Synthesis and Antitumor Activity of Tropolone Derivatives. 5[1]

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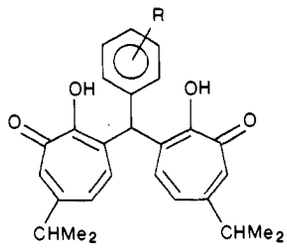
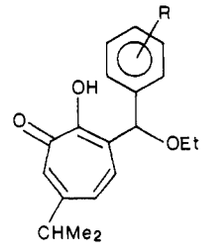
As part of a study on the structure–activity relationship of antitumor-active tropolone derivatives, a series of bistropone analogues, related to potently active bistropolone **1a**, were synthesized and tested for their antitumor activity in in vitro (KB cell) and in vivo (leukemia P388 in mice) systems. The methoxytropones **3** and **5**, hydroxytropothione **10**, and (*N*-methylamino)tropones **12** and **13** were inactive in both systems. Methoxytropone **6** exhibited weak activity, whose potency was equal to that of monotropolone **2a**.

We have previously reported the syntheses of mono- and bistropolones (**1** and **2**) and their antitumor activities.[2] Although both derivatives exhibit nearly equal potency in inhibitory activity against the growth of KB cells, the activity of bistropolone **1a** is about 200 times that of monotropolone **2a** in the survival test of P388 mice.



1a: R = *p*-OMe



2a: R = *p*-OMe

A recent study of ours has shown that the antitumor activity of bistropolones **1** with ortho-, meta-, and/or para-substituted benzene ring varied as the substituent was changed.[3]

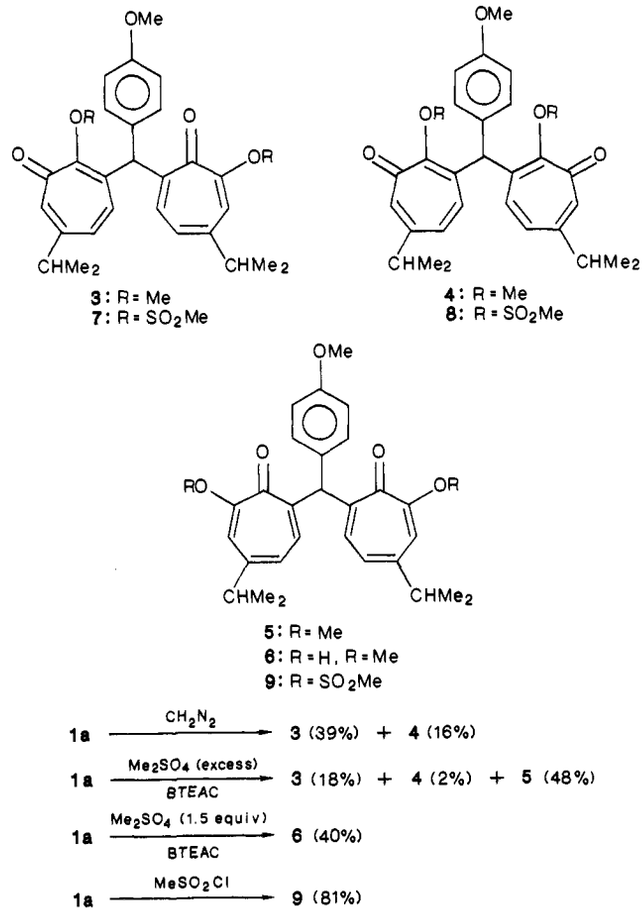
To explore the structure–activity relationships of bistropolones **1** and to gain insight into a possible mechanism of action, we have successfully modified the hydroxyl and carbonyl groups of the tropolone ring of **1a**.

Chemistry

Methoxytropones α-(6-isopropyl-2-methoxytropon-3-yl)-α-(4-isopropyl-2-methoxytropon-7-yl)-4-methoxytoluene (**3**) and α,α-bis(6-isopropyl-2-methoxytropon-3-yl)-4-methoxytoluene (**4**) were prepared by the reaction of **1a** with diazomethane[4] in 39% and 16% yields, respectively. The phase-transfer reaction of **1a** with excess dimethyl sulfate in the presence of benzyltriethylammonium chloride gave three regioisomers, **3**, **4**, and α,α-bis(4-isopropyl-2-methoxytropon-7-yl)-4-methoxytoluene (**5**), in 18%, 2%, and 60% yields, respectively.

Structures for these compounds were established on the basis of their NMR spectra. In the NMR spectrum of **3**, the signals due to the methyl protons of the two isopropyl groups on the two tropone rings appeared separately at δ 1.15 and 1.21. Similarly, the signals due to the two methoxy groups on the two tropone rings appeared at δ 3.80 and 3.89, respectively. These data implied that **3** contains two unsymmetric methoxytropone rings. In the case of **4** or **5**, signals due to the two isopropyl or the two methoxy groups on the two tropone rings exhibited the same chemical shift, respectively. In addition, when the two signals assigned to the respective α-methyne protons of **4** and **5** were compared, the former appeared at δ 6.17 and the latter appeared at δ 6.35. The peak at δ 6.35 could be assigned to the methyne proton of **5**, which would be deshielded by the two neighboring carbonyl groups on the two tropone rings. Consequently, the structures of **4** and **5** were established as shown in Scheme I.

Scheme I



When 1.5 equiv of dimethyl sulfate was used in a similar phase-transfer reaction of **1a**, the monomethylated derivative α-(2-methoxy-4-isopropyltropon-7-yl)-α-(2-hydroxy-6-isopropyltropon-3-yl)-4-methoxytoluene (**6**) was obtained. The structure of **6** was established by its conversion into **5**.

The syntheses of compounds **10**, **12**, and **13** were accomplished as shown in Scheme II.

2-Methoxytropones are known to give replacement

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(1) Yamato, M.; Hashigaki, K.; Sakai, J.; Kawasaki, Y.; Tsukagoshi, S.; Tashiro, T. *J. Med. Chem.* **1987**, *30*, 117.
(2) (a) Yamato, M.; Ishikawa T.; Ishikawa, S.; Hashigaki, K. *Chem. Pharm. Bull.* **1983**, *31*, 2952. (b) Yamato, M.; Hashigaki, K.; Kokubu, N.; Tsuruo, T.; Tashiro, T. *J. Med. Chem.* **1984**, *27*, 1749.
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(4) Nozoe, T.; Katsura, S. *Yakugaku Zasshi* **1944**, *64*, 181.

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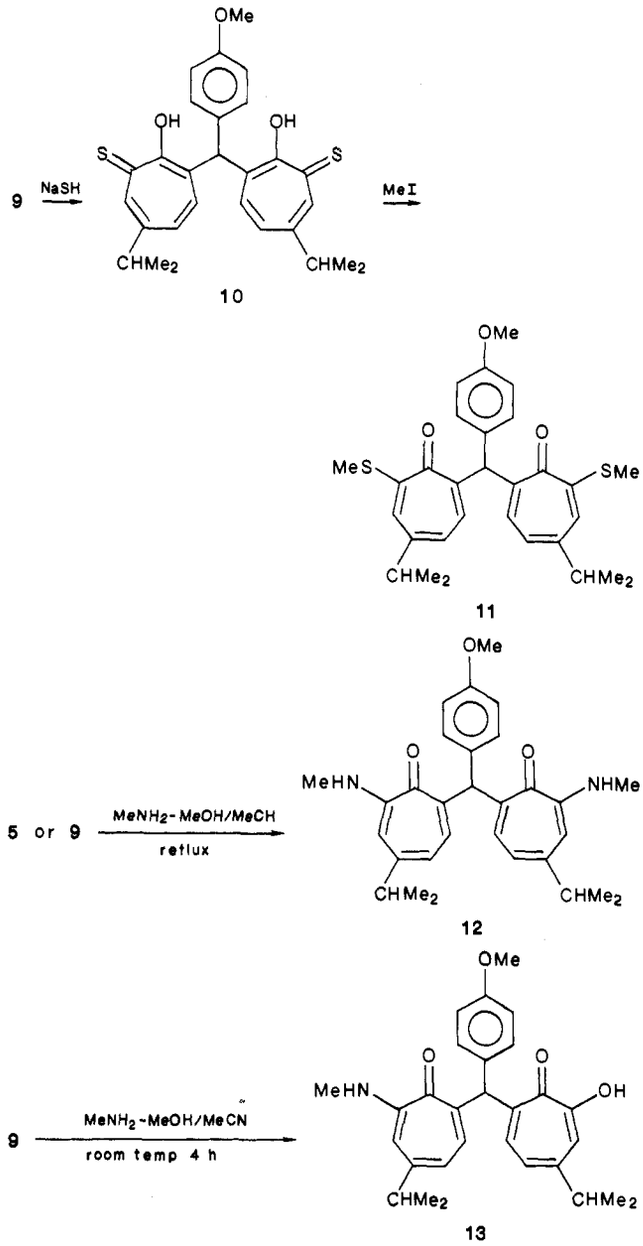
Scheme II



products of the methoxy group by reacting with thiol, amines, or other nucleophiles.[5] Therefore, we first designed a reaction scheme to use the bis(methoxytropone) **5** as the synthetic intermediate of **10**, **12**, or **13**. However, the preparation of **5** required the troublesome separation of the regioisomers **3**–**5** by column chromatography. An alternative intermediate was explored. Compound **1a**, on treatment with mesyl chloride, gave only bis[(mesyloxy)tropone] A. In its NMR spectrum, the signal due to the mesyl groups appeared at δ 3.32 as a singlet, implying that it contains two symmetric mesyloxytropone rings. Consequently, its structure was found to be **8** or **9** and not **7** (Scheme I). Of these two possibilities, we have selected **9**, because compound A gave selectively α,α-bis(2-hydroxy-5-isopropyl-3-thioxo-1,4,6-cycloheptatrienyl)-4-methoxytoluene (**10**) in 81% yield by the treatment with sodium hydrosulfide in dimethylformamide (Scheme II).

The structure of **10** was established by reference to the work of Nozoe and Matsui[6] on the structure of 2-hydroxytropothiones. 2-Hydroxytropothiones can be present in two tautomeric forms, the thioketo and enethiol forms. However, they determined the predominant tautomeric forms to be 2-hydroxytropothiones on the basis of their physicochemical properties.[6] They also reported that the NMR spectra of 2-hydroxytropothiones show the characteristic low field signal at about δ 8.4, which is assigned to the proton adjacent to the thiocarbonyl group.[6] For example, the C(7)-H proton signal for 6-isopropyl-2-hydroxytropothione appears at about δ 8.42 as a singlet. On the other hand, in the NMR spectra of 2-(methylthio)tropones, the peak appearing at δ 8.40 for 2-hydroxytropothiones upshifts to δ 6.7–7.0.[6] In the NMR spectrum of **10**, the characteristic low field signal due to the two protons adjacent to the thiocarbonyl group could be observed at δ 8.50 as a singlet. This indicated that compound **10** contains two symmetric 6-isopropyl-2-hydroxytropothione moieties. Consequently, compound **10** could be assigned the structure shown in Scheme II. Moreover, S-methylation of **10** with methyl iodide gave bis[(methylthio)tropone] **11** without its regioisomer. In the NMR spectrum of **11**, the signal due to the two methylthio groups on the two tropone rings appeared at δ 2.33 and the proton signals of tropone and the aromatic rings appeared at δ 6.27–7.39. On the basis of these results, the structures of **10** and **11** in Scheme II were established.

α,α-Bis[4-isopropyl-2-(*N*-methylamino)tropon-7-yl]-4-methoxytoluene (**12**) was prepared by the reaction of **9** with methylamine in refluxing acetonitrile in 63% yield. The assignment of structure of **12** was based on the NMR signals due to the two isopropyl and the two methylamino groups [1.33 (12 H, d), 3.01 (6 H, d)]. Furthermore, the product obtained by the reaction of **5** with methylamine was identical with compound **12**, implying the structure of **12** in Scheme II to be correct.

Treatment of **9** with methylamine in acetonitrile at room temperature gave α-[4-isopropyl-2-(*N*-methylamino)tropon-7-yl]-α-(2-hydroxy-6-isopropyltropon-3-yl)-4-methoxytoluene (**13**) in 71% yield. Its structure was confirmed by conversion into **12**.

Table I. Antitumor Activities of Tropone Derivatives

compd	inhibn of KB cell growth: IC_{50}, μg/mL	antitumor act. P388 in mice, ip[a] doses, mg/kg	T/C, %
1a	0.5	5	173
		2.5	134
		0.6	127
2a	0.5	400	140
		200	128
		100	140
		50	119
3	5.4	inactive[b]	
4	NT[c]	inactive[b]	
5	23.5	inactive[b]	
6	1.7	400	60
		200	148
		100	133
10	5.3	inactive[b]	
12	100	inactive[b]	
13	50	inactive[b]	

[a] The doses listed were given once a day for 1 and 5 days. [b] Dose: 400 mg/kg. [c] NT = not tested.

Biological Results and Discussion

The compounds listed in Table I were evaluated for growth inhibition of KB cells and antitumor activity against leukemia P388 in mice.[2b]

Methoxy analogues **3** and **5** were inactive even in the in vitro system as expected. The monomethoxytropone **6**

(5) Francesco, P. *Chem. Rev.* **1973**, *73*, 293.
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showed activity in the in vivo system, whose potency was nearly equal to monotropolone **2a**. The EC_{50} value of the inhibitory activity against KB cells of the bistropolone **10** was borderline; however, it was inactive in the in vivo system. Replacement of the hydroxyl of one or two tropolone rings by a methylamino group gave **13** or **12**, which were inactive in the in vitro system.

On the basis of our previous findings, we have presumed that the antitumor activity of the tropolone series relates to their ability to form a chelate with a metal ion.[1] Namely, these tropolones, which could act as potent chelators, were considered to be inhibitors of the metal-dependent enzyme, such as ribonucleoside diphosphate reductase[7] and hence the synthesis of DNA.

The present result that the methoxytropone analogues **3** and **5** have no ability to form a chelate and thus are inactive supports the above presumption. On the other hand, the question as to why the thio (**10**) and methylamino (**12** and **13**) analogues were inactive is raised, because they are able to form a chelate.

In summary, the modification of the tropolone moiety of potently active bistropolone **1a** resulted in a loss of activity in the in vivo system. This demonstrated that there is a rigorous structural requirement for antitumor activity.

Experimental Section

Melting points were determined on a Yanagimoto micromelting apparatus and are uncorrected. NMR spectra were run on a Hitachi R-24 spectrometer 60 MHz, 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 solutions were dried over anhydrous $MgSO_4$. Column chromatographic separations were performed by flash technique on 200–300-mesh silica gel (Wako C-300).

Reaction of 1a with CH_2N_2. To a solution of **1a** (5 g, 11 mmol) in tetrahydrofuran (100 mL) was added a solution of excess diazomethane in ether and the mixture was allowed to stand at room temperature for 3 h. The remaining diazomethane was decomposed with acetic acid and the solvent was evaporated. A solution of the residue in CH_2Cl_2 was washed with saturated $KHCO_3$ solution and water and concentrated. The residue, of which TLC showed two spots, was column chromatographed. First elution with hexane–AcOEt (1:1) gave **α,α-bis(6-isopropyl-2-methoxytropon-3-yl)-4-methoxytoluene (4)** (0.9 g, 16%): recrystallized from ether, mp 114–116 °C; IR (Nujol) 1620 cm^{-1}; NMR ($CDCl_3$) δ 1.20 (d, J = 7 Hz, 12 H, 2 CHMe_2), 2.3–3.1 (m, 2 H, 2 CHMe$_2$), 3.67 (s, 6 H, 2 OMe), 3.78 (s, 3 H, OMe), 6.10 (s, 1 h, CH), 6.6–7.2 (m, 10 H, aromatic H, tropolone H); MS, m/z 474 (M^+). Anal. ($C_{30}H_{34}O_5$) C, H. Second elution with hexane–AcOEt (1:2) gave **α-(6-isopropyl-2-methoxytropon-3-yl)-α-(4-isopropyl-2-methoxytropon-7-yl)-4-methoxytoluene (3)** (2.1 g, 39%): recrystallized from MeOH–CH_2Cl_2 (5:1), mp 188–189 °C; IR (Nujol) 1620 cm^{-1}; NMR ($CDCl_3$) δ 1.14 (d, J = 7 Hz, 6 H, CHMe_2), 1.25 (d, J = 7 Hz, 6 H, CH 2), 2.4–3.3 (m, 2 H, 2 CHMe$_2$), 3.80 (s, 3 H, OMe), 3.85 (s, 3 H, OMe), 3.95 (s, 3 H, OMe), 6.37 (s, 1 H, CH), 6.7–7.3 (m, 10 H, tropolone H, aromatic H); MS, m/z 474 (M^+). Anal. ($C_{30}H_{34}O_5$) C, H.

Reaction of 1a with Dimethyl Sulfate. A mixture of **1a** (2 g, 4.5 mmol), powder KOH (0.75 g, 13.4 mmol), dimethyl sulfate (1.3 mL, 13.4 mmol), benzyltriethylammonium chloride (BTEAC; 0.34 g, 1.3 mmol), and CH_2Cl_2 (50 mL) was stirred at room temperature for 8 h, washed with water, and concentrated. The residue, of which TLC showed three spots, was column chromatographed. First elution with hexane–AcOEt (3:1) gave **4** (40 mg, 2%) and second elution with AcOEt (1:1) gave **3** (390 mg, 18%). The third elution with CH_2Cl_2 gave **α,α-bis(4-isopropyl-2-methoxytropon-7-yl)-4-methoxytoluene (5)** (1.02 g, 48%): recrystallized from CH_2Cl_2, mp 229–230 °C; IR (Nujol) 1590 cm^{-1}; NMR ($CDCl_3$) δ 1.22 (d, J = 7 Hz, 12 H, 2 CHMe_2), 2.4–3.1 (m, 2 H, 2 CHMe$_2$), 3.75 (s, 3 H, OMe), 3.88 (s, 6 H, 2 OMe), 6.35 (s, 1 H, CH), 6.5–7.3 (m, 10 H, aromatic H, tropolone H); MS, m/z 474 (M^+). Anal. ($C_{30}H_{34}O_5$) C, H.

α-(2-Methoxy-4-isopropyltropon-7-yl)-α-(2-hydroxy-6-isopropyltropon-3-yl)-4-methoxytoluene (6). A mixture of **1a** (1 g, 2.2 mmol), powder KOH (0.19 g, 3.4 mmol), dimethyl sulfate (0.32 mL, 3.4 mmol), benzyltriethylammonium chloride (85 mg, 0.45 mmol), and CH_2Cl_2 (20 mL) was stirred at room temperature for 4 h, made acidic with 10% HCl solution, and extracted with AcOEt. The organic layer was washed with water and concentrated. The residue was purified by column chromatography (hexane–AcOEt, 1:1) to afford **6** (410 mg, 40%): mp 196–198 °C; IR (Nujol) 3400, 1590 cm^{-1}; NMR ($CDCl_3$) δ 1.28 (d, J = 7 Hz, 12 H, 2 CHMe_2), 2.5–3.1 (m, 2 H, 2 CHMe$_2$), 3.80 (s, 3 H, OMe), 3.93 (s, 3 H, OMe), 6.50 (s, 1 H, CH), 6.6–7.4 (m, 10 H, aromatic H, tropolone H); MS, m/z 460 (M^+). Anal. ($C_{29}H_{32}O_5$) C, H.

α,α-Bis[4-isopropyl-2-(mesyloxy)tropon-7-yl]-4-methoxytoluene (9). Mesyl chloride (0.9 mL, 11.6 mmol) was added dropwise to a solution of **1a** (2 g, 4.5 mmol) and triethylamine (3.1 mL, 22.4 mmol) in CH_2Cl_2 (20 mL) with cooling. The mixture was stirred at room temperature for 1 h, made acidic with 10% HCl solution, and extracted with AcOEt. The AcOEt layer was washed water and concentrated to give **9** (2.18 g, 81%): recrystallized from hexane–AcOEt, mp 192–195 °C; IR (Nujol) 1590 cm^{-1}; NMR ($CDCl_3$) δ 1.24 (d, J = 7 Hz, 12 H, 2 CHMe_2), 2.5–3.1 (m, 2 H, 2 CHMe$_2$), 3.32 (s, 6 H, 2 SO_2Me), 3.78 (s, 3 H, OMe), 6.27 (s, 1 H, CH), 6.5–7.5 (m, 10 H, aromatic H, trpolone H). Anal. ($C_{30}H_{34}O_9S_2$) C, H.

α,α-Bis(2-hydroxy-5-isopropyl-3-thioxo-1,4,6-cycloheptatrienyl)-4-methoxytoluene (10). To a solution of sodium hydrosulfide (0.52 g, 9.3 mmol) in dimethylformamide was added dropwise a solution of **9** (0.56 g, 0.9 mmol) with cooling. The mixture was stirred at room temperature for 9 h and extracted with AcOEt. The organic layer was washed with water and concentrated. The residue was purified by column chromatography (hexane–AcOEt, 1:1) to give **10** (0.36, 81%), mp 138–140 °C, as a yellow crystal: NMR ($CDCl_3$) δ 1.22 (d, J = M Hz, 12 H, 2 CHMe_2), 2.5–3.1 (m, 2 H, 2 CHMe$_2$), 3.75 (s, 3 H, OMe), 6.51 (s, 1 H, CH), 6.7–7.5 (m, 8 H, aromatic H, tropolone H), 8.50 (s, 2 H, tropolone H), 9.7 (br, 2H, 2 OH). Anal. ($C_{28}H_{30}O_3S_2$) C, H.

α,α-Bis[4-isopropyl-2-(methylthio)tropon-7-yl]-4-methoxytoluene (11). A mixture of **10** (360 mg, 0.75 mmol), K_2CO_3 (0.25 g, 1.8 mmol), CH_3I (0.11 mL, 1.8 mmol), and dry dimethylformamide (7 mL) was stirred at room temperature for 1.5 h, poured into ice–water, and extracted with AcOEt. The AcOEt layer was washed with water and concentrated. The residual oil was crystallized from CH_2Cl_2–ether (1:1) to give **11** (68%): mp 219–221 °C; IR (Nujol) 1580 cm^{-1}; NMR ($CDCl_3$) δ 1.24 (d, J = 7 Hz, 12 H, 2 CHMe_2), 2.33 (s, 6 H, 2 SMe), 2.5–3.1 (m, 2 H, 2 CHMe$_2$), 3.76 (s, 3 H, OMe), 6.29 (s, 1 H, CH), 6.5–7.3 (m, 10 H, tropolone H, aromatic H); MS, m/z 506 (M^+), 5 08 (M^+ + 2). Anal. ($C_{30}H_{34}O_3S_2$) C, H.

α,α-Bis[4-isopropyl-2-(*N*-methylamino)tropon-7-yl]-4-methoxytoluene (12). Method A. From 9. A solution of **9** (0.3 g, 0.5 mmol) and 30% methylamine–MeOH solution (1.4 mL, 10.1 mmol) in acetonitrile (10 mL) was allowed to reflux for 1 h. After the solvent was removed, the residue was dissolved in $CHCl_3$. The $CHCl_3$ layer was washed with water and concentrated. The residue was purified by column chromatography (hexane–AcOEt, 1:1) to give **12** (0.15 g, 62%) as a light yellow powder: mp 287–289 °C; IR (Nujol) 3450, 1600 cm^{-1}; NMR ($CDCl_3$) δ 1.33 (d, J = 7 Hz, 12 H, 2 CHMe_2), 2.6–3.1 (m, 2 H, 2 CHMe$_2$), 3.01 (d, J = 5 Hz, 6 H, 2 NHMe), 3.78 (s, 3 H, OMe), 6.3–7.4 (m, 11 H, CH, aromaticH, tropolone H); MS, m/z 472 (M^+). Anal. ($C_{30}H_{36}N_2O_3$) C, H, N.

Method B. From 5. A solution of **5** (470 mg, 1 mmol) and 30% methylamine–MeOH solution (2.8 mL, 20.2 mmol) in acetonitrile (15 mL) was allowed to reflux for 3.5 h and worked up as described for method A to give **12** (346 mg, 74%), mp 288–289 °C.

α-[4-Isopropyl-2-(*N*-methylamino)tropon-7-yl]-α-(2-hydroxy-6-isopropyltropon-3-yl)-4-methoxytoluene (13). A solution of **9** (1.04 g, 1.73 mmol) and 30% methylamine–MeOH

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solution (2.4 mL, 17.4 mmol) in acetonitrile (35 mL) was stirred for 4 h at room temperature and concentrated in vacuo. The residue was dissolved in $CHCl_3$ and then the $CHCl_3$ solution was washed with water and concentrated. The resulting oil (0.41 g) after chromatography was dissolved in THF, to which 10% KOH solution (40 mL) was added. The solution was allowed to reflux for 10 min, made acidic with 10% HCl solution, and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water and concentrated. Recrystallization of the crude product by AcOEt gave 13 (0.25 g, 71%) as a light yellow powder: mp 233–235 °C; IR (Nujol) 3300, 3250, 1600 cm^{-1}; NMR ($CDCl_3$) δ 1.23 (d, J = 7 Hz, 6 H, $CHMe_2$), 1.27 (d, J = 7 Hz, $CHMe_2$), 2.7–3.3 (m, 2 H, 2 $CHMe_2$), 3.02 (d, J = 5 Hz, 3 H, NH*Me*), 3.79 (s, 3 H, OMe), 6.4–7.5 (m, 11 H, CH, aromatic H, tropolone H). Anal. ($C_{29}H_{33}NO_4$) C, H, N.

Biological Assays. Assays of antitumor activity were carried out as described previously.[2b]

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Registry No. **1a**, 92832-17-2; **2a**, 92832-11-6; **3**, 108149-77-5; **4**, 108149-78-6; **5**, 108149-79-7; **6**, 108149-80-0; **9**, 108149-81-1; **10**, 108149-82-2; **11**, 108149-83-3; **12**, 108149-84-4; **13**, 108149-85-5.

Synthesis of β-Carboline–Benzodiazepine Hybrid Molecules: Use of the Known Structural Requirements for Benzodiazepine and β-Carboline Binding in Designing a Novel, High-Affinity Ligand for the Benzodiazepine Receptor

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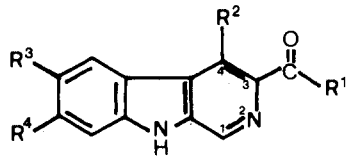
Hybrid molecules incorporating pharmacologically important structural features of both 3-carboxy-β-carbolines and 1,4-benzodiazepines were synthesized, and their affinities for the benzodiazepine receptor were determined in vitro. One of these hybrids, 8,14-dioxo-13,14-dihydro-8*H*-indolo[3′,2′:4,5]pyrido[2,1-*c*][1,4]benzodiazepine (**13**), demonstrated high affinity for the receptor, displacing both benzodiazepines (IC_{50} = 23 nM) and β-carbolines (IC_{50} = 47 nM) from their binding sites. Of the compounds synthesized, **13** also most closely satisfied the structural requirements that generally ensure a high affinity of both β-carbolines and benzodiazepines for the receptor (e.g., aromaticity of the β-carboline, presence of a carbonyl at C-3 of the β-carboline and of a π_2-region on the benzodiazepine). The hybrids not fulfilling these requirements had no affinity for the receptor. In vivo pharmacological properties of **13** could not be demonstrated because of its metabolic instability and/or its poor transport into the brain. The results are discussed in terms of a possible overlapping of β-carboline binding sites with those of benzodiazepines on the receptor.

The benzodiazepine receptor, which has been shown to mediate the sedative, anxiolytic, muscle-relaxant, and anticonvulsant effects of 1,4-benzodiazepines in the central nervous system,[1-5] is known to recognize a wide variety of apparently unrelated structures besides the benzodiazepines (represented by diazepam (**1**), Figure 1). For example, β-carbolines such as β-CCM (**2**) also display high



2 R^1 = OCH_3; R^2=R^3=R^4=H (β-CCM)
3 R^1 = OCH_2CH_3; R^2=R^3=R^4=H (β-CCE)
4 R^1 = $NHCH_3$; R^2=R^3=R^4=H (FG 7142)
7 R^1 = R^3 = R^4 = OCH_3; R^2 = CH_2CH_3 (DMCM)

affinities for the benzodiazepine receptor equivalent or superior to those of the benzodiazepines themselves.[6,7] However, though β-CCM can displace diazepam from its receptor sites (and vice versa), this compound also possesses pharmacological properties mediated by the benzodiazepine receptor that are completely opposite to those generally associated with benzodiazepines. Thus, β-CCM is a potent convulsant in various species[9-12] and demonstrates anxiogenic properties in mice.[13] Other analogues of β-CCM also display such inverse properties to various degrees. The ethyl ester, β-CCE (**3**), is proconvulsant in mice[14] and baboons[15] and anxiogenic in rats.[16] The methyl amide derivative, FG 7142 (**4**), also has attenuated convulsant properties[17] and is anxiogenic in certain para-

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