), 128.7 (C-3a), 130.8 (C-7, 4a), 134.5 (C-1′), 134.8 (C-8a), 148.6 (C-2), 150.5 (C-4′), 179.5 (C-3). *Anal.* Calcd for $C_{16}H_9N_3O_3$ (M.W. 291.27): C, 65.98; H, 3.11; N, 14.43. Found: C, 66.32; H, 3.01; N, 14.60.

2-(4-Methoxyphenyl)-3-oxo-3*H*-pyrazolo[1,5-*a*]indole (1c) 98%. Dark red crystals, dec. 178—183 °C (EtOAc, sublimed at 167 °C). MS *m/z*: 276 (M^+, 73%), 143 (100), 115 (50), 88 (6). IR (KBr): 1688 (C=O), 1603 (C=N), 1353, 1307, 1287, 1254, 1161, 823, 750 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 3.87 (3H, s, OMe), 6.98 (2H, m, 3′, 5′-H), 7.09 (1H, td, J=8.1, 1.0 Hz, 6-H), 7.17 (1H, s, 4-H), 7.43 (1H, t, J=8.1 Hz, 7-H), 7.53 (1H, d, J=8.1 Hz, 8-H), 7.61 (1H, d, J=8.1 Hz, 5-H), 8.21 (2H, m, 2′, 6′-H). ^{13}C-NMR ($CDCl_3$) δ: 55.4 (OMe), 109.9 (C-8), 110.3 (C-4), 114.3 (C-3′, 5′), 121.0 (C-1′), 122.2 (C-6), 125.6 (C-5), 128.3 (C-3a), 129.3 (C-2′, 6′), 129.8 (C-7), 130.4 (C-4a), 134.5 (C-8a), 152.6 (C-2), 161.8 (C-4′), 181.3 (C-3). *Anal.* Calcd for $C_{17}H_{12}N_2O_2$ (M.W. 276.3): C, 73.90; H, 4.38; N, 10.14. Found: C, 74.29; H, 4.54; N, 9.99.

2-(4-Dimethylaminophenyl)-3-oxo-3*H*-pyrazolo[1,5-*a*]indole (1d) 97%. Black needles, dec. 179—180 °C (EtOAc–pentane, sublimed at 185 °C). MS *m/z*: 289 (M^+, 68%), 146 (85), 143 (69), 129 (9), 115 (53), 86 (38), 84 (67), 71 (31), 69 (31), 57 (59), 55 (38), 51 (31), 49 (88), 43 (100). IR (KBr): 1694, 1606, 1370, 1307, 1227, 1158, 810, 744 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 3.05 (6H, s, NMe_2), 6.75 (2H, m, 3′, 5′-H), 7.05 (1H, td, J=8.1, 1.1 Hz, 6-H), 7.12 (1H, d, J=0.9 Hz, 4-H), 7.40 (1H, td, J=8.1, 1.1 Hz, 7-H), 7.53 (1H, dd, J=8.1, 0.9 Hz, 8-H), 7.60 (1H, d, J=8.1 Hz, 5-H), 8.17 (2H, m, 2′, 6′-H). ^{13}C-NMR ($CDCl_3$) δ: 40.0 (NMe_2), 109.0 (C-4), 109.9 (C-8), 111.8 (C-3′, 5′), 115.7 (C-1′), 121.7 (C-6), 125.5 (C-5), 128.2 (C-3a), 129.0 (C-2′, 6′), 129.3 (C-7), 130.3 (C-4a), 134.2 (C-8a), 151.9 (C-4′), 153.0 (C-2), 182.4 (C-3). *Anal.* Calcd for $C_{18}H_{15}N_3O$ (M.W. 289.34): C, 74.72; H, 5.23; N, 14.52. Found: C, 74.50; H, 5.13; N, 14.31. A less expensive oxidant, chloranil was also applied to the oxidation of **5d**, though it was not effective in the oxidation of **5a**. A mixture of **5d** (408 mg, 1.40 mmol) and chloranil (738 mg, 3.00 mmol) in dry tetrahydrofuran (THF) (100 ml) was stirred at room temperature overnight. The solution was evaporated, the residue was dissolved in ethyl acetate, and the organic solution was washed with aqueous 1 M NaOH solution twice, water twice and saturated brine twice. The crude product (370 mg) thus obtained was purified by chromatography (silica gel, hexane–ethyl acetate 5:1) to give **1d** (280 mg, 69%).

3-Oxo-2-*E*-styryl-3*H*-pyrazolo[1,5-*a*]indole (7) The alcohol **6** (1.751 g, 6.3 mmol)[4a] in dry THF was treated with DDQ (3.025 g, 13.3 mmol) under an argon atmosphere at room temperature for 2 h. After dilution with ether (100 ml), the solution was washed successively with 10% aqueous NaOH solution once, water three times and brine once. The crude product thus obtained was flash-chromatographed (silica gel, *n*-hexane–ethyl acetate, 6:1) to give **7** (1.222 g, 70.8%), mp 164.0—166.0 °C (CH_2Cl_2–*n*-hexane). MS *m/z*: 274 (M^++2, 17%), 273 (M^++1, 88), 144 (11), 143 (100), 115 (61), 114 (13). HRMS Calcd for $C_{18}H_{12}N_2O$: 272.0949. Found: 272.0972. IR ν_{max} cm^{-1}: 1695, 1618, 1156, 978, 967, 748, 736. ^{1}H-NMR ($CDCl_3$) δ: 6.98 (1H, d, J=16.6 Hz, 11-H), 7.07 (1H, t-like m, 6-H), 7.13 (1H, s, 4-H), 7.32—7.48 (5H, m, Ar-H), 7.52—7.60 (3H, m, Ar-H), 7.99 (1H, d, J=16.4 Hz, 10-H). ^{13}C-NMR ($CDCl_3$) δ: 109.9 (C-8), 110.7 (C-4), 115.8 (C-10), 122.4 (C-6), 125.7 (C-5), 127.3 (C-2′, 6′), 128.3 (C-3a), 128.8 (C-3′, 5′), 129.4 (C-7), 129.9 (C-4′), 130.6 (C-4a), 134.4 (C-8a), 136.3 (C-1′), 139.2 (C-11), 152.9 (C-2), 180.7 (C-3). *Anal.* Calcd for $C_{18}H_{12}N_2O$: C, 79.39; H, 4.44; N, 10.29. Found: C, 79.09; H, 4.30; N, 10.09.

3-Hydroxy-2-(2-phenylethyl)-4*H*-pyrazolo[1,5-*a*]indole (8) The ketone **7** (200 mg, 0.73 mmol) in ethyl acetate (100 ml) was hydrogenated over 5% Pd–C (30 mg) in a hydrogen atmosphere at room temperature for 10 h. The catalyst was removed, and the filtrate was evaporated. The crude product was purified by column chromatography (silica gel, dichloromethane–ethyl acetate, 65:35) to give **8** (115 mg, 56.9%), mp 180.5—182.0 °C (CH_2Cl_2–*n*-hexane). MS *m/z*: 276 (M^+, 21%), 275 (100), 185 (40), 183 (38), 143 (86), 115 (63), 91 (64). IR ν_{max} cm^{-1}: 1625, 1245, 1140, 1008, 744, 698. ^{1}H-NMR (DMSO-d_6) δ: 2.82—3.02 (4H, m, 10-H, 11-H), 3.85 (2H, s, 4-H), 7.80—7.52 (9H, m, Ar-H), 8.64 (1H, s, OH). ^{13}C-NMR (DMSO-d_6) δ: 27.3 (4), 27.5 (C-10), 34.2 (C-11), 108.6 (C-8), 123.0 (C-6), 125.7 (C-5 or C-4′), 126.2 (C-4′ or C-5), 127.7 (C-7), 128.1 (C-2′, 3′, 5′, 6′), 128.5 (C-3), 132.9 (C-4a), 134.0 (C-3a), 140.1 (C-8a), 141.7 (C-2), 145.4 (C-1′).

3-Oxo-2-(2-phenylethyl)-3*H*-pyrazolo[1,5-*a*]indole (9) Crude **8** obtained above (330 mg, 1.21 mmol) was oxidized with DDQ (270 mg, 1.21 mmol) in methanol (20 ml), and work-up as described above gave **9** (188 mg, 56% overall), mp 126.5—127.0 °C (isopropyl ether). MS *m/z*: 275 (100%), 274 (M^+, 6), 183 (39), 143 (82), 115 (60), 91 (52). HRMS Calcd for $C_{18}H_{14}N_2O$: 274.1105. Found: 274.1068. IR ν_{max} cm^{-1}: 1687, 1150, 749. ^{1}H-NMR ($CDCl_3$) δ: 2.84 (2H, t with small splits, J=8.1 Hz, $C\underline{H}_2$–CH_2), 3.05 (2H, t with small splits, J=8.1 Hz, CH_2–$C\underline{H}_2$), 7.06 (1H, t-like m, 6-H), 7.10 (1H, s, 4-H), 7.18—7.46 (7H, m, Ar-H), 7.57 (1H, d, J=8.1 Hz, 8-H). ^{13}C-NMR ($CDCl_3$) δ: 28.7 (C-10), 32.4 (C-11), 109.7 (C-8), 110.6 (C-4), 122.0 (C-6), 125.5 (C-5), 126.3 (C-4′), 127.6 (C-3a), 128.4 (C-2′, 6′), 128.5 (C-3′, 5′), 129.9 (C-7), 130.3 (C-4a), 134.5 (C-8a), 140.5 (C-1′), 158.9 (C-2), 180.7 (C-3). *Anal.* Calcd for $C_{18}H_{14}N_2O$: C, 78.81; H, 5.14; N, 10.21. Found: C, 78.92; H, 5.06; N, 10.05.

Cytotoxic Activities Cytotoxicity was measured by the microculture

tetrazolium assay as described.[8] Cell lines of human lung cancer (PC-1), breast cancer (MCF-7), colon cancer (LoVo), uterus cervical cancer (HeLa), gastric cancer (KATOIII), and murine leukemia (L1210) were maintained in RPMI 1640 (Nissui Pharmaceutical Co., Ltd., Tokyo) supplemented with 10% fetal bovine serum (Hyclone Laboratories, Inc. Utah, U.S.A.). Growth inhibition experiments were carried out in 96-well flat-bottomed microplates, and the amount of viable cells at the end of the incubation was determined using the MTT assay. Thus, tumor cells (L1210 5×10^3 cells/well, HeLa 2×10^3 cells/well, and other cells 3×10^3 cells/well) were incubated in RPMI-1640 supplemented with 10% fetal calf serum. Drugs were dissolved in DMSO (2 mg/ml), diluted with culture medium (20 μg/ml) and introduced to the cell culture by gradual dilution. The cells were exposed to drugs for 3 d at 37 °C in air containing 5% carbon dioxide.

MTT (10 μl, 5 mg/ml in phosphate-buffered saline) was added to each well. The plates were incubated for an additional 4 h, and the medium was removed. The formazan product was dissolved in DMSO (150 μl/well) and the absorbance was measured at 540 nm using a Microplate Reader (Molecular Devices Corporation, California, U.S.A.). Each data point on growth curves was the average of three wells. The 50% growth-inhibitory concentration (IC_{50}) was calculated by the probit method.

Antitumor Activity The assay for L1210 leukemia in mice was performed as specified in the standard NCI protocols.[10] L1210 cells (1×10^5) were inoculated intraperitoneally (i.p.) into BDF1 mice (male, 6 week old) on day 0 and the mice were divided into several groups (5 mice per group) on day 1. Test compounds were suspended in 0.5% CMC and given i.p. on day 1. Survival was monitored daily and the increase in life span (*ILS*) was calculated using the following formula: $ILS\ (\%) = [(\text{mean survival time of treated group})/(\text{mean survival time of control group}) - 1] \times 100$.

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References and Notes

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