Synthesis and Antitumor Activity of Tropolone Derivatives. 6.[1] Structure–Activity Relationships of Antitumor-Active Tropolone and 8-Hydroxyquinoline Derivatives

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The bis derivative **6** of 8-hydroxyquinoline, which, like tropolones, readily forms a chelate, was synthesized and found to have high potency (dose = 12.5 mg/kg, T/C % = 164) against leukemia P388 in mice approximately equivalent to that of the bistropolone **1b**. 8-Hydroxyquinoline analogues with broad structural variation were synthesized and their structure–activity relationships followed the same pattern as in the tropolone series. In addition, the bistropolones **1a–e** were tested for their ability to bind to tubulin and found to have no such property. The results of this study suggested that bistropolone and bis(8-hydroxyquinoline) derivatives must form a chelate with the metal necessary for the enzyme, such as ribonucleotide reductase, which catalyzes the DNA biosynthetic pathways.

Mono- and bistropolones **1** and **2**, synthesized from hinokitiol[2] in our laboratory, have been found to have antitumor activity (Chart I).[3] Although both derivatives exhibit nearly equal potency in inhibitory activity against the growth of KB cells, the activity of bistropolone **1** is about 100–200 times that of the monotropolones, such as **2** and **3**, in the survival test of P388 in mice.

An earlier study[4] on the molecular requirement for this activity showed that the tropolone moiety is essential. However, the reason why two tropolone rings in a molecule are necessary for producing potent activity in the in vivo system remained unknown. In order to gain insight into this problem, new types of tropolone derivatives, such as **4**, in which the one tropolone ring in **1b** is replaced by an aromatic ring, or heteroaromatic compounds that contain a hydroxyl and/or a carbonyl group as found in tropolones, have been synthesized.[5] Their structure–activity relationships showed that two pairs of an acidic hydroxyl and a proton accepting group situated in the neighboring position, which permits the formation of a chelate with a metal ion, contributed to enhanced activity.

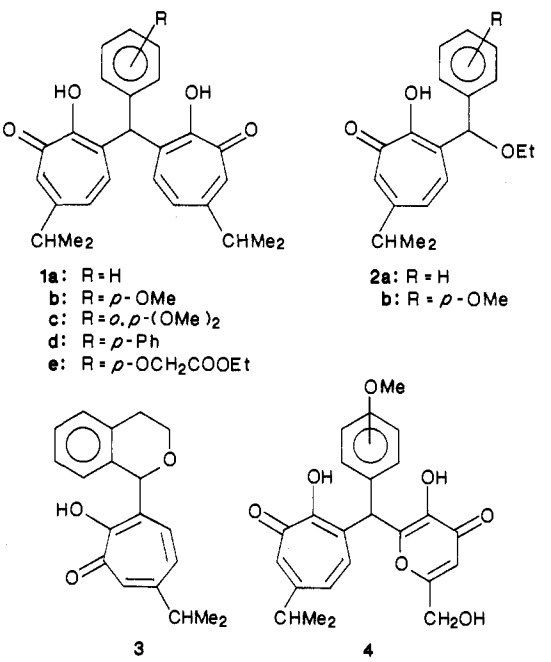
From these studies, the bis derivative **6** of 8-hydroxyquinoline, well known as a potent chelator, was designed, synthesized, and found to be almost as potent as bistropolone **1b** in the in vivo system.[6] In order to see if the structure–activity relationships for **6** followed the same pattern as in the tropolone series, the quinoline analogues listed in Table I were synthesized.

Chemistry

Compound **6** was prepared by heating of 2 equiv of 8-hydroxyquinoline with 4-anisaldehyde diethyl acetal (**5**) in refluxing *p*-cymene in 26% yield. In the establishment of its structure, the substituted position was found to be position 7 and not position 5 of the quinoline ring from the following facts: Compound **6** was first brominated according to the method of Pearson et al.[7] The resulting bromo derivative **9** was converted to the acetate **10**, because **9** is only slightly soluble in all NMR solvents. The acetate **10** was found to be identical with an alternative sample prepared by the reaction of 5-bromo-8-hydroxyquinoline[8] with **5** followed by acetylation by comparison of IR and ^{1}H NMR spectra and melting points (Scheme I).

Thiophene and furan analogues (**7** and **8**) were synthesized in a way similar to the preparation for **6**, but their yields were very poor.

Chart I



Heating of 8-hydroxyquinoline with 4 equiv of **5** in refluxing xylene gave the mono(8-hydroxyquinoline) **11** in 20% yield (Scheme II). Compound **12** was prepared by heating **11** with kojic acid (38% yield). The reaction of 1-ethoxyisochroman with 8-hydroxyquinoline in refluxing *p*-cymene gave the isochromanyl (8-hydroxyquinoline) **13** in 42% yield.

In order to confirm that the ability to form a chelate of **6** was closely correlated with the antitumor activity, mono- and bis(6-hydroxyquinoline) derivatives (**14** and **15**) were synthesized (Scheme III). Treatment of 2 equiv of 6-

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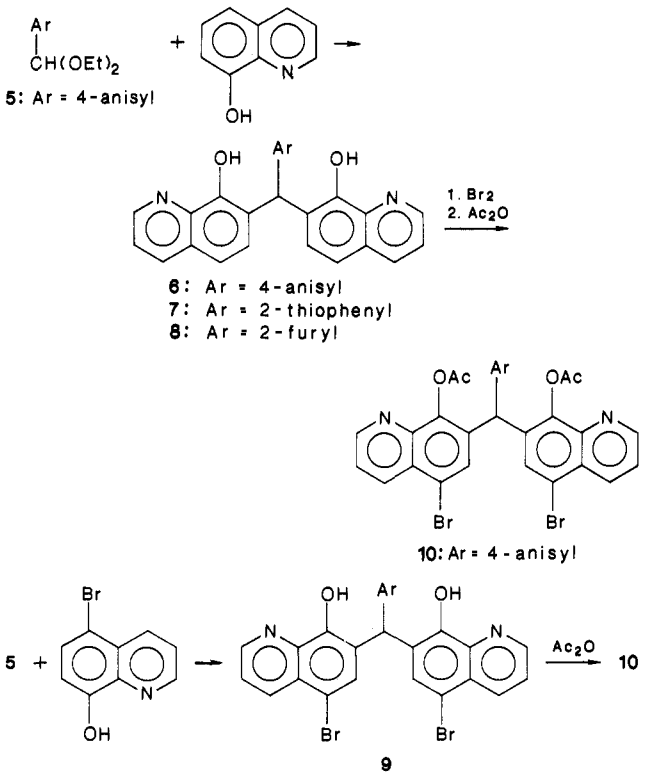
0022-2623/87/1830-1897$01.50/0

Table I. Antitumor Activities of Quinoline Derivatives and Related Tropolone Derivatives

compd	inhibn of KB cell growth: IC_{50}, μg/mL	antitumor act. P388 in mice	
		dose,[a] mg/kg ip	% T/C
1b	<0.3	10	170
		5	173
		2.5	134
2a	<0.3		*b*
2b	0.5	400	140
		200	128
		50	119
3	0.5	400	108
		200	126
		100	112
4	0.5	50	152
		25	146
		12.5	128
6	<0.3	12.5	164
		6.3	128
		3.1	111
7	<0.3	20	136
		10	125
		2.5	108
8	<0.3	40	138
		20	120
		10	113
11	0.8	400	129
		100	112
12	<0.3	50	135
		25	138
		12.5	120
13	1.3		*b*
14	17		*b*
15	44		*b*

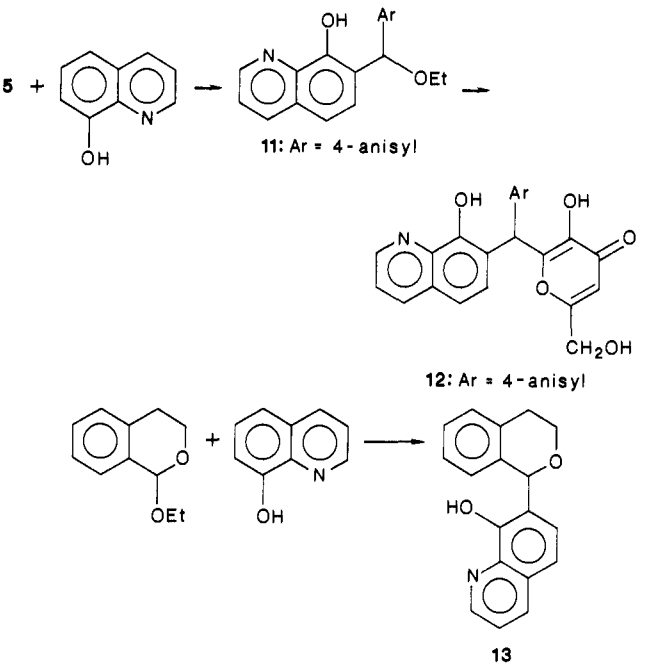
[a] The doses listed were given once a day for 1 and 5 days. [b] Dose = 400 mg/kg; inactive.

Scheme I

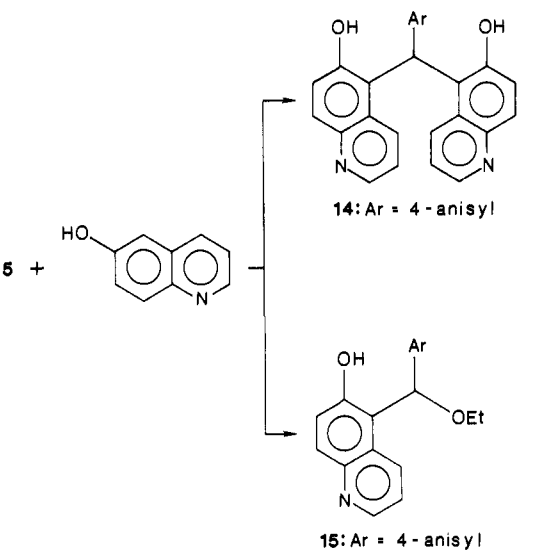


hydroxyquinoline with **5** in refluxing *p*-cymene gave the bis(6-hydroxyquinoline) **14** in 48% yield. In the ^1H NMR spectrum, the protons due to C-7 and C-8 of the quinoline ring were observed at δ 7.41 and 8.00 as doublets (J = 9.2 Hz), respectively, indicating the substituted position to be

Scheme II



Scheme III



the 5-position and not 7-position of the quinoline ring. When 0.7 equiv of 6-hydroxyquinoline was used in a similar reaction, the mono(6-hydroxyquinoline) **15** was obtained in 29% yield.

Biological Results

Compounds **6–8** and **11–15** were evaluated for inhibition of growth of KB cells (Table I). As expected, the 6-hydroxyquinolines **14** and **15** were inactive, while mono- and bis(8-hydroxyquinoline) derivatives (**6–8** and **11–13**) exhibited potent inhibitory activity.

Antitumor effect was tested against leukemia P388 in mice. The bis(8-hydroxyquinoline) **6** was found to have remarkable potency approximately equivalent to that of the bistropolone **1b**. The potencies of other bis(8-hydroxyquinolines) **7** and **8**, which contain a heteroaromatic ring, were weaker than that of **6**; however, they exhibited significant activity at relatively low doses. The mono(8-hydroxyquinoline) **11** exhibited significant activity at high doses, but the isochromanyl derivative **13** was inactive. Replacement of the ethoxy group of **11** by kojic acid, which was selected as a typical compound containing

an acidic hydroxyl and a carbonyl group in the neighboring position, resulted in remarkably enhanced activity, as found in the tropolone series, such as **4**.[5]

These structure–activity relationships in the in vivo and in vitro systems were found to be the same as those in the tropolone series. This suggested that the antitumor activities of both tropolone and 8-hydroxyquinoline derivatives are closely correlated with their ability to form a chelate.

The possibility that the biological mechanism of antitumor activity of the tropolones might be similar to that of colchicine[9-11] which contains a tropolone moiety, could not be neglected. The antitumor effect of colchicine is known to be due to binding to tubulin. Consequently, the bistropolones **1a–e**[12] were tested for the ability to inhibit colchicine binding to tubulin. The compounds, at concentrations of 10 μM, were unable to inhibit the binding of 10 μM [^{3}H]colchicine to tubulin. Also, at 10 μM concentrations, the compounds were unable to inhibit the polymerization of 15 μM tubulin into microtubules. Therefore, we concluded that the antitumor activity of the tropolone series does not involve the microtubule system.

Conclusion

An important finding of this study was the development of a new type of antitumor-active bis(8-hydroxyquinolines) **6–8**. The structure–activity relationships of the tropolone and 8-hydroxyquinoline series provided evidence that the antitumor effects of both series are a consequence of their abilities to form a chelate, and that bis-type structure is required for producing potent activity. In addition, biological mechanism of antitumor activity of bistropolones **1** was found not to be due to binding to tubulin.

These findings led us to assume that they must form a chelate with the metal necessary for the enzyme that catalyzes the DNA biosynthetic pathways. A possible enzyme, ribonucleotide reductase,[13] was considered because the enzyme contains two iron ions which are required for enzyme activity.[14] On the basis of this assumption, we have recently investigated the biological mechanism of antitumor activity of **1b** and found that **1b** inhibits intracellular ribonucleotide reductase. The detailed results will be reported elsewhere.[15]

Experimental Section

Chemistry. Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. ^{1}H NMR spectra were run on a Hitachi R-24 60-MHz spectrometer, with Me_4Si as an internal standard. MS spectra were recorded on a Shimadzu LKB-9000 spectrometer. IR spectra were taken on a Nippon Bunko A-102 spectrometer. The elemental analyses (C, H, and N) were within ±0.4% of the theoretical values. The extracted solution were dried over anhydrous $MgSO_4$. Column chromatographic separations were performed by flash technique on 200–300-mesh silica gel (Wako C-300).

α,α-Bis(8-hydroxyquinolin-7-yl)-4-methoxytoluene (6). A solution of 8-hydroxyquinoline (16 g, 110 mmol) and 4-anisaldehyde diethyl acetal (**5**; 12 g, 57 mmol) in *p*-cymene (50 mL) was allowed to reflux for 8 h. After the solvent was removed under reduced pressure, the resulting precipitates were filtered off and recrystallized from AcOEt–hexane to give **6** (8.8 g, 38%): mp 200–203 °C; ^{1}H NMR (Me_2SO-d_6) δ 3.66 (s, 3 H, OMe), 6.50 (s, 1 H, CH), 6.77 (d, J = 7.5 Hz, 2 H, 2-H, 6-H), 6.90 (d, J = 7.5 Hz, 2 H, 3-H, 5-H), 7.08 (d, J = 8.0 Hz, 2 H, 6′-H, 6″-H), 7.26 (d, J = 8.0 Hz, 2 H, 5′-H, 5″-H), 7.45 (dd, J = 4.0, 8.0 Hz, 2 H, 3′-H, 3″-H), 8.21 (dd, J = 2.0, 8.0 Hz, 2 H, 4′-H, 4″-H), 8.76 (dd, J = 2.0, 4.0, Hz, 2 H, 2′-H, 2″-H); MS, m/z 408 (M^+). Anal. ($C_{26}H_{20}N_2O_3$) C, H, N.

2-[Bis(8-hydroxyquinolin-7-yl)methyl]thiophene (7). A solution of 8-hydroxyquinoline (3 g, 10 mmol), 2-thiophenealdehyde diethyl acetal (1.9 g, 10 mmol), *t*-BuOK (0.1 mg, 1 mmol), and hexamethylphosphorictriamide (HMPA) (3 mL) in xylene (10 mL) was allowed to reflux for 13 h. After the solvent was removed under reduced pressure, the residue was column chromatographed (AcOEt–hexane, 3:1) to give **7** (0.19 g, 5%), recrystallized from THF–EtOH: mp 253–255 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 5.77 (s, 1 H, CH), 6.70 (d, J = 2 Hz, 1 H, 3-H), 6.85 (d, J = 1.7 Hz, 1 H, 4-H), 7.05 (d, J = 1.7 Hz, 1 H, 5-H), 7.3–7.7 (m, 8 H, quinoline H), 8.33 (dd, J = 2.0, 4.5 Hz, 2 H, 4′-H, 4″-H), 8.72 (dd, J = 2.0, 3.0 Hz, 2 H, 2′-H, 2″-H); MS, m/z 384 (M^+). Anal. ($C_{23}H_{16}N_2O_2S$) C, H, N.

Similarly, **2-[bis(8-hydroxyquinolin-7-yl)methyl]furan (8)** was prepared in 4% yield: mp 222–225 °C; ^{1}H NMR (Me_2SO-d_6) δ 5.88 (s, 1 H, CH), 6.3–6.4 (m, 2 H, 1-H, 4-H), 6.57 (d, J = 2.5 Hz, 1 H, 3-H), 7.1-7.7 (m, 8 H, quinoline H), 8.30 (dd, J = 2.0, 4.0 Hz, 2 H, 4′-H, 4″-H), 8.75 (dd, J = 2.0, 3.0 Hz, 2 H, 2′-H, 2″-H); MS, m/z 368 (M^+). Anal. ($C_{23}H_{16}N_2O_3$) C, H, N.

α,α-Bis(8-acetoxy-5-bromoquinolin-7-yl)-4-methoxytoluene (10). Method A. Bromine (0.9 g, 5.5 mmol) was added dropwise to a solution of **6** (0.96 g, 24 mmol) in $CHCl_3$ (17 mL)–AcOH (11 mL) at room temperature,[7] and then the mixture was stirred for 15 min at the same temperature. The resulting precipitates were filtered off and recrystallized from THF to give **α,α-bis(5-bromo-8-hydroxyquinolin-7-yl)-4-methoxytoluene (9**; 0.88 g, 77%): mp 262–264 °C; IR (Nujol) 3360 (OH) cm^{-1}; MS, m/z 566 (M^+).

A solution of **9** (39 mg, 0.08 mmol) and Ac_2O (40 μL) in dry pyridine (0.5 mL) was stirred for 1 day at room temperature, made acidic with 10% HCl, and extracted with AcOEt. The AcOEt layer was washed with H_2O and concentrated. The resulting precipitates were recrystallized from AcOEt–Et_2O to give **10** (30 mg, 76%): mp 144–146 °C; IR (Nujol) 1770 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.34 (s, 6 H, 2 COMe), 3.84 (s, 3 H, OMe), 6.28 (s, 1 H, CH), 6.8–7.6 (m, 8 H, aromatic H), 8.58 (dd, J = 1.5, 5.0 Hz, 2 H, 4′-H, 4″-H), 9.00 (dd, J = 1.5, 2.5 Hz, 2 H, 2′-H, 2″-H); MS, m/z 650 (M^+). Anal. ($C_{30}H_{22}Br_2N_2O_5$) C, H, N.

Method B. A solution of 5-bromo-8-hydroxyquinoline[8] (0.65 g, 3.5 mmol) and **5** (0.37 g, 1.8 mmol) in *p*-cymene (15 mL) was allowed to reflux for 4 h. The resulting precipitates were filtered off and recrystallized from THF-EtOH to give **9** (45 mg, 5%).

By a procedure similar to the one described above, **9** was converted to **10**, which was identical (melting point, TLC, and spectral data) with the sample prepared by method A.

7-(α-Ethoxy-4-methoxybenzyl)-8-hydroxyquinoline (11). A solution of 8-hydroxyquinoline (1 g, 6.8 mmol) and **5** (6.3 g, 30 mmol) in xylene (10 mL) was allowed to reflux for 5 h. After unreacted 8-hydroxyquinoline and excess **5** were removed by distillation in vacuo, the residue was column chromatographed to give **11** (0.5 g, 20%) as a viscous oil: IR (neat) 3100 (OH) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.31 (t, J = 7.8 Hz, 3 H, OCH_2CH_3), 3.63 (q, J = 7.8 Hz, 2 H, OCH_2CH_3), 3.77 (s, 3 H, OMe) 6.09 (s, 1 H, CH), 6.88 (d, J = 9 Hz, 2 H, 2′-H, 6′-H), 7.3–7.8 (m, 5 H, aromatic H), 8.11 (dd, J = 1.2, 5.0 Hz, 1 H, 4-H), 8.76 (dd, J = 1.2, 2.0 Hz, 1 H, 2-H); MS, m/z 309 (M^+). Anal. ($C_{19}H_{19}NO_3$) C, H, N.

8-Hydroxy-7-[α-[3-hydroxy-6-(hydroxymethyl)-4-oxo-4*H*-pyran-2-yl]-4-methoxybenzyl]quinoline (12). A mixture of **11** (0.3 g, 0.97 mmol) and kojic acid (0.2 g, 1.4 mmol) was heated at 140 °C for 1 h and column chromatographed (AcOEt) to give **12** (0.15 g, 38%), recrystallized from AcOEt–$CHCl_3$: mp 205–207 °C; ^{1}H NMR ($CDCl_3$) δ 3.75 (s, 3 H, OMe), 4.40 (s, 2 H, CH_2OH),

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5.58 (1 H, br, 5′-H) 6.3–7.5 (m, 11 H, aromatic H, OH), 8.05 (dd, J = 2.0, 8.0 Hz, 1 H, 4-H), 8.70 (dd, J = 2.0, 5.0 Hz, 1 H, 2-H); MS, m/z 405 (M^+). Anal. ($C_{23}H_{19}NO_6$) C, H, N.

8-Hydroxy-7-isochroman-1-ylquinoline (13). A solution of 8-hydroxyquinoline (1.6 g, 11 mmol) and 1-ethoxyisochroman (2.0 g, 11 mmol) in *p*-cymene (20 mL) was allowed to reflux for 8 h, and the solvent was removed under reduced pressure. Recrystallization of the residue from EtOH gave **13** (1.3 g, 43%): mp 166–167 °C; IR (Nujol) 3330 (OH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.7–3.3 (m, 2 H, 4′-H_2), 4.0–4.5 (m, 2 H, 3′-H_2), 6.58 (s, 1 H, 1′-H), 6.8–7.6 (m, 7 H, aromatic H), 8.16 (dd, J = 1.5, 5.0 Hz, 1 H, 4-H), 8.89 (dd, J = 1.5, 3.0 Hz, 1 H, 2-H); MS, m/z 276 (M^+). Anal. ($C_{18}H_{15}NO_2$) C, H, N.

α,α-Bis(6-hydroxyquinolin-5-yl)-4-methoxytoluene (14). A solution of 6-hydroxyquinoline (3 g, 21 mmol) and **5** (2.1 g, 10 mmol) in *p*-cymene (10 mL) was allowed to reflux for 2 h. After the solution was cooled, the resulting precipitates were filtered off and recrystallized from EtOH to give **14** (2 g, 49%): mp 238–240 °C dec; ^1H NMR (Me_2SO-d_6) δ 3.68 (s, 3 H, OMe), 6.77 (d, J = 8.8 Hz, 2 H, 2-H, 6-H), 6.90 (d, J = 8.8 Hz, 2 H, 3-H, 5-H), 6.95 (s, 1 H, CH), 7.20 (dd, J = 4.4, 8.8 Hz, 2 H, 3′-H, 3″-H), 7.34 (d, J = 9.2 Hz, 2 H, 7′-H, 7″-H), 7.80 (d, J = 9.2 Hz, 2 H, 8′-H, 8″-H), 8.37 (dd, J = 2.2, 8.8 Hz, 2 H, 4′-H, 4″-H), 8.55 (dd, J = 2.2, 4.4 Hz, 2 H, 2′-H, 2″-H), 9.94 (br, 2 H, 2 OH); MS, m/z 408 (M^+). Anal. ($C_{26}H_{20}N_2O_3$) C, H, N.

5-(α-Ethoxy-4-methoxybenzyl)-6-hydroxyquinoline (15). A solution of 6-hydroxyquinoline (3 g, 21 mmol) and **5** (6 g, 29 mmol) in *p*-cymene (20 mL) was allowed to reflux for 2 h. After the solution was cooled, the resulting precipitates were filtered. The filtrate was concentrated and column chromatography (AcOEt–hexane, 3:1) of the residue gave **15** (1.9 g, 29%), recrystallized from EtOH: mp 118–120 °C; ^1H NMR (Me_2SO-d_6) δ 1.33 (t, J = 7 Hz, 3 H, OCH_2CH_3), 3.72 (q, J = 7.0 Hz, 2 H, OCH_2CH_3), 3.75 (s, 3 H, OMe) 6.20 (s, 1 H, CH), 6.66 (d, J = 8.0 Hz, 2 H, 2′-H, 6′-H), 7.28 (2H, d, J = 8.0 Hz, 3′-H, 5′-H), 7.30 (dd, J = 4.0, 8.0 Hz, 1 H, 3-H), 8.02 (dd, J = 2.0, 8.0 Hz, 1 H, 4-H), 8.71 (dd, J = 2.0, 4.0 Hz, 1 H, 2-H). Anal. ($C_{19}H_{19}NO3$) C, H, N.

Pharmacology. Antitumor Activity in Vitro and in Vivo. Assays of antitumor activity were carried out as previously described.[3]

Tubulin Binding Assays. Materials. [^{3}H]Colchicine was obtained from New England Nuclear, Cambridge, MA. All other materials were purchased or obtained as previously described.[16] Microtubules were purified by a cycle of assembly and disassembly.[17] Heat-stable microtubule-associated proteins (MAPs) were obtained from the microtubule pellets as previously described.[17] Tubulin was purified by phosphocellulose chromatography.[17] Test compounds **1a–e** were prepared as previously described.[12]

Microtubule Assembly. All assembly experiments were performed in the buffer of Fellous et al.[17] Tubulin (1.5 mg/mL) was incubated in the presence or absence of the tropolone derivative at 37 °C in cuvettes in a Gilford Model 250 spectrophotometer. The tropolone derivatives were dissolved in dimethyl sulfoxide (Me_2SO). Equivalent amounts of Me_2SO were added to the control cuvette. The Me_2SO concentration in the samples was 2%. To initiate assembly, MAPs were added to the tubulin solution to a final MAP concentration of 0.3 mg/mL. Microtubule assembly was measured by turbidimetry.

Compounds **1a–e** exhibited no effect on the assembly of microtubule protein at 10^{-5} M.

Tubulin Binding Assay. The binding of the tropolone derivative to microtubule protein was determined by measuring its ability to inhibit binding of [^{3}H]colchicine. The binding to tubulin of [^{3}H]colchicine was measured by the filter disk method of Borisy.[18] The protein content was determined by the method of Lowry et al.[19]

Compounds **1a–e** exhibited no effect on [^{3}H]colchicine binding to tubulin at 5.1^{-5} M.

Acknowledgment. This study was supported in part by a grant from the Ministry of Education, Science and Culture, Japan (Grant No. 61015066) and by Grant AQ-726 from the Robert Welch Foundation to R.F.L.

Registry No. **1b**, 92832-17-2; **2a**, 87864-23-1; **2b**, 92832-11-6; **3**, 87864-22-0; **4**, 104598-52-9; **5** (4-anisyl), 2403-58-9; **5** (4-thiophenyl), 13959-97-2; **5** (2-furyl), 13529-27-6; **6**, 105192-47-0; **7**, 105192-48-1; **8**, 105192-49-2; **9**, 109976-85-4; **10**, 109976-86-5; **11**, 109976-87-6; **12**, 109976-88-7; **13**, 109976-89-8; **14**, 109976-90-1; **15**, 109976-91-2; 8-hydroxyquinoline, 148-24-3; 5-bromo-8-hydroxyquinoline, 1198-14-7; kojic acid, 501-30-4; 1-ethoxyisochroman, 75802-22-1; 6-hydroxyquinoline, 580-16-5.

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