Synthesis and Antitumor Activity of Tropolone Derivatives. 7.[1] Bistropolones Containing Connecting Methylene Chains

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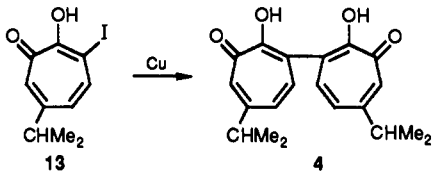
Bistropolone derivatives (4–12) containing differing lengths of linkage between the two tropolone rings were prepared and examined for their antitumor activity in in vitro (KB cell) and in vivo (leukemia P388 in mice) systems. Parent compound 3, related compounds previously prepared, and the new compounds 4–12 were evaluated for inhibitory activity against ribonucleotide reductase by indirect means to measure their effects on the dNTP pool imbalance. Present structure–activity relationship results would suggest that potently active bistropolones in vivo inhibit intracellular ribonucleotide reductase through chelating with the two irons at the two active sites of the enzyme.

We have previously reported the syntheses[2] and antitumor activities[3] of monotropolone (2) and bistropolone (3) derivatives (Figure 1[4]). These two types of compounds exhibit nearly equal potency in inhibitory activity against the growth of KB cells (in vitro system). However, the potency of bistropolone 3 is about 200 times of that of monotropolone 2 in the survival test of P388-induced mice (in vivo system) (Table I). The structure–activity relationships of mono- and bistropolones (2 and 3) provide evidence that the antitumor effects of this series are consequences of their metal-chelating properties and that the bis-type structure is required for producing potent activity.[1,5-9]

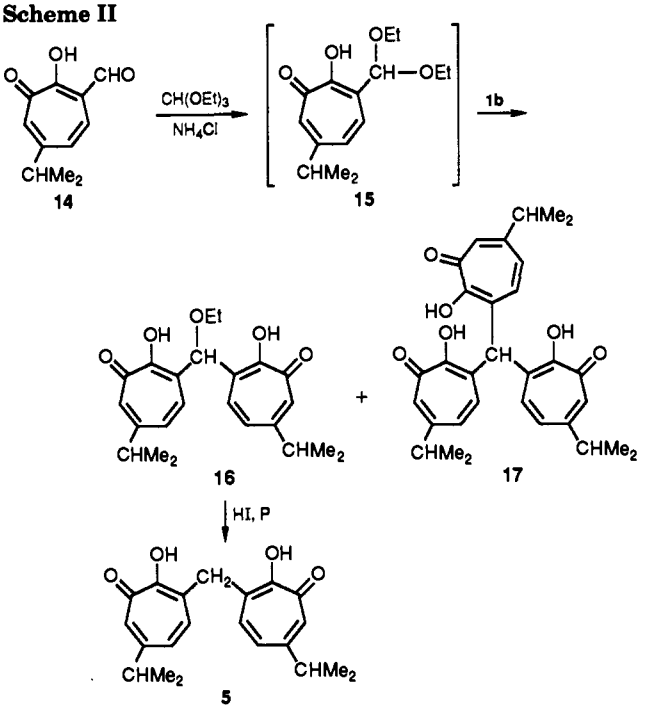
The mechanism of the antitumor action of this series of compounds has remained unknown. However, we[1] have previously assumed that this series of compounds may inhibit intracellular metal–enzymes such as ribonucleotide reductase[10] through their metal-chelating properties. This enzyme contains two nonheme irons and a tyrosyl free radical as part of its primary structure.[11] Hydroxyurea, which has antitumor activity and which is a well-known inhibitor for the enzyme, interacts with this radical, thereby inhibiting the enzyme action.[11,12] The inactivation of the enzyme with hydroxyurea causes intracellular deoxyribonucleoside triphosphate pool imbalance (dNTP pool imbalance),[13] depleting the intracellular pool of dATP and dGTP and increasing dTTP. Ganeshaguru et al.[14] have screened a number of potential chelators as inhibitors of ribonucleotide reductase by studying their effect on the dATP and dTTP concentrations in normal stimulated lymphocytes. They found that this method is more satisfactory than direct ribonucleotide reductase assay for inhibitor studies and that tropolone (1a) is a strong inhibitor for the enzyme.

Based on this relevant background information, it can be inferred that the antitumor activity of this series of compounds is the consequence of their inhibitory activities against ribonucleotide reductase and that bistropolone 3 simultaneously binds two irons at the two active sites of the enzyme, whereas monotropolone 2 binds only one iron at one active site of the enzyme. We assumed that these difference in the interaction mode with the enzyme between the mono- and bistropolones (2 and 3) must cause the remarkable difference in the potency of their in vivo antitumor activity. In order to examine the validity of the mechanism, we presently examined whether or not mono- and bistropolone induce dNTP pool imbalance. Moreover, among the compounds previously prepared, several antitumor-active and inactive compounds were selected as typical examples and examined for their abilities to induce dNTP pool imbalance. We presently studied the relationship between their antitumor activity and the ability

Scheme I



Scheme II



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(1) Part 6: Yamato, M.; Hashigaki, K.; Yasumoto, Y.; Sakai, J.; Luduena, F. R.; Banerjee, A.; Tsukagoshi, S.; Tashiro, T.; Tsuruo, T. Synthesis and Antitumor Activity of Tropolone Derivatives. 6. *J. Med. Chem.* **1987**, *30*, 1897–1900.
(2) Yamato, M.; Hashigaki, K.; Kokubu, N.; Nakato, Y. Synthesis of Tropolone Derivatives. *J. Chem. Soc., Perkin Trans. 1* **1984**, 1301–1304.
(3) Yamato, M.; Hashigaki, K.; Kokubu, N.; Tsuruo, T.; Tashiro, T.; Tsukagoshi, S. Synthesis and Antitumor Activity of Tropolone Derivatives. 1. *J. Med. Chem.* **1984**, *27*, 1749–1753.
(4) Tropolones exist as A and B forms of tautomers. The tropolone derivatives reported in this paper are represented as A or B form of tautomer.
(5) Yamato, M.; Hashigaki, K.; Ishikawa, S.; Kokubu, N.; Inoue, Y.; Tsuruo, T.; Tashiro, T. Synthesis and Antitumor Activity of Tropolone Derivatives. 2. *J. Med. Chem.* **1985**, *28*, 1026–1031.

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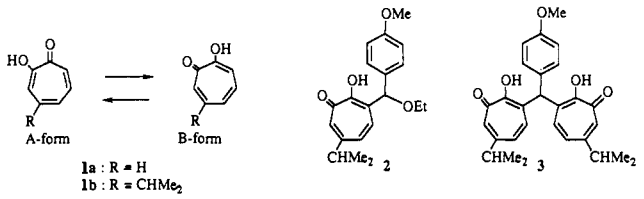
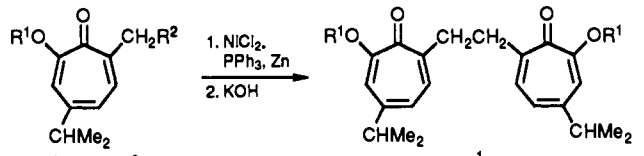


Figure 1.

Scheme III



18: R^1 = Me; R^2 = OH
19: R^1 = Me; R^2 = Cl
6: R^1 = H

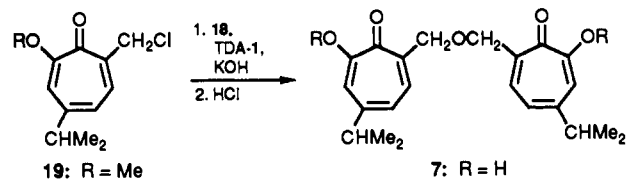
to induce dNTP pool imbalance.

We then turned our attention to a new series of bistropolones (**4–12**) containing differing lengths of linkage between the two tropolone rings. The enzyme contains two active sites having iron(III) ions, although the distance between the two active sites is not clear. We thought that varying the distance between the two tropolone rings might have a pronounced effect on the inhibitory activity of the enzyme and on the antitumor activity. Therefore, we presently synthesized the new type of bistropolones (**4–12**) and examined their antitumor activities and abilities to induce dNTP pool imbalance.

Chemistry

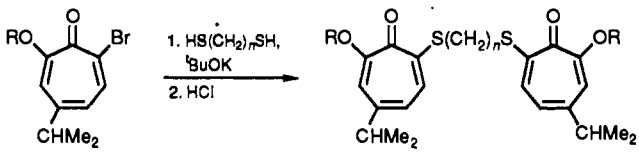
6,6′-Diisopropyl-3,3′-bistropolone (**4**) was prepared according to the method of Nozoe et al.[15] (Scheme I), and other new compounds (**5–12**) were synthesized as shown in Schemes II–VI.

Scheme IV



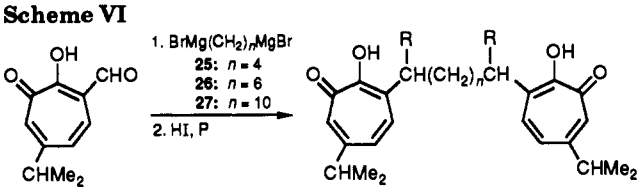
19: R = Me
7: R = H

Scheme V



22: R = Me
8: R = H; n = 2
9: R = H; n = 3

Scheme VI



14
10: R = H; n = 4
11: R = H; n = 6
12: R = H; n = 10

Bis(2-hydroxy-6-isopropyltropon-3-yl)methane (**5**) was prepared from 3-formyl-6-isopropyltropolone[16] (**14**) (Scheme II). Compound **14** was converted to diethyl acetal **15**, which, on heating with hinokitiol (**1b**), gave bis(2-hydroxy-6-isopropyltropon-3-yl)methyl ethyl ether (**16**) accompanied with small amount of tris(2-hydroxy-6-isopropyltropon-3-yl)methane (**17**). Reductive cleavage of the ethoxyl group of **16** with hydroiodic acid in the presence of red phosphorus gave **5**.

1,2-Bis(2-hydroxy-6-isopropyltropon-3-yl)ethane (**6**) was prepared by the self-coupling reaction of 7-(chloromethyl)-4-isopropyl-2-methoxytropone[17] (**19**) (Scheme III). Compound **19** was heated in the presence of activated nickel–zinc catalyst[18] to give bis(4-isopropyl-2-methoxytropon-7-yl)ethane (**20**). Hydrolysis of the methoxyl groups of **20** with potassium hydroxide gave **6**.

Syntheses of 1,ω-bis(2-hydroxy-6-isopropyltropon-3-yl) analogues containing trimethylene, tetramethylene, or pentamethylene groups as a linkage did not succeed by the means presently attempted. Therefore, their isosteric analogues (**7–9**), in which the carbon atom of the linkage portion was replaced by an oxygen or sulfur atom, were synthesized. 1,1′-Bis(2-hydroxy-6-isopropyltropon-3-yl)-dimethyl ether (**7**) was prepared by heating of 7-(chloromethyl)-2-methoxytropone (**19**) with 7-(hydroxymethyl)-2-methoxytropone[17] (**18**) in the presence of potassium hydroxide and phase-transfer catalyst (TDA-1) followed by hydrolysis with hydrochloric acid (Scheme IV). 1,2-Bis[(2-hydroxy-6-isopropyltropon-3-yl)thio]ethane (**8**) was prepared from 7-bromo-4-isopropyl-2-methoxytropone[19] (**22**) (Scheme V). Compound **22** was treated

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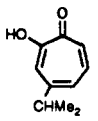
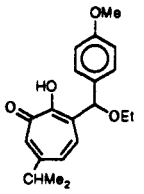
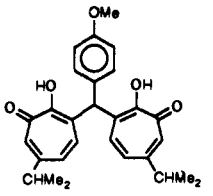
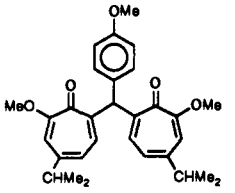
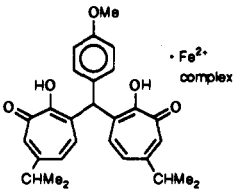
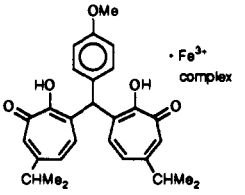
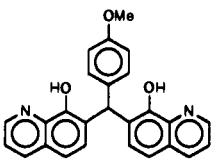
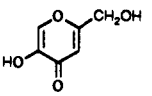
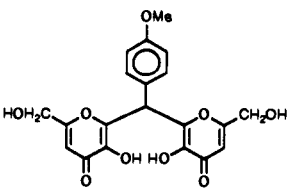
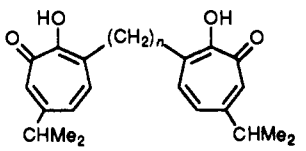
(17) Teitei, T. Amino Acids of the Tropolone Series: The Synthesis of DL-α-Amino-β-(2-hydroxy-5-methyl-3-oxocyclohepta-1,4,6-trienyl)propanoic Acid. *Aust. J. Chem.* **1979**, *32*, 1631–1634.
(18) Colon, I.; Keisey, R. D. Coupling of Aryl Chlorides by Nickel and Reducing Metals. *J. Org. Chem.* **1986**, *51*, 2627–2637.

Table I. Antitumor Activities and Abilities To Induce dNTP Pool Imbalance of Tropolone Derivatives and Others

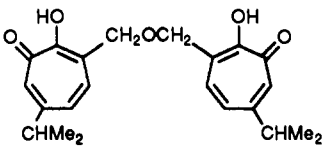
compd	structure	inhibn of KB cells growth IC_{50}, μM	inhibn of FM3A cells growth IC_{50}, μM	dNTP pool		antitumor act. P388 in mice, ip	
				dose, μM	imbalance[a]	doses,[b] mg/kg	T/C, %
1		3.5	1.1	41	+	inactive[c]	
2		1.6	1.1	6.1	+	400 200	140 140
3		1.1	0.4	9.0	+	5 2.5 0.6	173 134 127
31		114	14.1	21	-	inactive	
32		NT[d]	0.3	6.0	+	5 0.6	180 169
33		NT	>199	6.0	-	inactive	
34		<2.1	2.1	41	+	200 100	97 92
35		<0.7	0.01	0.1	+	12.5 6.3 3.1	164 128 111
36		NT	NT	41	-	NT	
37		>249	>249	9.0	-	inactive	

[a] (+) Significant imbalance in the intracellular dNTP pool in FM3A cells was induced at the dose listed. (-) Significant imbalance in the intracellular dNTP pool in FM3A cells was not induced at the dose listed. [b] The dose listed were given once a day for 1 and 5 days. [c] Dose = 400 mg/kg. [d] NT = not tested.

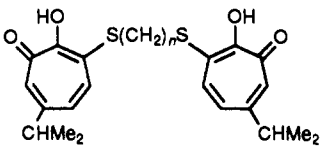
Table II. Antitumor Activities and Abilities To Induce dNTP Pool Imbalance of 1,ω-Bis(2-hydroxy-6-isopropyltropon-3-yl)alkanes



4–6, 10–12



7



8, 9

compd	n	inhibn of KB cells growth IC_{50}, μM	inhibn of FM3A cells growth IC_{50}, μM	dNTP pool dose, μM	dNTP pool imbalance[a]	antitumor act. P388 in mice, ip[b] doses, mg/kg	antitumor act. P388 in mice, ip[b] T/C, %
4	0	9.5	3.7	6.1	+	100	143
						50	171
						25	152
5	1	<0.9	1.0	7.9	+	25	123
						12.5	152
						6.3	137
6	2	8.0	1.2	15.0	+	100	165
						25	150
						6.3	121
7		6.2	0.08	3.5	+	40	156
						20	186
						10	164
8	2	1.6	4.8	16.4	+	400	80
						200	171
						100	151
9	3	1.6	NT[c]	NT		400	169
						200	131
						100	93
10	6	1.5	0.9	7.3	+	400	70
						200	80
						100	128
						50	137
						25	110
11	8	4.3	0.9	6.2	+	400	110
						200	110
						100	107
12	12	27.3	1.3	20.2	+	400	105
						200	107
						100	106
				10.1	−		

[a] See Table I. [b] See Table I. [c] NT = not tested.

with the dipotassium salt of 1,2-ethanedithiol at room temperature to give 1,2-bis[(4-isopropyl-2-methoxytropon-3-yl)thio]ethane (23). Hydrolysis of the methoxyl group of 23 with hydrochloric acid gave 8. Similarly, 1,3-bis[(2-hydroxy-6-isopropyltropon-3-yl)thio]propane (9) was prepared from 22 with 1,3-propanedithiol.

Bistropolone analogues containing linkages longer than a hexamethylene group were prepared via the Grignard reaction, because the Grignard reagents of a 1,ω-dihaloalkane having groups larger than a tetramethylene group are easily prepared (Scheme VI).[20] 3-Formyl-6-isopropyltropolone (14) was treated with the Grignard reagent prepared from 1,4-dibromobutane to give 1,6-bis(2-hydroxy-6-isopropyltropon-3-yl)hexane-1,6-diol (28). The hydroxyl groups of 28 were reduced with hydroiodic acid in the presence of red phosphorus to give 10. Analogues (11 and 12) containing linkages of octamethylene and dodecamethylene groups were prepared by the same method.

Antitumor Activity. All compounds, except iron complexes (32 and 33) of bistropolone 3, listed in Table I have previously been evaluated for growth inhibition of KB cells (in vitro system)[3] and for antitumor activity against leukemia P388 in mice (in vivo system).[3] The iron(II) complex (32) of parent compound 3 showed potent activity equivalent to that of 3. On the other hand, the iron(III) complex (33) of 3 was found to be inactive in both systems. This was a very interesting finding, because ribonucleotide reductase has two iron(III) ions at the two active sites.

The compounds listed in Table II were presently evaluated in the in vitro and in vivo systems. All test compounds (4–12) were found to be cytotoxic in the in vitro system, whereas the in vivo activities of these compounds varied in relation to the variation in length of the linkage between the two tropolone rings. Compounds 4 without linkage and 5–7 containing a short alkyl linkage showed potent antitumor activity at low dose. On the other hand, the potency of compounds 8–12, which have a linkage longer than a tetramethylene group, decreased with increasing length of the linkage between the two tropolone rings. Thus, compounds 8–10 were active but their potencies were approximately equal to that of monotropolone 2. Compounds 11 and 12 did not retain the activity.

Ability To Induce dNTP Pool Imbalance. Parent compound 3 and related compounds were evaluated in mouse mammary tumor FM3A cells (wild type F28-7) for inhibition of cell growth and dNTP pool imbalance. The

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(20) Nenitzescu, D. C.; Necsoiu, I. The Synthesis of Cyclic Alcohols and Olefines by the Interaction of Dimagnesium Halides and Esters. *J. Am. Chem. Soc.* **1950**, *72*, 3483–3486.

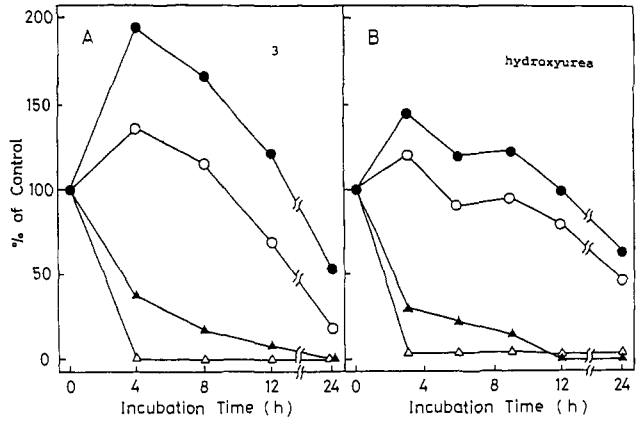

Figure 2. dNTP pool changes in FM3A (F28-7) cells treated with compound **3** or hydroxyurea. F28-7 cells at a density of 2×10^5 cells/mL were treated with 3.7 μM of **3** (A) or 10 mM of hydroxyurea (B). At the indicated times, aliquots of 50 mL were removed and dNTP pools were measured as described in the Experimental Section. (▲) dATP, (△) dGTP, (○) dCTP, (●) dTTP.

IC_{50} and IC_{90} (M) were defined as the concentration of the test compounds required to reduce the growth rate of the control. The compounds were tested for ability to induce dNTP pool imbalance at the concentration of IC_{90}.

Their activities were compared with those of hydroxyurea. Cell culture, preparation of cell extracts, quantitative determination of deoxyribonucleoside triphosphate and cell extracts were used according to the previous work.[21]

The IC_{50} values for bistropolone **3** and hydroxyurea were 0.57 and 60 μM, respectively. In terms of the trypan blue staining, their cell death began at 15 h of exposure to **3**. At 36 h, about 30% of the cells were unstained (i.e. viable).

FM3A cells were seeded into the culture medium at 5×10^4 cells/mL, and when the cell density became 2×10^5 cells/mL, **3** (3.7 μM) or hydroxyurea (10 mM) was added to the culture. Figure 2 shows the cellular dNTP pool as a function of the time of treatment with **3** or hydroxyurea. These treatments resulted in significant changes in the intracellular dNTP pools. With **3**, the dATP pool size at 12 h was about 10% of the zero-time control, and the dGTP pool size became lower than the limit of measurement within 4 h. A large increase in dTTP and a slight increase in dCTP were observed at 4 h. Figure 2 also shows the similarity in the pool changes between the treatments with **3** and hydroxyurea. Intracellular ribonucleoside triphosphate (rNTP) pools did not change with the **3** treatment (data not shown).

Similarly, the other compounds listed in Tables I and II were evaluated for this ability to induce dNTP pool imbalance. As shown in the tables, all compounds having potent cytotoxicity in the in vitro system induced dNTP pool imbalance in a manner similar to that with **3** and hydroxyurea. However, compounds without cytotoxicity did not induce dNTP pool imbalance even at a high concentration.

Discussion

We have previously assumed that antitumor activities of this series of compounds are consequences of their inhibitory activities of ribonucleotide reductase through their metal-chelating property. If they readily inhibit the enzyme, dNTP pool imbalance should be induced. In order to verify this assumption, we presently selected several antitumor-active and inactive compounds among the compounds previously prepared as typical examples and examined their ability to induce dNTP pool imbalance (Table I). We have previously synthesized several binary non-troponoid derivatives including **35**[1,9] and **37**.[5] They have been designed to have an acidic hydroxyl and proton-acceptable groups situated in the near position which permit chelation with a metal. Among them, only compound **35** has potent activity equivalent to that of parent compound **3** in the in vivo system, and the structure–activity relationship for **35** follows the same pattern as in the tropolone series.[1,9] However, all other binary non-troponoid compounds including **37** are inactive in the vitro system as well as in vivo,[5] although they have chelating properties. In the present dNTP pool screening, the antitumor-active compound **35** induced dNTP pool imbalance, while the inactive compound **37** did not induce the imbalance. Moreover, bismethoxytropone **31**,[8] which, of course, has neither chelating properties nor antitumor activity, did not induce dNTP pool imbalance. The present result that there is complete correlation between cytotoxicity and ability to induce dNTP pool imbalance provides evidence that the target enzyme of this series is ribonucleotide reductase.

Bistropolones having various lengths of linkage listed in Table II were synthesized to determine whether or not the distance between the two tropolone rings has an effect on the cytotoxicity or on the ability to induce dNTP pool imbalance or on the in vivo antitumor activity. In the in vitro assays, such as cytotoxicity and dNTP pool imbalance, a significant difference between those compounds was not observed. However, their in vivo antitumor activities varied with the length of the linkage. Thus, compounds **4–6** having a short linkage exhibited potent activity equivalent to that of parent compound **3**. Compounds **8–10** having a moderate length of linkage exhibited weak activity equivalent to that of monotropolone **2**. Compounds **11** and **12** having a linkage longer than an octamethylene group did not retain the activity. These results demonstrated that the distance between the two tropolone rings is an important factor for the in vivo antitumor activity.

From present findings, we assume as follows: Parent compound **3** and other potently active bistropolones in vivo easily bind the two irons at the two active sites of the ribonucleotide reductase which causes complete or irreversible inhibition of the enzyme action. Monotropolones **1** and **2** bind one iron at one active site of the enzyme, which causes partial or reversible inhibition of the enzyme action. The weak activity or inactivity of bistropolones containing long linkages may be due to the fact that their structures are not proper for efficient binding to the two irons of the enzyme. Without other factors associated with drug delivery, it is difficult to correlate the in vivo antitumor activity with inhibitory activity against ribonucleotide reductase. However, it is likely that the difference in the mode of interaction with the enzyme resulted in the difference in the in vivo antitumor activity.

Experimental Section

Chemistry. Melting points were determined on a Yanagimoto micromelting apparatus and are uncorrected. ^1H NMR spectra were run on a Hitachi R-24 60-MHz spectrometer, with Me_4Si as an internal standard. MS spectra were recorded on Shimadzu LKB-9000 or VG-70SE spectrometer. IR spectra were taken on a Nippon Bunko A-102 spectrometer. The elemental analyses (C, H, and S) 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

(21) Tanaka, K.; Yoshioka, A.; Tanaka, S.; Wataya, Y. An Improved Method for the Quantitative Determination of Deoxyribonucleoside Triphosphates in cell Extracts. *Anal. Biochem.* 1984, *139*, 35–41.

on 230–400-mesh silica gel (Merck Silica gel 60).

1,1-Bis(2-hydroxy-6-isopropyltropon-3-yl)-1-ethoxymethane (16). A mixture of 3-formyl-6-isopropyltropolone[16] (14; 2.27 g, 11.8 mmol), triethyl orthoformate (2.05 g, 17.7 mmol), NH_4Cl (0.06 g, 1.1 mmol), and absolute EtOH (30 mL) was heated to reflux for 30 min. The solvent was evaporated off, and the residue was dissolved in dry toluene (30 mL), to which hinokitiol (7; 1.95 g, 11.9 mmol) and potassium *tert*-butoxide (0.12 g, 1.1 mmol) were added. The solution was heated to reflux for 6 h. After removal of the solvent, the residue was made acidic with 10% HCl solution and extracted with AcOEt. The organic extract was washed with water and concentrated. The residue was chromatographed on silica gel (hexane–AcOEt, 3:1 v/v) to give **16** (2.03 g, 49%): mp 140–143 °C; IR (Nujol) 3170 cm^{-1}; ^1H NMR (CCl_4) δ 1.24 (t, 3 H, J = 7 Hz), 1.27 (d, 12 H, J = 7 Hz), 2.65–3.10 (m, 2 H), 3.61 (q, 2 H, J = 7 Hz), 6.27 (s, 2 H), 6.82 (dd, 2 H, J = 10, 1.6 Hz), 7.15 (d, 2 H, J = 1.6 Hz), 7.62 (d, 2 H, J = 10 Hz), 9.25 (br, 2 H); MS m/z 384 (M^+). Anal. Calcd for $C_{23}H_{28}O_5$: C, H. A second elution with hexane–AcOEt (1:1 v/v) gave tris(2-hydroxy-6-isopropyltropon-3-yl)methane (17; 280 mg, 5%): mp 265–268 °C; IR (Nujol) 3150 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.25 (d, J = 7 Hz, 18 H), 2.50–3.15 (m, 3 H), 6.60–7.35 (m, 9 H); MS m/z 502 (M^+). Anal. Calcd for $C_{31}H_{34}O_6$: C, H.

Bis(2-hydroxy-6-isopropyltropon-3-yl)methane (5). A mixture of **8** (1.2 g, 3.1 mmol), 54% HI (10 mL), red phosphorus (0.01 g, 0.3 mmol), and AcOH (20 mL) was stirred at 50 °C for 20 min. The mixture was made basic with cooling in ice bath and extracted with AcOEt. The organic extract was washed with saturated $Na_2S_2O_3$ solution and water. After removal of the solvent, the residue was chromatographed on silica gel (hexane–AcOEt, 3:1 v/v) to give **9** (0.31 g, 29%): mp 97–99 °C; IR (Nujol) 3180 cm^{-1}; ^1H NMR (CCl_4) δ 1.23 (d, 12 H, J = 7 Hz), 2.61–3.05 (m, 2 H), 4.16 (s, 2 H), 6.78 (dd, 2 H, J = 10, 1.6 Hz), 7.09 (d, 2 H, J = 1.6 Hz), 7.88 (d, 2 H, J = 10 Hz), 9.04 (br, 2 H); MS m/z 340 (M^+). Anal. Calcd for $C_{21}H_{24}O_4$: C, H.

1,2-Bis(4-isopropyl-2-methoxytropon-7-yl)ethane (20). A mixture of zinc powder (1.17 g, 17 mmol), NaBr (0.13 g, 1.3 mmol), PPh_3 (1.17 g, 4.5 mmol), anhydrous $NiCl_2$ (0.058 g, 0.45 mmol), and degassed dry *N,N*-dimethylformamide (DMF) (5.4 mL) was stirred at 100 °C and changed to reddish brown.[18] A solution of 7-chloromethyl-4-isopropyl-2-methoxytropone (11; 2.02 g, 8.8 mmol) in degassed dry DMF was added dropwise to the cooled mixture. The reaction mixture was stirred at 70 °C for 1 h and diluted with $CHCl_3$. The $CHCl_3$ solution was washed with water and concentrated. The residue was chromatographed on silica gel with AcOEt to give **20** (0.40 g, 23%): mp 114–117 °C; ^1H NMR ($CDCl_3$) δ 1.21 (d, 12 H, J = 7 Hz), 2.41–3.02 (m, 2 H), 3.00 (s, 4 H), 3.88 (s, 6 H), 6.58 (dd, 2 H, J = 10, 1.6 Hz), 6.61 (d, J = 1.6 Hz), 7.34 (d, 2 H, J = 10 Hz); MS m/z 382 (M^+). Anal. Calcd for $C_{24}H_{30}O_4$: C, H.

1,2-Bis(2-hydroxy-6-isopropyltropon-3-yl)ethane (6). A mixture of **20** (200 mg, 0.52 mmol), 10% aqueous KOH (20 mL), and MeOH (20 mL) was heated to reflux for 3 h, made acidic with 10% HCl solution, and extracted with AcOEt. The organic extract was washed with water and concentrated. The residue was crystallized from AcOEt–Et_2O to give **6** (53 mg, 25%): mp 152–155 °C; IR 3150 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.25 (d, 12 H, J = 7 Hz), 2.33–3.08 (m, 2 H), 3.10 (s, 4 H), 6.01 (dd, 2 H, J = 10, 1.6 Hz), 7.29 (d, 2 H, J = 1.6 Hz), 7.40 (d, 2 H, J = 10 Hz), 9.03 (br, 2 H); MS m/z 354 (M^+). Anal. Calcd for $C_{22}H_{26}O_4$: C, H.

Bis[(4-isopropyl-2-methoxytropon-7-yl)methyl] Ether (21). A mixture of 7-(hydroxymethyl)-4-isopropyl-2-methoxytropone[17] (18; 9.60 g, 46.1 mmol), powder KOH (5.18 g, 92.3 mmol), tris(3,6-dioxaheptyl)amine (TDA-1; 310 μL), and dry CH_2Cl_2 (110 mL) was stirred at room temperature for 6 h and washed with water. The solvent was evaporated. The resulting precipitates were recrystallized from AcOEt–EtOH to give **21** (1.70 g, 53%): mp 120–123 °C; ^1H NMR ($CDCl_3$) δ 1.32 (d, 12 H, J = 7 Hz), 2.50–3.15 (m, 2 H), 3.99 (s, 6 H), 4.63 (s, 4 H), 6.78 (d, 2 H, J = 1.6 Hz), 6.91 (dd, 2 H, J = 10, 1.6 Hz), 7.68 (d, 2 H, J = 10 Hz); MS m/z 354 (M^+). Anal. Calcd for $C_{22}H_{30}O_5$: C, H.

Bis[(2-hydroxy-6-isopropyltropon-3-yl)methyl] Ether (7). A mixture of **21** (1.7 g, 4.3 mmol), THF (10 mL), and 10% HCl (15 mL) was heated to reflux for 1 h and extracted with AcOEt. The organic extract was washed with water and concentrated. The residue was chromatographed on silica gel (hexane–AcOEt, 1:1 v/v) to give **7** (1.25 g, 80%): mp 160–162 °C; IR (Nujol) 3220 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.24 (d, 12 H, J = 7 Hz), 2.68–3.14 (m, 2 H), 4.82 (s, 4 H), 7.01 (dd, 2 H, J = 10, 1.6 Hz), 7.34 (d, 2 H, J = 1.6 Hz), 7.87 (d, 2 H, J = 10 Hz), 8.00 (br, 2 H). Anal. Calcd for $C_{22}H_{26}O_5$: C, H.

1,2-Bis[(4-isopropyl-2-methoxytropon-7-yl)thio]ethane (23). Potassium *tert*-butoxide (2.16 g, 19.5 mmol) was added to a solution of ethanedithiol (970 μL, 1.67 mmol) in absolute MeOH (10 mL) at 0 °C for 30 min. The reaction mixture was added dropwise to a solution of 7-bromo-2-methoxy-4-isopropyltropone[19] (22; 6 g, 2.34 mmol). The resultant mixture was stirred at room temperature for 6 h and diluted with CH_2Cl_2. The CH_2Cl_2 solution was washed with water and concentrated. The residue was chromatographed on silica gel with AcOEt to give **23** (2.7 g, 52%): viscous oil; ^1H NMR ($CDCl_3$) δ 1.26 (d, 12 H, J = 7 Hz), 2.80–3.30 (m, 2 H), 3.14 (s, 6 H), 6.81 (dd, 2 H, J = 10, 1.6 Hz), 6.86 (d, 2 H, J = 1.6 Hz), 7.34 (d, 2 H, J = 10 Hz); FAB-MS (positive ion mode) m/z 447 ($M^+ + 1$).

1,3-Bis[(4-isopropyl-2-methoxytropon-7-yl)thio]propane (24) was prepared in a similar manner that described for the synthesis of **23**: viscous oil; yield 54%; ^1H NMR ($CDCl_3$) δ 1.24 (d, 12 H, J = 7 Hz), 1.65–2.30 (m, 2 H), 2.60–3.15 (m, 6 H), 3.95 (s, 6 H), 6.78 (dd, 2 H, J = 10, 1.6 Hz), 6.82 (d, 2 H, J = 1.6 Hz), 7.24 (d, 2 H, J = 10 Hz); FAB-MS (positive ion mode) m/z 461 ($M^+ + 1$).

1,2-Bis[(2-hydroxy-6-isopropyltropon-3-yl)thio]ethane (8). A mixture of **23** (1.30 g, 2.91 mmol), 10% HCl (15 mL), and THF (10 mL) was heated to reflux for 30 min and extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with water and concentrated. The resulting precipitates were recrystallized from AcOEt–EtOH to give **8** (280 mg, 23%): mp 148–150 °C; IR (Nujol) 3210 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.30 (d, 12 H, J = 7 Hz), 2.65–3.10 (m, 2 H), 3.27 (s, 4 H), 6.98 (dd, 2 H, J = 10, 1.6 Hz), 7.38 (d, 2 H, J = 1.6 Hz), 7.53 (d, 2 H, J = 10 Hz); FAB-MS (positive ion mode) m/z 419 ($M^+ + 1$). Anal. Calcd for $C_{22}H_{26}O_4S_2$: C, H, S.

1,3-Bis[(2-hydroxy-6-isopropyltropon-3-yl)thio]propane (9) was prepared in the similar manner described for the synthesis of **8**: mp 145–147 °C; yield 40%; IR (Nujol) 3200 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.31 (d, 12 H, J = 7 Hz), 1.60–2.54 (m, 2 H), 2.56–3.30 (m, 6 H), 7.05 (dd, 2 H, J = 10, 1.6 Hz), 7.39 (d, 2 H, J = 1.6 Hz), 8.02 (d, 2 H, J = 10 Hz); FAB-MS (positive ion mode) m/z 433 ($M^+ + 1$). Anal. Calcd for $C_{23}H_{28}O_4S_2$: C, H, S.

1,6-Bis(2-hydroxy-6-isopropyltropon-3-yl)hexane-1,6-diol (28). Magnesium turning (5.9 g, 243 mmol) and dry THF (30 mL) were stirred under an Ar atmosphere. 1,4-Dibromobutane (7.3 mL, 60.9 mmol) was added dropwise at 0 °C, and the mixture was heated to reflux for 1 h. The resulting supernatant was added to a solution of 3-formyl-6-isopropyltropolone (14; 3.9 g, 20.3 mmol) in dry THF (20 mL). The reaction mixture was stirred at room temperature for 10 min, made acidic with 10% HCl solution, and extracted with AcOEt. The organic extract was washed with water and concentrated. The residue was chromatographed on silica gel (hexane–AcOEt, 1:1 v/v) to give **28** (1.6 g, 35%): mp 92–93 °C; IR (Nujol) 3390 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.24 (d, 12 H, J = 7 Hz), 1.57 (s, 8 H), 2.43–2.97 (m, 2 H), 4.83–5.07 (m, 2 H), 5.60 (br, 4 H), 6.90 (dd, 2 H, J = 10, 1.6 Hz), 7.23 (d, 2 H, J = 1.6 Hz), 7.60 (d, 2 H, J = 10 Hz); MS m/z 443 (M^+), 407 ($M^+ - 36$). Anal. Calcd for $C_{26}H_{34}O_6$: C, H.

Compounds **29** and **30** were prepared in the similar manner.

1,8-Bis(2-hydroxy-6-isopropyltropon-3-yl)octane-1,8-diol (29): viscous oil; yield 39%; IR (neat) 3410 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.30 (d, 12 H, J = 7 Hz), 1.35 (s, 12 H), 2.68–3.14 (m, 2 H), 4.87–5.07 (m, 2 H), 6.07 (br, 4 H), 7.00 (dd, 2 H, J = 10, 1.6 Hz), 7.25 (d, 2 H, J = 1.6 Hz), 7.60 (d, 2 H, J = 10 Hz); MS m/z 434 ($M^+ - 36$).

1,12-Bis(2-hydroxy-6-isopropyltropon-3-yl)dodecane-1,12-diol (30): viscous oil; yield 29%; IR (neat) 3410 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.25 (s, 20 H), 1.26 (d, 12 H, J = 7 Hz), 2.58–3.11 (m, 2 H), 4.91–5.12 (m, 2 H), 6.14 (br, 4 H), 7.00 (dd, 2 H, J = 10, 1.6 Hz), 7.30 (d, 2 H, J = 1.6 Hz), 7.68 (d, 2 H, J = 10 Hz); MS m/z 490 ($M^+ - 36$).

1,6-Bis(2-hydroxy-6-isopropyltropon-3-yl)hexane (10). Red phosphorus (0.16 g, 0.5 mmol) and 54% HI (6 mL) were added to a solution of **28** (2.20 g, 5.04 mmol) in AcOH (30 mL). The mixture was stirred at 50 °C for 30 min, made basic with saturated

K_2CO_3 solution, and extracted with AcOEt. The organic extract was washed with saturated $Na_2S_2O_3$ solution and water, and then the solvent was evaporated. The residue was chromatographed on silica gel (hexane–AcOEt, 1:1 v/v) to give **10** (0.73 g, 35%): mp 90–92 °C; IR (Nujol) 3170 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.31 (d, 12 H, J = 7 Hz), 1.62 (s, 8 H), 2.69–3.28 (m, 6 H), 6.98 (dd, 2 H, J = 10, 1.6 Hz), 7.40 (d, 2 H, J = 1.6 Hz), 7.56 (d, 2 H, J = 10 Hz), 9.14 (br, 2 H); MS m/z 410 (M^+). Anal. Calcd for ($C_{26}H_{34}O_4$) C, H.

Compounds **11** and **12** were prepared in the similar manner.

1,8-Bis(2-hydroxy-6-isopropyltropon-3-yl)octane (11): mp 69–72 °C; yield 58%; IR (Nujol) 3180 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.36 (s, 12 H), 1.38 (d, 12 H, J = 7 Hz), 2.75–3.21 (m, 6 H), 6.95 (dd, 2 H, J = 10, 1.6 Hz), 7.37 (d, 2 H, J = 1.6 Hz), 7.44 (d, 2 H, J = 10 Hz), 9.01 (br, 2 H); MS m/z 438 (M^+). Anal. Calcd for $C_{28}H_{38}O_4$: C, H.

1,12-Bis(2-hydroxy-6-isopropyltropon-3-yl)dodecane (12): mp 35–37 °C; yield 46%; IR (Nujol) 3190 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.23 (d, 12 H, J = 7 Hz), 1.28 (s, 20 H), 2.65–3.10 (m, 6 H), 6.88 (dd, 2 H, J = 10, 1.6 Hz), 7.30 (d, 2 H, J = 1.6 Hz), 7.47 (d, 2 H, J = 10 Hz), 9.01 (br, 2 H); MS m/z 494 (M^+). Anal. Calcd for $C_{32}H_{46}O_4$: C, H.

α,α-Bis(2-hydroxy-6-isopropyltropon-3-yl)-4-methoxytoluene–Fe(II) Complex (32). A solution of $FeSO_4 \cdot 7H_2O$ (800 mg, 2.9 mmol) in degassed water (5 mL) was added dropwise to a solution of **3** (200 mg, 0.45 mmol) in degassed $CHCl_3$ (5 mL). The mixture was stirred for 20 min at room temperature under an Ar atmosphere. The $CHCl_3$ solution was separated, washed with water, and concentrated. The resulting precipitate was filtered and dried in vacuo at 50 °C to give a quantitative yield of **32** as an ocher precipitate: mp 165–168 °C.

α,α-Bis(2-hydroxy-6-isopropyltropon-3-yl)-4-methoxytoluene–Fe(III) Complex (33). A solution of $FeCl_3 \cdot 6H_2O$ (240 mg, 0.89 mmol) in MeOH (15 mL) was added dropwise to a solution of **3** (600 mg, 1.34 mmol) in $CHCl_3$ (10 mL) and MeOH (10 mL). The mixture was stirred for 5 min at room temperature. After removal of the solvent, the residue was dissolved in $CHCl_3$. The $CHCl_3$ solution was washed with water and concentrated to give a quantitative yield of **33** as a reddish brown precipitate: mp >300 °C.

Pharmacology. Materials. The compounds listed in Tables I and II were synthesized as previously described.[1-3,8] Mouse mammary tumor FM3A cells (wild type, F28-7) were generously given by Dr. T. Seno (Saitama Cancer Center Research Institute, Japan).

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

Assay for Ability To Induce dNTP Pool Imbalance. Cell Culture. FM3A cells were grown in ES medium containing 2% heat-inactivated fetal bovine serum at 37 °C under 5% CO_2.[22] The cell cultures were maintained by twice weekly passage into fresh media. Cell numbers were measured using a micro cell counter CC-108 (Toa Medical Electric Co.). For growth-inhibition studies, cells were seeded at about 5×10^4 cells/mL and treated with various concentrations of the drugs. Cell numbers were determined 48 h after the start of incubation. IC_{50} value refers to the concentration of drug necessary to reduce the growth rate of cells by 50% of the control. Cell viability during treatment with test compound was determined by staining with trypan blue.

Preparation of Cell Extract. Test compound or hydroxyurea was added to an exponentially growing number of cells in suspension culture at the concentration of drug necessary to reduce the growth rate of cells by 90% of the control, respectively. The drug addition was done when the cell density reached 2.5×10^5 cells/mL. The volume of the cell suspension being examined was 1000 mL. At a desired time, an aliquot of 50 mL was removed from the culture bottle and was centrifuged at 100g and at 4 °C for 4 min. The cells thus collected were washed twice with 25 mL of ice-cold phosphate-buffered saline (containing 0.1% glucose) and were suspended at 4 °C in ca. 100 μL of the phosphate-buffered saline. After the cell number was determined, the suspension was transferred to a 1.5-mL microtest tube 3810 (Eppendorf), and cold 100% trichloroacetic acid was added to the suspension to give a final concentration of 0.3 M. The mixture was vortexed and kept for 30 min at 4 °C. After centrifugation, the acid supernatant was separated and added to 1.1 volume of cold Freon-Amine solution (0.5 M trioctylamine in 1,1,2-trichlorotrifluoroethane). The aqueous upper layer containing nucleotides was separated and analyzed by an HPLC procedure for determination of dNTP. The subsequent procedure for the preparation of cell extracts was described previously.[21]

Quantitative Determination of Deoxyribonucleoside Triphosphates in Cell Extract. To 80 μL of cell extract in a 1.5 mL microtest tube were added 20 μL of 20 mM deoxyguanosine and 20 μL of 0.2 M $NaIO_4$. After vortexing and centrifugation (15600g at 4 °C for 10 s), the suspension was incubated at 37 °C for 2 min. The tube was placed on ice, and then 2 μL of 1 M rhamnose and 30 μL of 4 M CH_3NH_2 (neutralized to pH 6.5 with H_3PO_4) were added to the reaction mixture. The suspension was well mixed and centrifuged at 15600g at 4 °C for 10 s. After incubation at 37 °C for 30 min, the sample was cooled on ice.

Chromatography on Partisil-10 SAX (4.6 × 250 mm, Whatman) was done as previously described.[21] HPLC analysis was performed using a Waters 6000A pump with a Waters 440 absorbance detector (using A_{254nm}) and a Hewlett-Packard 3390A integrator. Ribonucleoside triphosphate (rNTP) pools were determined by the method previously described.[21]

Registry No. **1**, 499-44-5; **2**, 92832-11-6; **3**, 92832-17-2; **4**, 137568-73-1; **5**, 137568-74-2; **6**, 137568-75-3; **7**, 137568-76-4; **8**, 137568-77-5; **9**, 137568-78-6; **10**, 137626-09-6; **11**, 137593-86-3; **12**, 137593-87-4; **14**, 137568-79-7; **16**, 137626-10-9; **17**, 136578-31-9; **18**, 137568-80-0; **19**, 137568-81-1; **20**, 137568-82-2; **21**, 137568-83-3; **22**, 137568-84-4; **23**, 137568-85-5; **24**, 137568-86-6; **28**, 137568-87-7; **29**, 137568-88-8; **30**, 137568-89-9; **31**, 137626-11-0; **32**, 137568-90-2; **33**, 137593-88-5; **34**, 148-24-3; **35**, 105192-47-0; **36**, 501-30-4; **37**, 96306-51-3; ribonucleotide reductase, 9040-57-7; ethanedithiol, 540-63-6; propanedithiol, 109-80-8; 1,8-dibromooctane, 4549-32-0; 1,4-dibromobutane, 110-52-1; 1,6-dibromohexane, 629-03-8; hinokitiol, 499-44-5.

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