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Design and Synthesis of Novel C14-Hydroxyl Substituted Triptolide Derivatives as Potential Selective Antitumor Agents

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It has long been considered that the free β hydroxyl group at C14 of triptolide (**1**) is essential to its potent anticancer activity. In this study, we synthesized novel derivatives of **1** with a hydroxyl group substituted by epoxy groups (**4**−**8**) or a five-membered ring (**11**−**13**). Compounds (**4**−**8**) showed significant in vitro anticancer activity although less potent than **1**. Although with an α oxygen configuration at the C14 position, (14*S*)-14,21-epoxytriptolide (**4**) exhibited the highest potency among all these derivatives, clearly challenging the traditional viewpoint on the necessity of C14β-hydroxyl group of compound **1**. Further studies revealed that while displaying broad spectrum in vitro anticancer activity, compound **4** demonstrated prominent selective in vivo anticancer activity, particularly against human ovarian SK-OV-3 and prostate PC-3 cancers with obviously lower toxicity than **1**. Noticeably, compound **4** was also highly effective against multidrug resistant cancer cells. Therefore, our study gives new insights into the structure−activity relationship of **1** and also produces a promising anticancer drug candidate with unique anticancer activities.

Introduction

Tripterygium wilfordii Hook. f. (TWHF[a]), commonly known as Lei Gong Teng (Thunder God Vine), has been used in Traditional Chinese Medicine to treat autoimmune and inflammatory diseases such as rheumatoid arthritis for centuries.[1−3] Triptolide (**1**) (Figure 1), the major component responsible for the clinical properties of TWHF, was first isolated from TWHF extracts and characterized as a diterpenoid triepoxide lactone containing an 18 (4→3) abeo-abietane skeleton in 1972.[4] Right after its isolation, **1** was shown to possess high antileukemic activity in murine models[4] besides its remarkable efficacy in rheumatoid arthritis,[5] and this led to extensive studies regarding its antitumor properties.

Compound **1** induces growth inhibition and apoptosis against various cultured human cancer cell lines[6−9] as well as on primary cultures of human tumor samples.[10,11] In addition to its impressive in vivo activity against murine L-1210 leukemia,[4] **1** could also inhibit the growth of xenografts formed by different solid tumor cells[6,9,12] and the experimental metastasis of B16F10 cells.[9] Meanwhile, **1** was shown to sensitize cells to Apo/Trail, tumor necrosis factor-α, or DNA-damaging chemotherapeutic agents.[13−15] Compared with some conventional chemotherapeutic drugs, **1** has similar or even superior anticancer activity, especially over p53 mutated or multidrug resistant cells.[9] All of these antitumor properties mentioned above suggest that **1** should be a promising anticancer drug, however, its clinical development has been discontinued. One major obstacle for this is the severe toxicity of **1**, and the tissues and organs being inflicted include gastrointestinal tracts, liver, kidney, heart, blood cells, bone marrow, testes, and ovaries as well as skin.[16,17] In addition, the therapeutic window of **1** is so narrow that the toxic dose is just about twice the effective dose[6] or even equal in some reports.[18]

Previous studies on the structure−activity relationship of **1** indicated that the characteristic hydrogen-bonded C9,C11-epoxy-C14β-hydroxy system may account for its antitumor effect,[19] meanwhile, the configuration of the hydroxyl group should be the β orientation.[2,20] On the basis of these principles, for a long time the aim of C14 modification was just to improve the lead compound's water solubility by carboxylation of C14β−OH to introduce water solubility enhancing moieties.[21,22] However, most of these derivatives are enzymatically converted into **1** in vivo, thus retaining many of its side effects. For example, 14-succinyl triptolide sodium salt (PG490-88)[23] (Figure 1), a water-soluble prodrug converted to **1** in serum, entered into phase I clinical trials in Europe for the treatment of solid tumors in 2003,[23] but later this clinical trial was discontinued.[24] On the other hand, some medicinal chemists have found recently that substituting C14-hydroxyl with other groups such as fluoride while keeping β orientation of the substituents could retain the cytotoxicity of **1**,[25] and the anticancer activity of compound **1** analogues may not be

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[a] Abbreviations: TWHF, *Tripterygium wilfordii* Hook. f.; SRB, sulforhodamine B; MDR, multidrug resistance; VER, verapamil; ADR, adriamycin; VCR, vincristine; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; RTV, relative tumor volume.

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explained as simply as before.[19] Therefore, there is still big confusion on the role of C14-hydroxyl and any derivative of **1** that retains its anticancer activity but lowers risk in toxicity is still the concern of anticancer drug development.

In this study, to explore whether the C14β-hydroxyl group of **1** is faithfully critical to its anticancer activity and to find novel C14 substituted compound **1** derivatives as more promising antitumor drug candidates, we designed two series of C14-spiro-triptolide derivatives with the C14-hydroxyl substituted by a chiral epoxy group or five-membered ring, which were impossible to form intramolecular hydrogen bond and assessed their anticancer activity. Our results significantly challenge the traditional learnings and, in particular, give new insights into the structure−activity relationship of **1**. Moreover, we discovered that a novel derivative of **1**, compound **4**, possesses unique selective in vivo anticancer activity and potential low systematic toxicity, which favorably makes it a very promising anticancer drug candidate.

Chemistry

The synthetic strategy followed for the preparation of the analogues **4**−**8** and **10**−**13** of compound **1** is depicted in Schemes 1 and 2. We used triptonide (**3**), which was extracted from TWHF of our region, as starting material and initially focused our attention on introducing another epoxide group at C14 position in order to obtain a series of 14,21-epoxytriptolide derivatives (Scheme 1). Quad-epoxy compound **4** was obtained from triptonide through a modified Corey−Chaykovsky methylenation reaction.[26] We found that treatment of triptonide with NaH and Corey methylenation reagent in anhydrous DMSO at room temperature cleanly furnished **4** in 85% yield, while in the presence of *t*-BuOK under the same reaction conditions provided a 61:39 mixture of **4** and its diastereoisomer **5**. Nucleophilic ring-opening at the C12 position of compound **4** by 1.67% HCl in acetone provided chloride **6**. Alternatively, treatment of quad-epoxy **4** with SeO_2 in 1,4-dioxane under reflux introduced a hydroxyl group at C5 to give compound **7** as the main product (50% yield) and a dehydration compound **8** in 10% yield. The absolute stereochemistry of C14 in compound **4** was determined by X-ray crystallography (Figure 2).

Then, we switched to the construction of the five-membered ring derivatives of **1**. Treatment of triptonide with (isopropoxy-dimethylsilyl)methylmagnesium chloride followed by Tamao oxidation[27] provided C14β-hydroxymethylepitriptolide **10**. Modifying the two hydroxyl groups of **10** by cyclic sulfitation with thionyl chloride resulted in two diastereoisomers **11** and **12**, which are different in the configuration of sulfur atom. They can both be oxidized with $NaIO_4$ and a catalytic amount of $RuCl_3 \cdot 3H_2O$ to give sulfate **13** as the sole

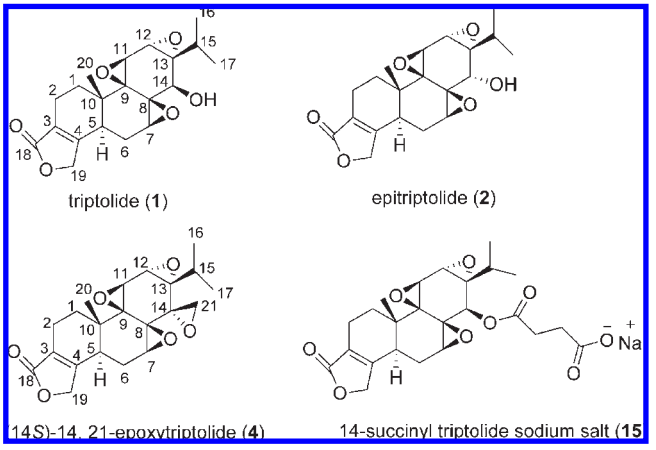


Figure 1. Triptolide, epitriptolide, (14*S*)-14,21-epoxytriptolide and 14-succinyl triptolide sodium salt.

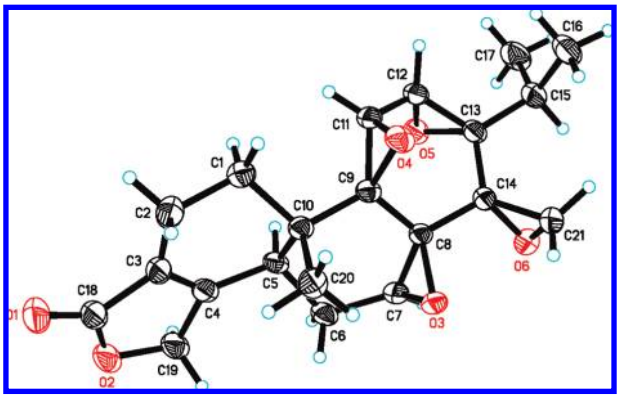
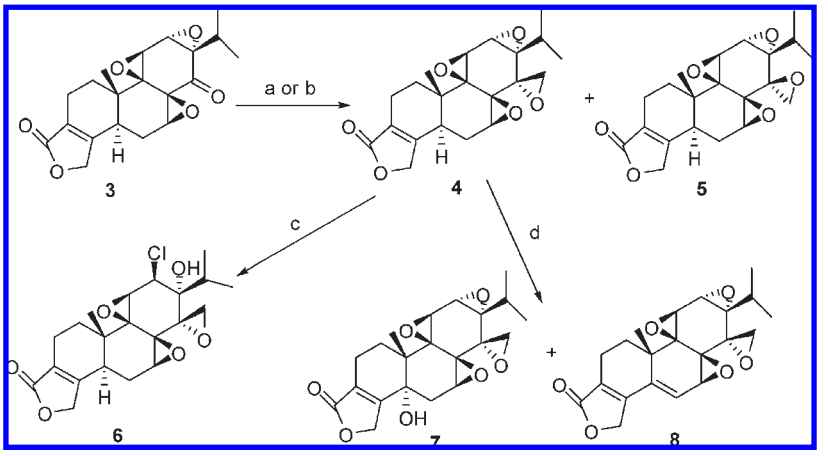


Figure 2. X-ray single crystal structure of (14*S*)-14,21-epoxytriptolide (**4**).

Scheme 1. Synthesis of Derivatives **4**, **5**, **6**, **7**, and **8**[a]



[a] Reagents and conditions: (a) $(CH_3)_3SOI$, NaH, DMSO, rt, 20 min, 85% (exclusively **4**); (b) $(CH_3)_3SOI$, *t*-BuOK, DMSO, rt, 20 min, 65%, (**4**/**5** = 1.6:1); (c) 1.67% HCl aq, acetone, reflux 7 h, 39%; (d) SeO_2, 1,4-dioxane, reflux 24 h, 60% (**7**/**8** = 5:1).

product. The absolute configuration of the sulfur atom in **11** was determined by X-ray crystallography (Figure 3). Herein, we also found another stereospecific route to synthesize compound **4** from dihydroxy **10** in two steps. Selective mesylation of the primary alcohol in **10** followed by cyclization with K_2CO_3 in MeOH solely afforded quad-epoxy compound **4** (Scheme 2).

Results and Discussion

Substitution of the C14*β*-Hhydroxyl Group of 1 with a Chiral Epoxy Group Retains Its in Vitro Anticancer Activity. As shown above, we obtained two series of new compound **1** analogues by substituting its C14*β*-hydroxyl group. To examine whether the substitution affected their biological activities, we first evaluated the in vitro anticancer effects of those target compounds (**4**−**8** and **11**−**13**) against three human tumor cell lines derived from ovary (SK-OV-3), breast (MDA-MB-468), and prostate (PC-3) using sulforhodamine B (SRB) assays.[28] The result revealed that although there is no free C14*β*-hydroxyl group in our new derivatives, the series of 14,21-epoxytriptolide derivatives were all effective against those three cell lines, with IC_{50} values ranging from 0.10 to 7.34 μM (Table 1). Among them, compound **4** exhibited the highest potency, with the lowest IC_{50} value (0.10 μM for SKOV-3 cells) (Table 1). Unexpectedly, compound **5**, the oxygen's direction of which was the same with that of **1**, exhibited much less potency than its

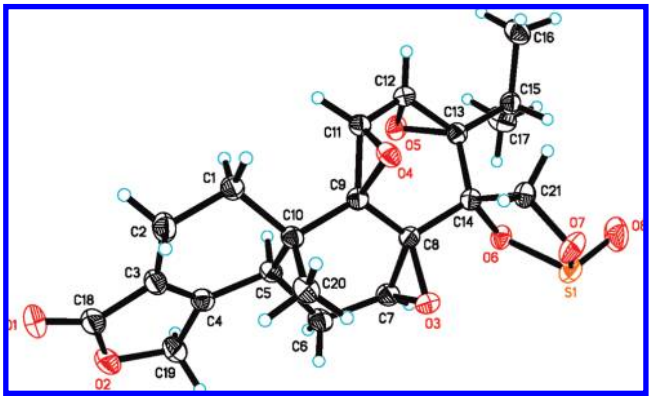


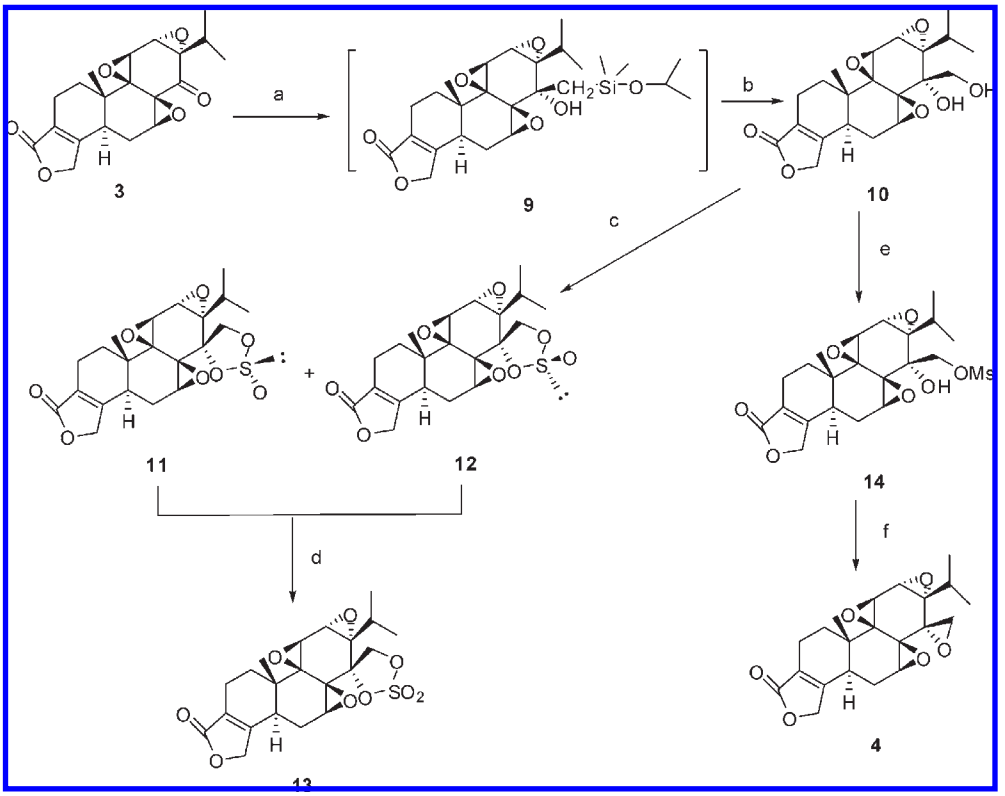
Figure 3. X-ray single crystal structure of (14*S*,S_S)-14-spiro-14α,21-sulfinyldioxytriptolide (**11**).

Table 1. In Vitro Anticancer Activity of Compound **1** Derivatives in SK-OV-3, MDA-MB-468, and PC-3 Cells

	IC_{50} (μM)[a]		
compd	SK-OV-3	MDA-MB-468	PC-3
1	0.009	0.01	0.02
2	0.79	1.32	1.60
4	0.10	0.18	0.27
5	1.56	1.92	2.70
6	0.14	0.21	0.47
7	1.86	2.37	7.34
8	0.57	1.14	1.80
10	> 100	> 100	> 100
11	> 100	> 100	20.01
12	5.10	> 100	6.52
13	> 100	> 100	13.93

[a] IC_{50}: The drug concentration required for 50% inhibition of cell proliferation, while the maximum concentration used here was 100 μM.

Scheme 2. Synthesis of Derivatives **4**, **10**, **11**, **12**, **13**, and **14**[a]



[a] Reagents and conditions: (a) (isopropoxy-dimethylsilyl) methyl chloride, Mg, Br-$(CH_2)_2$-Br, THF, reflux 45 min; (b) KF, $KHCO_3$, 30% H_2O_2, 0 °C, 2 h, 60% over 2 steps; (c) $SOCl_2$, Et_3N, CH_2Cl_2, 0 °C, 2 h, 79%, (**11**/**12** = 1.2:1); (d) $NaIO_4$, $RuCl_3 \cdot 3H_2O$, CH_3CN/H_2O = 4:1, rt, 15 min, 86%; (e) MsCl, Py, rt, 10 min, 84%; (f) K_2CO_3, CH_3OH, rt, 20 min, 90%.

diastereoisomer compound **4** even though the latter possesses an oxygen atom of α direction on C14. Compound **6**, which could easily transform to compound **4**, showed similar but a little less cytotoxicity against those three cell lines. The data indicates that the C14β-hydroxyl group of **1** is not unchangeable even in order to generate analogues of potent anticancer activity. Our result apparently challenges the classical structure−activity relationship of **1** that considers the C14β-hydroxyl group to be essential for its anticancer activity.[2,19,20] In the meantime, our result shows that introduction of a chiral epoxy group to this important site of the lead compound is an effective modification method to retain the potent anticancer activity.

On the other hand, the series of analogues (**11**, **12**, **13**) with five-membered spiro sulfate or sulfite substituents on C14 nearly completely lost their cytotoxicity against MDA-MB-468, and only compound **12** retained weak potency against SKOV-3 and PC-3 cell lines. We presumed that the large space taken by C14-five-membered ring might form a spatial obstacle that prevented the interaction between these derivatives and their target molecule(s) responsible for initiating their cytotoxic effects.

Table 2. IC_{50} Calues of Compound **4** and **1** in a Panel of Human Tumor Cell Lines

		IC_{50}(mean ± SD)[a], μM	
origins	cell lines	compd **4**	compd **1**
blood	Molt-4	0.251 ± 0.077	0.017 ± 0.002
	K562	0.421 ± 0.125	0.050 ± 0.010
lung	A549	0.702 ± 0.228	0.059 ± 0.012
stomach	SGC-7901	0.175 ± 0.032	0.015 ± 0.0004
	MKN-28	2.538 ± 0.215	0.200 ± 0.042
colon	HCT-116	0.219 ± 0.037	0.010 ± 0.003
	SW1116	0.907 ± 0.301	0.052 ± 0.023
	HCT-15	0.648 ± 0.218	0.029 ± 0.006
liver	SMMC-7721	0.350 ± 0.020	0.018 ± 0.004
	BEL-7402	0.332 ± 0.069	0.020 ± 0.005
kidney	786-O	0.386 ± 0.200	0.022 ± 0.009
breast	MDA-MB-231	0.280 ± 0.067	0.024 ± 0.002
	MCF-7	0.360 ± 0.076	0.019 ± 0.002
ovary	SK-OV-3	0.270 ± 0.023	0.010 ± 0.001
	HO-8910	0.538 ± 0.059	0.028 ± 0.007
prostate	PC-3	0.637 ± 0.218	0.043 ± 0.012
	DU-145	0.256 ± 0.024	0.024 ± 0.009
others	KB	0.615 ± 0.120	0.043 ± 0.019
	Rh30	0.293 ± 0.064	0.014 ± 0.001
	Hela	0.507 ± 0.195	0.047 ± 0.040
	U251	0.657 ± 0.235	0.049 ± 0.015

[a] IC_{50} (mean ± SD): Cells in 96-well plates were treated with various concentrations of compound **4** for 72 h to get the IC_{50} values which were determined from dose−response curves, using MTT or SRB assays; IC_{50} values were presented as mean ± SD of three independent experiments.

Compound 4 Elicits Broad-Spectrum in Vitro Antitumor Effects. To characterize the anticancer activity of compound **4**, we further extended its in vitro anticancer evaluation to a panel of 21 human tumor cell lines, which come from distinct human tissues, including leukemia, lung cancer, gastric cancer, hepatoma, colon cancer, breast cancer, ovarian cancer, prostate cancer, renal cancer, cervical cancer, rhabdomyosarcoma, and glioma cells. The data showed that compound **4** exhibited a similar in vitro anticancer spectrum but reduced anticancer activities when compared to compound **1** (Table 2). Compound **4** displayed apparent cytotoxicity with IC_{50} values ranging from 0.18 to 0.91 μM against almost all of the above human tumor cell lines, except that this compound appeared to be relatively less effective against MKN-28 with an IC_{50} value of 2.54 μM. The results indicate that compound **4** possesses a broad anticancer spectrum of in vitro anticancer actions.

Compound 4 Produces Direct Cytotoxic Effects on Multidrug Resistant Cancer Cell Lines. Multidrug resistance (MDR), especially to drugs of natural origin, is an important impediment to the effective chemotherapy of cancer. It has always been the focused area to develop new agents to circumvent MDR. To examine the anticancer activity of compound **4** against MDR tumor cells, we used three classical MDR cell lines (K562/A02, KB/VCR, and MCF-7/ADR) that were significantly resistant to the corresponding drugs used to establish these cell lines. The IC_{50} values of compound **4** in these MDR cell lines were 0.74, 0.47, and 0.91 μM, while in their corresponding sensitive cell lines (K562, KB, and MCF-7), its IC_{50} values were 0.42, 0.61, and 0.36 μM, respectively. The results indicated that compound **4** showed approximately equivalent cytotoxicity against each MDR subline as compared with their parental cell lines, with resistance factors of 1.76, 0.77, and 2.53, respectively (Figure 4A). However, the metabolism of compound **4** was not seen via the p-glycoprotein transportation pathway because verapamil (VER), a well-characterized MDR reversal agent, did not increase its cytotoxic effect on KB/VCR cells but enhanced the cytotoxicity of VCR approximately 10-fold as expected (Figure 4B).

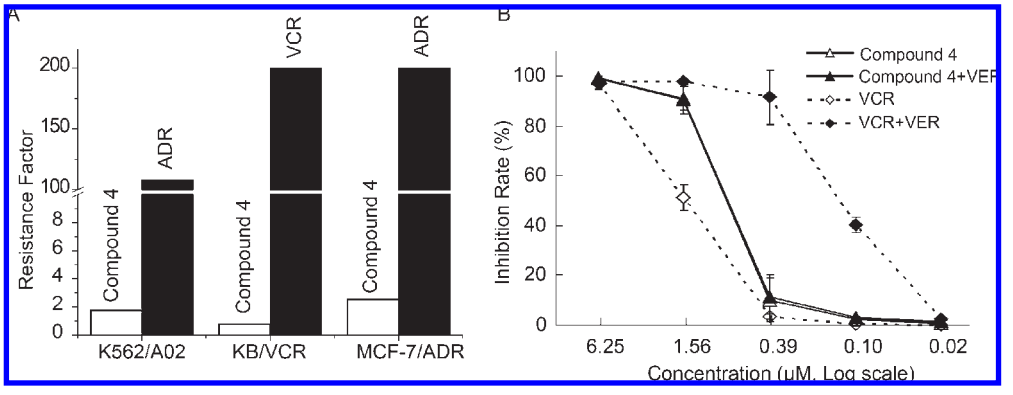


Figure 4. Anti-MDR activity of compound **4**. (A) Resistance factors of compound **4** and reference drugs. K562/A02, KB/VCR, and MCF/ADR cell lines are MDR cell lines derived from K562, KB, and MCF-7 cells, respectively. (B) The effect of VER on the cytotoxicity of compound **4** and VCR in KB/VCR cells. The inhibition rate was assessed by SRB assays, and each data point represents the mean of three independent experiments.

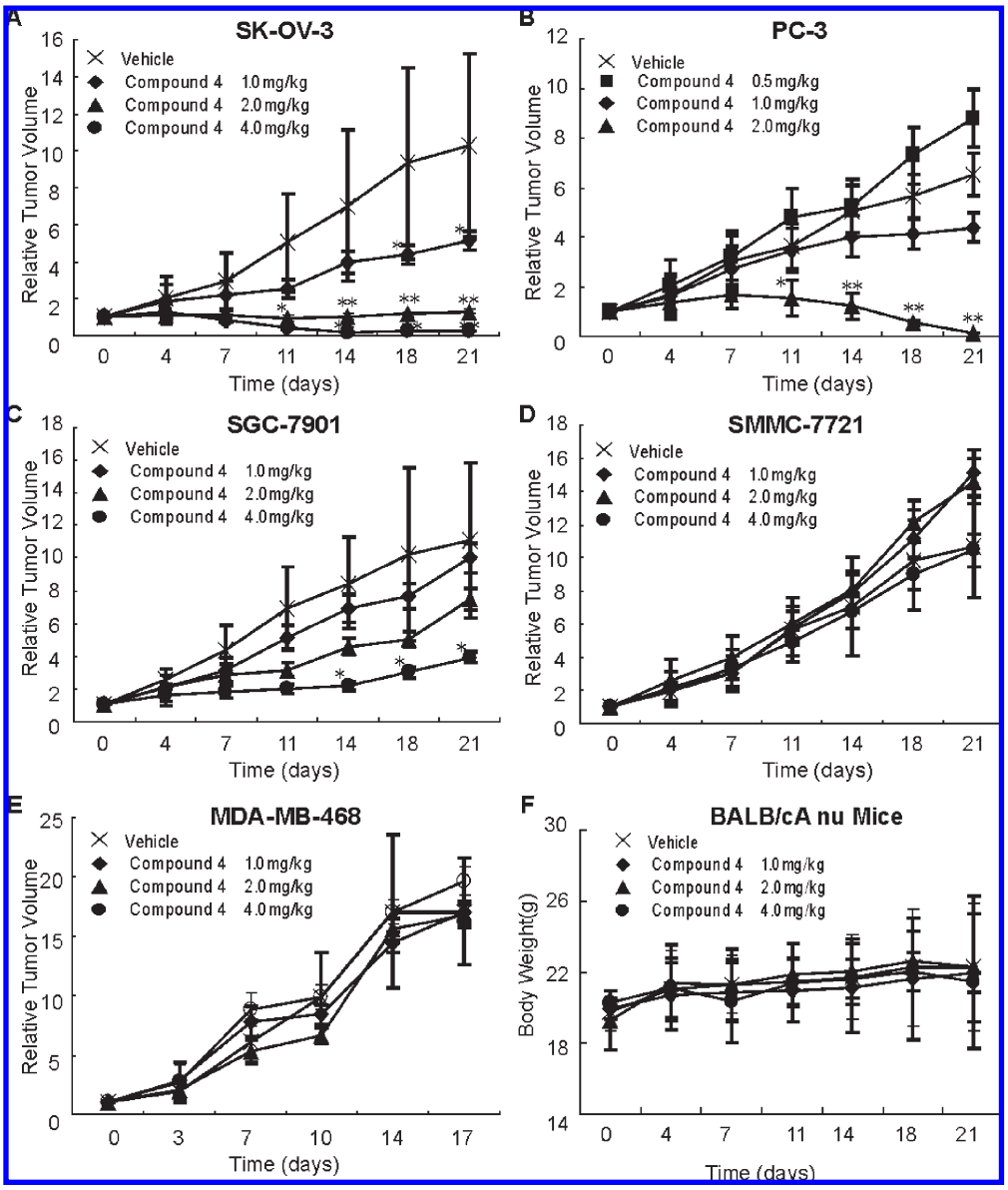


Figure 5. Antitumor effect of compound **4** in nude mouse models. The models were generated as described in the Experimental Section. Compound **4** displayed selective in vivo antitumor activity, as indicated by its special potent effect against ovarian SK-OV-3 (A) and prostate PC-3(B) cancers but moderate action in the SGC-7901 model (C) and no effect in the SMMC-7721 model (D) and in the MDA-MB-468 model (E). No body weight difference was seen between compound **4** and vehicle treated groups and the body weight curves were illustrated in (F). Significant differences between compound **4** treatment groups and control were determined using a Student *t* test. * $P < 0.05$; ** $P < 0.01$.

Compound 4 Exerts Selective in Vivo Anticancer Effects. Compound **1** has been reported to have a wide in vivo anticancer spectrum with apparent effects against human liver,[29] gastric,[9] and breast cancers[6,9] but to reveal a marginal effect against ovarian cancer in vivo.[30] Here we investigated the in vivo anticancer effects of compound **4** against human breast (MDA-MB-468), liver (SMMC-7721), stomach (SGC-7901), ovary (SK-OV-3), and prostate (PC-3) cancer xenografts in nude mice. Surprisingly, the results showed that compound **4**, orally administered, was highly effective against human ovarian and prostate cancer xenografts in contrast to its modest to minimal activity in the models of human gastric, hepatic, and breast cancer xenografts at the same dosage (Figure 5). The treatment with compound **4** resulted in a marked, even total, regression of tumors in the models of SK-OV-3 and PC-3 xenografts on the 21st day post the first drug administration. In the SK-OV-3 model, compound **4** at 2 and 4 mg/kg achieved a tumor growth inhibition rate of 87.5% and 97.4%, respectively. Moreover, tumor xenografts totally disappeared in 3 mice out of 6 at 4 mg/kg and in 1 mouse out of 6 at 2 mg/kg. The similar result was seen in the PC-3 models. However, compound **4** demonstrated relatively weak antitumor effect in the model of gastric cancer SGC-7901 xenografts, with a growth inhibition rate of only 75% even at the maximal dose used (4 mg/kg). More prominently, compound **4** led to no anticancer activity in the models of hepatic cancer SMMC-7721 and breast cancer MDA-MB-468 xenografts. These data indicate that compound **4** elicits selective anticancer effects, specifically against ovarian and prostrate cancers, although the mechanism(s) are still open to be clarified.

Noticeably, compound **4** was well tolerated, not causing death or serious systematic toxicity in mice. The change in the body weight of the tested mice in the groups treated with

compound **4** or with vehicle was basically identical (Figure 5F). There was no distinguishable gross anatomy differences observed in livers or kidneys between the compound **4** treated groups and the vehicle groups. In contrast, the lead compound **1** was reported to cause animal death within doses 4 times[9,31] or twice[6] over the effective doses, and the severe toxicity and narrow therapeutic window limited its clinical development as a anticancer agent.[16,17] Considering that there was a 4-time distance between the lowest effective dosage in SK-OV-3 xenografts model with T/C (%) value of 50.2 (1 mg/kg) and the highest dosage we used (4 mg/kg) in our experiments, compound **4** appears to have a larger safety window than compound **1**. Detailed toxicity evaluations on compound **4** are underway that will be helpful to precisely reveal its safety at least in animals.

Conclusions

A series of novel derivatives of **1** as potential anticancer agents were synthesized and tested for their cytotoxicity against three human tumor cell lines. Previous studies have indicated that the characteristic hydrogen-bonded C9,C11-epoxy-C14β-hydroxy system may account for the antitumor effect of compound **1** by selectively alkylating the thiol groups of key enzymes regulating tumor growth.[19] But in our study, the series of 14,21-epoxytriptolide derivatives (**4**−**8**) with C14-hydroxyl substituted by a chiral epoxy group which were impossible to form intramolecular hydrogen bond still retained moderate to good in vitro anticancer activity, especially compound **4**, with a different oxygen direction at C14 position from that of **1**, exhibited much higher potency than its diastereoisomer compound **5**. On the other hand, the cytotoxicity of C14 five-membered ring substituents analogues (**11**, **12**, **13**) reduced greatly against all three cell lines. On the basis of the above results, we presumed that the size and stereo configuration of functional groups at the C14 position may exert an important influence on interaction between the derivatives of compound **1** and their target molecule(s) responsible for initiating their cytotoxic effects although the exact mechanism is still unclear. Further studies revealed that although demonstrating broad -spectrum in vitro anticancer activity, compound **4** exhibited significant selectivity in its in vivo anticancer activity, as indicated by its specially highly effectiveness against human ovarian and prostate cancers. Moreover, compound **4** showed remarkable cell killing effect on MDR tumor cells. More importantly, its larger safety window seems to make it more applicable. Therefore, this study gives new insights into the structure−activity relationship of **1** and generates a new compound (compound **4**) as a promising anticancer drug candidate.

Experimental Section

Part of Chemistry. Mass spectra and high-resolution mass spectra were measured on a Finnigan MAT-95 mass spectrometer. Elemental analysis was performed on a Carlo Erba 1106 instrument. IR spectra were recorded on a Nicollet Magna FTIR-750 spectrometer using KBr pellets in cm^{-1}. ^1H and ^{13}C NMR spectra were determined on Bruker AM-300 and Bruker AM-400 instruments using tetramethylsilane as internal reference. The X-ray single crystal diffraction experiment was carried out by the Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences. Silica gel 60H (200−300 mesh), manufactured by Qingdao Haiyang Chemical Group Co. (China), was used for general chromatography. HPLC analysis was used a C18 reverse phase column (Agilent Eclipse XDB-C18; 5 μm; 4.6 mm × 250 mm) with an Agilent 1100 series system attached to a Hewlett-Packard chromatograph manager.

(14*S*)-14,21-Epoxytriptolide (4) and (14*R*)-14,21-epoxytriptolide (5). Method A. $(CH_3)_3SOI$ (374 mg, 1.7 mmol) and *t*-BuOK (174 mg, 1.5 mmol) were dissolved in dry DMSO (6.0 mL) under Ar atmosphere. The mixture was stirred for 20 min at room temperature. An anhydrous DMSO (6.0 mL) solution of triptonide (**3**) (360 mg, 1.0 mmol) was added all at once to the above mixture. The mixture was stirred at room temperature until TLC analysis revealed the absence of the starting material. H_2O (10.0 mL) was added to the mixture and then it was extracted with ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated. The crude product was purified first by chromatography on silica gel using ethyl acetate/cyclohexane (1/5). Then we use HPLC to separate the couple of diastereomers. Finally, we gained **4** (0.149 g, 0.40 mmol, 40%) and **5** (0.093 g, 0.25 mmol, 25%) separately.

Method B. $(CH_3)_3SOI$ (74.8 mg, 0.34 mmol) and NaH (60%) (12 mg, 0.3 mmol) were dissolved in dry DMSO (2.5 mL) under Ar atmosphere. The mixture was stirred for 20 min at room temperature. A solution of triptonide (71.6 mg, 0.2 mmol) in dry DMSO (2 mL) was added to the above mixture. The mixture was stirred at room temperature until starting material disappeared. H_2O (4.0 mL) was added to the mixture and then it was extracted with ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated. The crude product was purified by chromatography on silica gel using ethyl acetate/cyclohexane (1/5) to give exclusively **4** as white solide in 85% yield (63.2 mg, 0.17 mmol).

Method C. To a solution of **14** (504 mg, 1.1 mmol) in CH_3OH (20.0 mL) was added K_2CO_3 (1.33 g, 9.6 mmol). The mixture was stirred for 20 min under room temperature. After removal of the solvent under reduced pressure, the residue was diluted with water and then extracted with ethyl acetate, washed with brine, and dried with anhydrous Na_2SO_4. The crude product was purified via chromatography on silica gel using ethyl acetate/cyclohexane (1/5) to give **4** in 90% yield (368 mg, 0.99 mmol).

Compound 4. ^1H NMR($CDCl_3$, 300 MHz) δ 4.67 (s, 2 H), 3.88 (d, J = 2.9 Hz, 1 H), 3.52 (d, J = 3.1 Hz, 1 H), 3.40 (d, J = 5.5 Hz, 1 H), 2.84 (d, J = 5.2 Hz, 1 H), 2.80−2.69 (m, 2 H), 2.37−2.25 (m, 1 H), 2.21−2.05 (m, 2 H), 1.91−1.80 (m, 2 H), 1.57 (dd, J = 12.4, 4.9 Hz, 1 H), 1.29−1.17 (m, 1 H), 1.06 (s, 3 H), 0.86 (d, J = 6.8 Hz, 3 H), 0.80 (d, J = 6.8 Hz, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.2 (C), 160.2 (C), 125.2 (C), 69.9 (CH_2), 65.2 (C), 65.0 (C), 58.4 (C), 56.8 (CH), 55.9 (CH), 55.6 (C), 54.1 (CH), 47.9 (CH_2), 40.4 (CH), 35.6 (C), 30.2 (CH_2), 23.4 (CH_2), 23.2 (CH), 19.8 (CH_3), 17.6 (CH_3), 17.0 (CH_2), 13.5 (CH_3). IR (KBr) 3427, 3016, 2981, 2929, 1745, 1674, 1442, 1022 cm^{-1}. MS (EI, 70 eV) m/z (%) 373 ($[M+1]^+$, 2), 372 (M^+, 1), 357 (5), 343 (21), 91 (100). HRMS (EI) calcd for $C_{21}H_{24}O_6$ 372.1573, found 372.1578. Anal. ($C_{21}H_{24}O_6$) C, H.

Compound 5. ^1H NMR ($CDCl_3$, 300 MHz) δ 4.79 (dt, J = 16.8, 2.6 Hz, 1 H), 4.60 (dd, J = 16.8, 2.8 Hz, 1 H), 3.81 (d, J = 3.1 Hz, 1 H), 3.52 (d, J = 3.1 Hz, 1 H), 3.38 (d, J = 4.2 Hz, 1 H), 2.89 (d, J = 4.7 Hz, 1 H), 2.85 (d, J = 4.7 Hz, 1 H), 2.81−2.72 (m, 1 H), 2.39−2.27 (m, 1 H), 2.17 (dt, J = 14.3, 4.2 Hz, 1 H), 2.11−1.94 (m, 1 H), 1.94−1.81 (m, 2 H), 1.47−1.29 (m, 2 H), 0.96 (s, 3 H), 0.89 (d, J = 6.8 Hz, 3 H), 0.77 (d, J = 6.8 Hz, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.2 (C), 161.6 (C), 125.4 (C), 70.8 (CH_2), 65.9 (C), 64.6 (C), 58.3 (C), 57.2 (CH), 56.2 (CH), 55.9 (C), 54.7 (CH), 48.2 (CH_2), 36.9 (CH), 36.1 (C), 28.4 (CH_2), 26.9 (CH_2), 23.0 (CH), 21.7 (CH_3), 20.2 (CH_3), 17.3 (CH_3), 16.6 (CH_2). IR (KBr) 3433, 2947, 2929, 1768, 1755, 1689, 1442 cm^{-1}. MS (EI, 70 eV) m/z (%) 372 (M^+, 1), 357 (9), 343 (18), 325 (34), 259 (100). HRMS (EI) calcd for $C_{21}H_{24}O_6$ 372.1573, found 372.1586. Anal. ($C_{21}H_{24}O_6$) C, H.

(12*R*,13*R*,14*S*)-12β-Chloro-13α-hydroxy-14,21-epoxytriptolide (6). To a solution of compound **4** (40 mg, 0.11 mmol) in acetone (6.0 mL) was added aq HCl (1.67%, 6.0 mL, 2.7 mmol).

The mixture was gently refluxed for 7 h. After removal of most of the solvent under reduced pressure, the residue was extracted with ethyl acetate, washed with brine, and dried over anhydrous Na_2SO_4. The crude product was purified via chromatography on silica gel using ethyl acetate/cyclohexane (1/3) to provide **6** in 39% yield (17.5 mg, 0.043 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.69 (s, 2 H), 4.16 (d, J = 5.7 Hz, 1 H), 3.75 (d, J = 5.7 Hz, 1 H), 3.46 (d, J = 6.0 Hz, 1 H), 2.93−2.83 (m, 2 H), 2.78 (d, J = 5.1 Hz, 1 H), 2.36−2.25 (m, 1 H), 2.21−2.04 (m, 2 H), 1.94−1.74 (m, 2 H), 1.59 (dd, J = 12.3, 4.7 Hz, 1 H), 1.35−1.21 (m, 1 H), 1.03 (s, 3 H), 0.99 (d, J = 2.2 Hz, 3 H), 0.96 (d, J = 2.2 Hz, 3 H). ^{13}C NMR (acetone-d_6, 100 MHz) δ 173.7 (C), 162.2 (C), 124.6 (C), 76.6 (C), 70.7 (CH_2), 69.9 (C), 60.3 (CH), 59.8 (C), 58.3 (CH), 58.2 (C), 58.2 (CH), 48.0 (CH_2), 40.2 (CH), 35.8 (C), 31.3 (CH_2), 28.8 (CH), 23.1 (CH_2), 18.0 (CH_3), 17.4 (CH_2), 16.3 (CH_3), 13.9 (CH_3). IR (KBr) 3462, 2933, 2252, 1743, 1674, 1439, 1003 cm^{-1}. MS (EI, 70 eV) m/z (%) 408 (M^+, 9), 390 (3), 373 (8), 365 (100). HRMS (EI) calcd for $C_{21}H_{25}ClO_6$ 408.1339, found 408.1339.

(5*R*,14*S*)-5α-Hydroxy-14,21-epoxytriptolide (7) and (14*S*)-$\Delta^{5,6}$-dehydro-14,21-epoxytriptolide (8). To a solution of compound **4** (74.4 mg, 0.20 mmol) in 1,4-dioxane (8.0 mL) was added SeO_2 (111 mg, 1.0 mmol). The mixture was gently refluxed for 24 h. Then the mixture was cooled down to room temperature, filtered through a short pad of silicon gel, and rinsed with ethyl acetate. The solvent was removed under reduced pressure. To the residue was added ethyl acetate and saturated Na_2CO_3. After vigorous extraction, the organic layer was washed with water and brine and dried over anhydrous Na_2SO_4. After concentration, the residue was purified by chromatography on silica gel using CH_2Cl_2 to provide **7** in 50% yield (38.8 mg, 0.1 mmol) and **8** in 10% yield (7.4 mg, 0.02 mmol).

Compound 7. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.92 (dt, J = 17.1, 3.1 Hz, 1 H), 4.71 (dd, J = 17.1, 3.9 Hz, 1 H), 3.92 (d, J = 3.3 Hz, 1 H), 3.63 (d, J = 3.3 Hz,1 H), 3.40 (d, J = 4.8 Hz, 1 H), 2.86 (d, J = 5.1 Hz, 1 H), 2.79 (d, J = 5.1 Hz, 1 H), 2.41−2.04 (m, 4 H), 1.95−1.74 (m, 2 H), 1.29 (dd, J = 12.7, 4.9 Hz, 1 H), 1.12 (s, 3 H), 0.87 (d, J = 6.8 Hz, 3 H), 0.84 (d, J = 6.8 Hz, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.2 (C), 159.0 (C), 128.0 (C), 72.3 (C), 68.7 (CH_2), 65.8 (C), 63.2 (C), 58.4 (C), 56.4 (CH), 55.5 (C), 55.0 (CH), 54.5 (CH), 48.0 (CH_2), 40.6 (C), 31.0 (CH_2), 24.6 (CH_2), 23.4 (CH), 19.7 (CH_3), 17.8 (CH_3), 17.4 (CH_2), 16.2 (CH_3). IR (KBr) 3479, 2956, 1736, 1668, 1442, 1037 cm^{-1}. MS (EI, 70 eV) m/z (%) 389 ($[M + 1]^+$, 2), 373 (2), 359 (100), 341 (18). HRMS (EI) calcd for $C_{21}H_{25}O_7$ $(M + H)^+$ 389.1601, found 389.1606. Anal. ($C_{21}H_{24}O_7$) C, H.

Compound 8. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.03 (d, J = 3.7 Hz, 1 H), 4.95 (d, J = 16.0 Hz, 1 H), 4.83 (dd, J = 16.0, 2.5 Hz, 1 H), 3.86 (d, J = 3.0 Hz, 1 H), 3.56−3.48 (m, 2 H), 2.92 (d, J = 4.9 Hz, 1 H), 2.86 (d, J = 4.9 Hz, 1 H), 2.51−2.40 (m, 1 H), 2.36−2.21 (m, 1 H), 1.90 (sept, J = 6.8 Hz, 1 H), 1.52−1.34 (m, 2 H), 1.29 (s, 3 H), 0.92 (d, J = 6.8 Hz, 3 H), 0.78 (d, J = 6.8 Hz, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.0 (C), 153.1 (C), 140.4 (C), 126.9 (C), 121.8 (CH), 68.9 (CH_2), 65.1 (C), 64.4 (C), 61.3 (C), 56.6 (CH), 55.8 (C), 54.2 (CH), 53.7 (CH), 48.4 (CH_2), 37.2 (C), 30.3 (CH_2), 23.1 (CH), 22.8 (CH_3), 20.3 (CH_3), 17.2 (CH_3), 17.1 (CH_2). IR (KBr) 3433, 2979, 2927, 1747, 1657, 1358, 1024 cm^{-1}. MS (EI, 70 eV) m/z (%) 370 (M^+, 13), 355 (22), 341 (91), 327 (95), 115 (100). HRMS (EI) calcd for $C_{21}H_{22}O_6$ 370.1416, found 370.1404. Anal. ($C_{21}H_{22}O_6$) C, H.

(14*S*)-14β-Hydroxymethylepitriptolide (10). Under Ar atmosphere, a portion (1.0 mL) of a solution of (isopropoxydimethylsilyl) methyl chloride (0.72 mL, 4.0 mmol) in anhydrous THF (7.0 mL) was added to Mg turnings (108 mg, 4.5 mmol). To the stirred mixture was added a few drops of 1,2-dibromoethane at 60 °C and an exothermic reaction started in several minutes. The remaining solution was added dropwise over 5 min. After the addition was completed, the gray mixture was refluxed for 45 min and then cooled to 0 °C. A solution of triptonide (**3**) (358 mg, 1.0 mmol) in anhydrous THF (10.0 mL) was added to the Grignard reagent (freshly prepared) at the same temperature over a few minutes. After stirring at 0 °C for 2 h, the mixture was quenched with a saturated NH_4Cl solution and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give a single adduct. To a stirred mixture of colorless crude adduct, MeOH (5.0 mL), THF (8.0 mL), $KHCO_3$ (416 mg, 4.2 mmol), and KF (464 mg, 4.9 mmol) was added H_2O_2 (30%, 1.1 mL, 9.71 mmol) dropwise at room temperature. The mixture was stirred at room temperature until starting material disappeared. Aqueous sat. $Na_2S_2O_3$ solution (50%) was added slowly to the mixture and stirred until a negative starch/iodide test was observed. The mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The crude product was purified by chromatography on silica gel using ethyl acetate/cyclohexane (1/3) to provide **10** in 60% yield (234 mg, 0.6 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.67 (s, 2 H), 4.26 (d, J = 11.8 Hz, 1 H), 3.87−3.80 (m, 2 H), 3.64 (d, J = 11.5 Hz, 1 H), 3.46 (d, J = 3.3 Hz, 1 H), 2.76−2.64 (m, 1 H), 2.45 (sept, J = 6.9 Hz, 1 H), 2.37−2.25 (m, 1 H), 2.23−2.04 (m, 2 H), 1.89 (t, J = 14.1 Hz, 1 H), 1.55 (dd, J = 12.6, 5.2 Hz, 1 H), 1.25−1.13 (m, 1 H), 1.07 (s, 3 H), 0.91 (d, J = 6.9 Hz, 3 H), 0.89 (d, J = 6.9 Hz, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.2 (C), 160.2 (C), 125.4 (C), 74.4 (C), 70.0 (CH_2), 67.5 (C), 65.3 (C), 65.2 (CH_2), 65.0 (C), 56.5 (CH), 56.1 (CH), 54.4 (CH), 40.3 (CH), 36.0 (C), 30.1 (CH_2), 25.5 (CH), 23.4 (CH_2), 20.9 (CH_3), 18.6 (CH_3), 17.1 (CH_2), 13.7 (CH_3). IR (KBr) 3415, 3361, 2966, 2927, 2875, 1755, 1724, 1672, 1439, 1074, 1018 cm^{-1}. MS (EI, 70 eV) m/z (%) 391 ($[M + 1]^+$, 2), 372 (1), 71 (100). HRMS (EI) calcd for $C_{21}H_{27}O_7$ $(M + H)^+$ 391.1757, found 391.1752. Anal. ($C_{21}H_{26}O_7$) C, H.

(14*S*,S_S)-14-Spiro-14α,21-sulfinyldioxytriptolide (11) and (14*S*,S_R)-14-Spiro-14α,21-sulfinyldioxytriptolide (12). To a solution of compound **9** (78 mg, 0.2 mmol) in anhydrous CH_2Cl_2 (6.0 mL) was added dry Et_3N (0.21 mL, 1.6 mmol) dropwise. The mixture was then cooled to 0 °C. Under Ar atmosphere, $SOCl_2$ (0.3 mL, 1.2 mmol) was added to the mixture carefully. After being stirred for 2 h, the mixture was quenched with water and extracted with CH_2Cl_2. The organic layer was washed with water and brine and dried over anhydrous Na_2SO_4. After removal of the solvent under reduced pressure, the crude product was purified by chromatography on silica gel using acetate/cyclohexane (1/5) to give **11** in 43% yield (39.2 mg, 0.09 mmol) and **12** in 36% yield (30.5 mg, 0.07 mmol).

Compound 11. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.93 (d, J = 9.6 Hz, 1 H), 4.68 (s, 2 H), 4.58 (d, J = 9.6 Hz, 1 H), 3.84 (d, J = 3.0 Hz, 1 H), 3.71 (d, J = 5.7 Hz, 1 H), 3.61 (d, J = 2.9 Hz, 1 H), 2.78−2.67 (m, 1 H), 2.61 (sept, J = 6.9 Hz, 1 H), 2.38−2.26 (m, 1 H), 2.25−2.05 (m, 2 H), 1.91 (t, J = 14.1 Hz, 1 H), 1.54 (dd, J = 12.6, 4.5 Hz, 1 H), 1.26−1.14 (m, 1 H), 1.07 (s, 3 H), 0.95 (d, J = 6.9 Hz, 3 H), 0.93 (d, J = 6.9 Hz, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.1 (C), 159.8 (C), 125.5 (C), 91.9 (C), 74.1 (CH_2), 69.9 (CH_2), 65.1 (C), 64.9 (C), 61.7 (C), 56.5 (CH), 55.6 (CH), 55.5 (CH), 40.4 (CH), 35.8 (C), 30.0 (CH_2), 25.7 (CH), 23.3 (CH_2), 20.6 (CH_3), 18.7 (CH_3), 17.1 (CH_2), 13.5 (CH_3). IR (KBr) 3475, 2972, 2933, 2875, 1745, 1680, 1441, 1219, 1018 cm^{-1}. MS (EI, 70 eV) m/z (%) 436 (M^+, 2), 407 (1), 393 (6), 241 (100). HRMS (EI) calcd for $C_{21}H_{24}SO_8$ 436.1192, found 436.1199.

Compound 12. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.80 (d, J = 9.6 Hz, 1 H), 4.68 (s, 2 H), 4.58 (d, J = 9.6 Hz, 1 H), 3.84 (d, J = 3.0 Hz, 1 H), 3.79 (d, J = 5.6 Hz, 1 H), 3.53 (d, J = 3.0 Hz, 1 H), 2.80−2.68 (m, 1 H), 2.38−2.26 (m, 1 H), 2.24−2.02 (m, 3 H), 1.96 (t, J = 14.1 Hz, 1 H), 1.54 (dd, J = 12.6, 5.1 Hz, 1 H), 1.28−1.15 (m, 1 H), 1.10 (s, 3 H), 0.92 (d, J = 6.8 Hz, 3 H), 0.90 (d, J = 6.8 Hz, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.1 (C), 159.9 (C), 125.3 (C), 92.5 (C), 74.1 (CH_2), 69.9 (CH_2), 66.5 (C), 65.0 (C), 61.0 (C), 58.3 (CH), 55.4 (CH), 54.7 (CH), 40.4 (CH), 35.7 (C), 30.2 (CH_2), 25.7 (CH), 23.3 (CH_2), 20.5 (CH_3), 18.2 (CH_3), 17.0 (CH_2), 13.4 (CH_3). IR (KBr) 3435, 2970, 2933, 2877, 2254, 1755, 1674, 1444, 1346, 1223, 1020 cm^{-1}. MS (EI, 70 eV) m/z (%)

436 (M^+, 7), 421 (9), 71 (100). HRMS (EI) calcd for $C_{21}H_{24}SO_8$ 436.1192, found 436.1182.

(14*S*)-14-Spiro-14α,21-sulfonyldioxytriptolide (13). To a solution of compound **11** (40 mg, 0.092 mmol) in CH_3CN (4.0 mL) was added $NaIO_4$ (31 mg, 0.14 mmol), $RuCl_3 \cdot 3H_2O$ (6 mg, 0.028 mmol), and H_2O (1.0 mL) in sequence. The mixture was stirred at room temperature for 15 min. After removal of the solvent under reduced pressure, the residue was diluted with ethyl acetate and washed with water and brine and dried over anhydrous Na_2SO_4. The crude product was purified by chromatography on silica gel using acetate/cyclohexane (1/4) to provide **13** in 86% yield (36 mg, 0.079 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.92 (d, J = 10.0 Hz, 1 H), 4.72−4.63 (m, 3 H), 3.87 (d, J = 3.0 Hz, 1 H), 3.83 (d, J = 5.6 Hz, 1 H), 3.65 (d, J = 3.0 Hz, 1 H), 2.79−2.68 (m, 1 H), 2.51 (sept, J = 6.8 Hz, 1 H), 2.39−2.07 (m, 3 H), 1.98 (t, J = 14.2 Hz, 1 H), 1.53 (dd, J = 12.6, 5.2 Hz, 1 H), 1.29−1.14 (m, 1 H), 1.09 (s, 3 H), 0.99 (d, J = 6.8 Hz, 3 H), 0.97 (d, J = 6.8 Hz, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.9 (C), 159.4 (C), 125.6 (C), 91.3 (C), 73.4 (CH_2), 69.9 (CH_2), 66.3 (C), 65.2 (C), 61.9 (C), 58.3 (CH), 55.7 (CH), 55.5 (CH), 40.3 (CH), 35.7 (C), 30.2 (CH_2), 25.5 (CH), 23.2 (CH_2), 20.4 (CH_3), 18.3 (CH_3), 17.0 (CH_2), 13.4 (CH_3). IR (KBr) 3442, 2970, 2941, 1749, 1676, 1441, 1394, 1217, 1001 cm^{-1}. MS (EI, 70 eV) m/z (%) 452 (M^+, 8), 437 (10), 423 (10), 111 (100). HRMS (EI) calcd for $C_{21}H_{24}SO_9$ 452.1141, found 452.1169.

(14*S*)-14β-(Hydroxymethyl)epitriptolide (14). Compound **10** (0.50 g, 1.3 mmol) was dissolved in dry pyridine (4.0 mL, 49.3 mmol). The solution was cooled to 0 °C, then MsCl (0.61 mL, 7.7 mmol) was added to the solution dropwise and stirred for 10 min at room temperature. After removal of the solvent under reduced pressure, the residue was diluted with water, then extracted with ethyl acetate, washed with brine, and dried with anhydrous Na_2SO_4. After concentration, the residue was purified by chromatography by ethyl acetate/cyclohexane (1/2) to provide **14** as colorless oil. Yield: 0.504 g (84%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.70−4.57 (m, 4 H), 3.80 (d, J = 3.2 Hz, 1 H), 3.77 (d, J = 5.6 Hz, 1 H), 3.47 (d, J = 3.2 Hz, 1 H), 3.10 (s, 3 H), 2.77−2.67 (m, 1 H), 2.54 (sept, J = 6.9 Hz, 1 H), 2.37−2.26 (m, 1 H), 2.25−2.06 (m, 1 H), 1.91 (dd, J = 14.8, 13.4 Hz, 1 H), 1.54 (dd, J = 12.9, 5.1 Hz, 1 H), 1.28−1.13 (m, 2 H), 1.07 (s, 3 H), 0.92 (d, J = 7.7 Hz, 3 H), 0.89 (d, J = 7.7 Hz, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.2 (C), 160.1 (C), 125.4 (C), 74.5 (C), 72.0 (CH_2), 69.9 (CH_2), 67.8 (C), 65.1 (C), 63.3 (C), 55.9 (CH), 55.4 (CH), 54.0 (CH), 40.5 (CH), 37.5 (CH), 36.0 (C), 29.7 (CH_2), 25.6 (CH_3), 23.5 (CH_2), 20.5 (CH_3), 18.5 (CH_3), 17.1 (CH_2), 13.5 (CH_3). IR (KBr) 3483, 3024, 2972, 2937, 1749, 1674, 1446, 1354, 1174 cm^{-1}. MS (EI, 70 eV) m/z (%) 468 (M^+, 1), 450 (3), 432 (1), 407 (1), 354 (17), 111 (100). HRMS (EI) calcd for $C_{22}H_{28}SO_9$ 468.1454, found 468.1457.

Biology. Cell Lines and Cell Culture. Human gastric adenocarcinoma SGC-7901, hepatocellular carcinoma BEL-7402, renal cell carcinoma 786-O, and ovarian epitheloid carcinoma HO-8910 cell lines were obtained from the cell bank of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences. Human chronic myelogenous leukemia K562, lung adenocarcinoma A549, breast cancer MCF-7, MDA-MB-231, and MDA-MB-468, colorectal cancer HCT-116 and HCT-15, oral squamous cell carcinoma KB, prostate cancer DU-145, and cervical cancer Hela cell lines were purchased from the American Type Culture Collection (Manassas, VA). Human acute lymphoblastic leukemia Molt-4, gastric adenocarcinoma MKN-28, prostate cancer PC-3, and ovarian cancer SK-OV-3 cell lines were from Japanese Foundation of Cancer Research (Tokyo, Japan). Human hepatocellular carcinoma SMMC-7721 was a gift from the Second Military Medical School (Shanghai, China). The rhabdomyosarcoma cell line Rh30 was a gift from St. Jude Children's Research Hospital (Memphis, TN). The doxorubicin-selected multidrug resistant (MDR) cell subline K562/A02[32] was purchased from the Institute of Hematology, Chinese Academy of Medical Science (Tianjin, China). The vincristine-selected MDR subline KB/VCR[33] and doxorubicin-selected MDR subline MCF-7/ADR[34,35] was obtained from Zhongshan University of Medical Sciences (Guangzhou, China). All these cell lines except MCF-7 and MCF-7/Adr were maintained in RPMI 1640 medium (Gibco, Grand Island, NE) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Gibco), 2 mmol/L glutamine, 100 IU/mL penicillin, 100 μg/mL streptomycin, and 10 mmol/L HEPS (pH 7.4) in a humidified atmosphere of 95% air with 5% CO_2 at 37 °C. MCF-7 and the MCF-7/ADR cells were grown in the same medium supplemented with 1 mmol/L sodium pyruvate and 0.01 mg/mL bovine insulin.

Cytotoxicity Assays. The cytotoxicity of compound **1** derivatives and the reference drugs was examined using a panel of human tumor cell lines with the methods described before.[36] Briefly, cells in 96-well plates were treated in triplicate with gradient concentrations of tested agents at 37 °C for 72 h, then assessed by the microculture tetrazolium [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, MTT] (Sigma, Saint Louis, MO) assay for leukemia cell lines, or by the sulforhodamin B (Sigma) assay for solid tumor cell lines. The cytotoxicity was expressed as an IC_{50}, defined as the concentration required for 50% inhibition of cell growth compared with control cells and calculated with the logit method. Resistance factor was calculated as the ratio of IC_{50} in MDR cells over IC_{50} in the corresponding parental cells. The reference drugs ADR, VCR, and the MDR reversal agent VER were all purchased from Sigma with purity over 95%.

In Vivo Antitumor Activity Assays. BALB/cA nu/nu mice aged 4−5 weeks were bred in the Shanghai Institute of Materia Medica (Shanghai, China), and all experiments were done according to the institutional ethical guidelines on animal care. Under sterile conditions, well developed tumors were cut into 1 mm^3 fragments and transplanted sc into the right flank of nude mice using a trocar. When the tumor reached a volume of 100−200 mm^3, the mice were randomly assigned into control and treatment groups. Treatment groups receiving compound **4** (po) dissolved in vehicles and control groups were given vehicles alone. Vehicles and compound **4** were administered daily, and each experimental group contained six animals. The sizes of the tumors were measured twice a week using microcalipers. The tumor volume (V) was calculated as (length × width^2)/2. The individual relative tumor volume (RTV) was calculated as follows: RTV = V_t/V_0, where V_t is the measured volume each day, and V_0 is the volume at the beginning of the treatment. The therapeutic effect of the compound was expressed as the volume ratio of treatment to control (T/C):[37] T/C (%) = 100% × (mean RTV of the treated group/mean RTV of the control group).

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Supporting Information Available: Elemental analysis data for **1**, **2**, and compounds **4**, **5**, **7**, **8**, **10**, HPLC results of compounds **6**, **11**, **12**, **13**, and two crystallographic files of compounds **4** and **11** in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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