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PAPER

Total synthesis of novel D-ring-modified triptolide analogues: structure–cytotoxic activity relationship studies on the D-ring of triptolide†

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The total synthesis of a *trans*-position butenolide analogue of triptolide **3** and the semi-synthesis of analogue **4**, with a furan ring, and compound **5**, without a planar D-ring, are described. Studies into the antitumor activity of these compounds suggest that the five-membered unsaturated lactone ring (D-ring) of triptolide is essential to its potent anticancer activity and the C18 carbonyl group may exert an important influence on the interaction between triptolide and the target molecule(s) responsible for initiating their cytotoxic effects.

Tripterygium wilfordii Hook. f. (TWHF), 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**) and triptonide (**2**) (Fig. 1), the major components responsible for the clinical properties of TWHF, were first isolated from TWHF extracts and characterized in 1972 as diterpenoid triepoxide lactones containing an 18 (4→3) abeo-abietane skeleton.[4] Right after their isolation, triptolide (**1**) and triptonide (**2**) were shown to possess potent antitumor, anti-inflammatory, immunosuppressive, and antifertility activities.[4–22] Compared to some conventional chemotherapy drugs, triptolide has a similar and even superior anticancer activity, especially against p53 mutated or multi-drug resistant cells.[17] All of the antitumor properties mentioned above suggest that triptolide should be a promising anticancer drug. However, no systematic structure–cytotoxic activity relationship (SAR) studies have been reported.

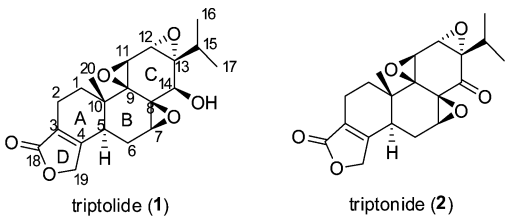

Fig. 1 Triptolide and triptonide from *Tripterygium wilfordii* Hook. f.

For a long time, there have been no studies on the structure–activity relationship of the D-ring of triptolide except for two patents,[23,24] describing some butenolide-modified triptolide analogues without any biological activity data, and our previous paper,[25] reporting that an analogue (compound **6**) (Fig. 2) with a five-membered unsaturated lactam ring has the same activity as the natural triptolide. So the structure–activity relationship of the D-ring is still obscure. To explore whether the five-membered unsaturated lactone ring of triptolide is completely critical to its anticancer activity, compound **3**, having a *trans*-position butenolide, compound **4**,which has a furan ring replacing the five-membered unsaturated lactone ring, and compound **5** without the planar D-ring were synthesized for SAR studies of the D-ring. The SAR studies of these tripolide analogues were performed by using ovary (SK-OV-3) and prostate (PC-3) tumor cells.

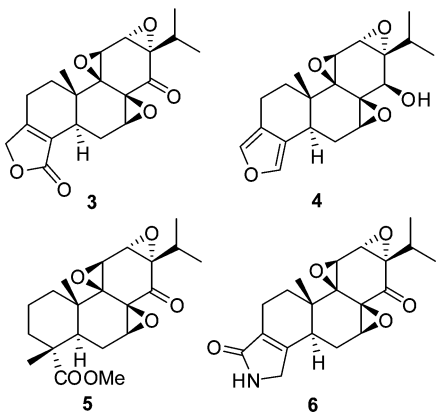

Fig. 2 Triptolide analogues **3–6**.

The synthetic strategy followed for the preparation of the triptonide analogue **3** is depicted in Scheme 1. The synthesis of compound **3** started with known abietic acid **7**, which was converted to C4-(18)-alkene **8** by a known procedure reported by us previously.[26] Allylic oxidation of **8** with SeO_2/TBHP provided a C-3 α-alcohol **9**, which was oxidized readily under Swern

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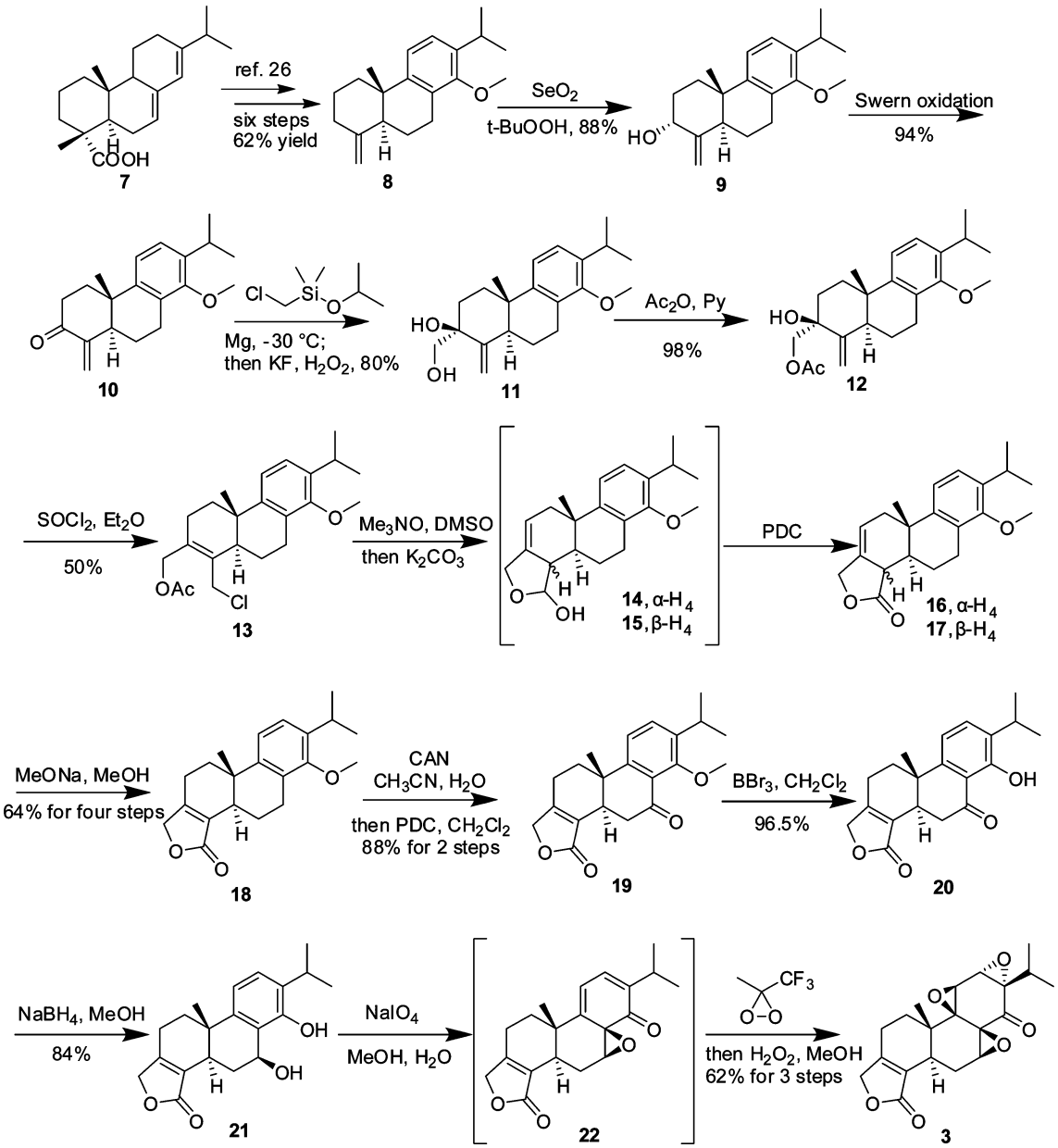


Scheme 1 The total synthesis of compound **3**.

conditions[27] to the enone **10** in 83% yield for the two steps. When compound **10** was treated with isopropoxydimethylsilylmethylmagnesium chloride in THF at –30 °C,[28] the nucleophilic addition proceeded smoothly, providing a β-hydroxysilane intermediate. This intermediate, without purification, was subjected to oxidative cleavage of the corresponding Si–C bond by potassium fluoride and 30% hydrogen peroxide to give the 1,2-diol **11** as a single stereoisomer in 80% yield. Protection of the primary hydroxyl group with acetic anhydride provided compound **12**, which was treated with $SOCl_2$ in Et_2O, affording **13** in 49% yield for the two steps. Ganem oxidation[29] of **13** with Me_3NO, including a double bond migration, gave an aldehyde intermediate, which, without purification, was treated with K_2CO_3 in MeOH and H_2O, providing hemiacetals **14** and **15** that, upon oxidation with pyridinium dichromate, delivered lactones **16** and **17** in 29% and 35% yields, respectively, for the three steps. Treatment of **16** and **17** with methoxide ion in methanol at room temperature for 10 min afforded butenolide **18**[30] in nearly quantitative yield. The X-ray crystallographic analysis of compound **18** confirmed its structure to be as shown in Fig. 3.[31]

With key intermediate **18** in hand, compound **18** was converted into compound **3** by a sequence similar to that developed previously for the construction of the C-ring functionality.[26,32] Treatment of **18** with ammonium ceric nitrate in H_2O–CH_3CN gave benzyl alcohol as the intermediate, which, without purification, upon oxidation of hydroxyl with pyridinium dichromate, delivered ketone **19** that was demethylated to afford phenol **20** in 85% yield for the three steps. Treatment of **20** with $NaBH_4$ in MeOH gave C-7 β-alcohol **21** in 84% yield, along with the C-7 α-alcohol isomer in 13% yield. Periodate oxidation[33] of **21** afforded epoxy dienone

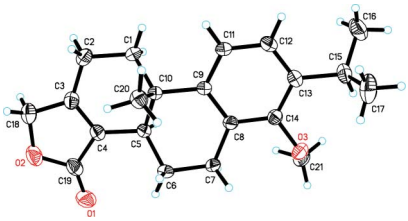


Fig. 3 The X-ray single crystal structure of compound **18**.

22. When methyl(trifluoromethyl)dioxirane generated *in situ* was used,[34] the second C-9,11 β-epoxide was introduced as a single diastereomer. Further epoxidation with basic H_2O_2 provided compound **3**, with triepoxides as the sole epoxidation product, in 62% yield for the three steps. The X-ray crystallographic analysis of compound **3** confirmed its structure to be as shown in Fig. 4.[31]

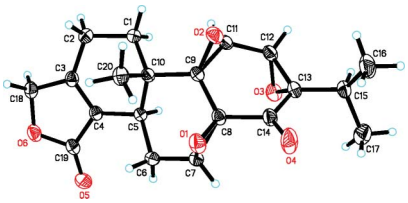
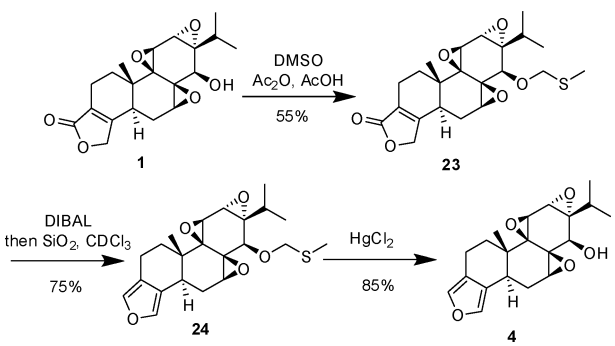


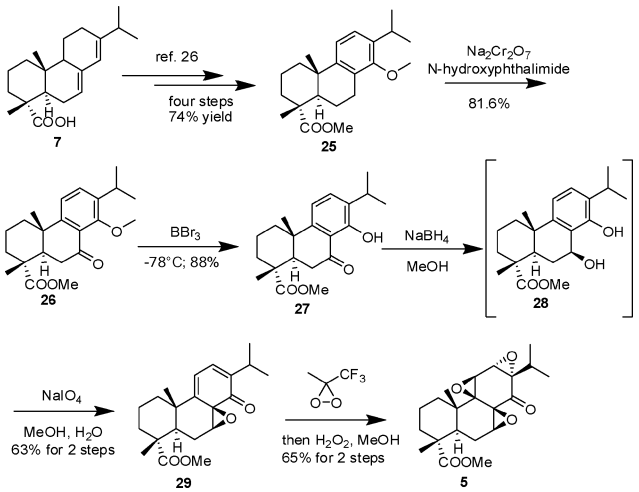
Fig. 4 The X-ray single crystal structure of compound 3.

We then switched to the synthesis of the furan ring triptolide analogue. We used triptolide (**1**), which was extracted from TWHF of our region, as the starting material and initially focused our attention on modifying the butenolide on the D-ring in order to obtain the furan ring analogues (Scheme 2). Protection of the hydroxyl group with DMSO and Ac_2O in AcOH gave the methylthiomethyl ether **23** in 55% yield. Reduction of the lactone of compound **23** with DIBAL in CH_2Cl_2 at −78 °C afforded a hemiacetal intermediate, which, without purification, upon treatment with silica gel[35] in $CDCl_3$, provided the furan ring in **24** in 75% yield. Deprotection of compound **24** with $HgCl_2$ in CH_3CN and H_2O gave target compound **4** in 85% yield.



Scheme 2 The synthesis of analogue **4**.

In order to obtain the analogues without a D-ring, we used abietic acid **7**, which was converted to compound **25** by a known procedure,[26] as the starting material (Scheme 3). Benzylic oxidation of **25** with $Na_2Cr_2O_7$ and *N*-hydroxyphthalimide[36] in acetone provided ketone **26** that was demethylated to afford the phenol **27** in 72% yield for two steps. Reduction of **27** with $NaBH_4$ in MeOH gave the C-7 β-alcohol **28**, along with the C-7 α-alcohol isomer. Periodate oxidation[32] of **28** afforded epoxy



Scheme 3 The synthesis of analogue **5**.

dienone **29** in 63% yield over the two steps. Epoxidation of **29** with methyl(trifluoromethyl)dioxirane[34] followed by treatment with basic H_2O_2 provided compound **5**, a triepoxide, as the sole epoxidation product, in 65% yield over two steps.

As shown above, we obtained four novel D-ring modified triptolide analogues with a *trans*-butenolide, with a furan ring, without a D-ring and with a five-membered unsaturated lactam ring and we evaluated the *in vitro* anticancer effects of those target compounds (**3–6**) against two human tumor cell lines derived from ovary (SK-OV-3) and prostate (PC-3) using sulforhodamine B (SRB) assays.[37] The results revealed that analogue **6**,[25] with a five-membered unsaturated lactam ring, which behaves as an ester isostere, had the same effect against the two cell lines as the natural triptonide (Table 1). However, compound **3** with a *trans*-butenolide was shown to be only weakly cytotoxic and analogue **4** with the furan ring and analogue **5** without the D-ring completely lost their cytotoxicity against PC-3 and SK-OV-3. Based on the above results, we presumed that the five-membered unsaturated lactone ring (D-ring) of triptolide is essential to its potent anticancer activity and that the C18 carbonyl group may exert an important influence on the interaction between triptolide and the target molecule(s) responsible for initiating their cytotoxic effects. However, the exact mechanism is still unclear as compounds **1**, **2** and **6**, having five-membered unsaturated lactone or lactam rings, are significantly cytotoxic while the furan ring analogue **4**, without the C18 carbonyl group, and analogue **5**, without the planar D-ring, nearly lost their cytotoxicity completely. Also, the cytotoxicity against the two cell lines of the *trans*-butenolide analogue **3** with the C19 carbonyl group was greatly reduced.

Table 1 The *in vitro* anticancer activity of the triptolide analogues in SK-OV-3 and PC-3 cells

Compound	IC_{50} (μM)	
	SK-OV-3	PC-3
1	0.006	0.02
2	0.008	0.6
3	6.5	> 100
4	> 100	> 100
5	> 100	> 100
6	0.02	1.4

In summary, four novel D-ring-modified triptolide analogues (**3**–**6**) with a *trans*-butenolide, a furan ring, without a D-ring and with a five-membered unsaturated lactam ring were synthesized and tested for their cytotoxicity against two human tumor cell lines. The effect of the five-membered unsaturated lactone ring (D-ring) on triptolide's potent anticancer activity has been unclear for a long time. From the current investigation, the structure–activity relationships of these compounds suggest that the five-membered unsaturated lactone ring (D-ring) of triptolide is essential to its potent anticancer activity and that the C18 carbonyl group may exert an important influence on the interaction between triptolide and the target molecule(s) responsible for initiating their cytotoxic effects. More importantly, it will give an impetus to the systematic structure–cytotoxic activity relationship studies of triptolide.

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References

1 Huang, S. The saint peasant's scripture of materia medica (18357), Beijing: Publishing house for Chinese medine classics, republished in 1982, pp 309-310.
2 X. X. Wang, R. Matta, G. Shen, L. D. Nelin, D. H. Pei and Y. S. Liu, *J. Mol. Med.*, 2006, **84**, 405.
3 G. S. Ding, *Clin. Ther.*, 1987, **9**, 345.
4 S. M. Kupchan, W. A. Court, R. G., Jr. Dailey, C. J. Gilmore and R. F. Bryan, *J. Am. Chem. Soc.*, 1972, **94**, 7194.
5 S. M. Kupchan and R. M. Schubert, *Science*, 1974, **185**, 791.
6 K. Y. Lee, W.-T. Chang, D.-M. Qiu, P. N. Kao and G. D. Rosen, *J. Biol. Chem.*, 1999, **274**, 13451.
7 Y. L. Zheng, J. F. Lin, C. C. Lin and Y. Xu, *ACTA Pharm. Sin.*, 1994, **15**, 540.
8 J. R. Zheng, K. X. Gu, L. F. Xu, J. W. Gao, Y. H. Yu and M. Y. Tang, *ACTA Acad. Med. Sin.*, 1991, **13**, 391.
9 W. Z. Gu, Chen, R. Brandwein, S. McAlpine, J. Burres and N. Int, *Int. J. Immunopharmacol.*, 1995, **17**, 351.
10 S. X. Yang, H. L. Gao, S. S. Xie, W. R. Zhang and Z.-Z. Long, *Int. J. Immunopharmacol.*, 1992, **14**, 963.
11 D. M. Qiu, G. H. Zhao, Y. Aoki, L. F. Shi, A. Uyei, S. Nazarian, J. C. H. Ng and P. N. Kao, *J. Biol. Chem.*, 1999, **274**, 13443.
12 Q. S. Zhen, X. Ye and Z. J. Wei, *Contraception*, 1995, **51**, 121.
13 J. R. Zheng, J. L. Fang, K. X. Gu, Y. Q. Yin, L. F. Xu, J. W. Gao, H. Z. Guo, Y. H. Yu and H. Z. Sun, *Acta. Acad. Med. Sin.*, 1987, **9**, 323.
14 L. A. Shamon, J. M. Pezzuto, J. M. Graves, R. R. Mehta, S. Wangcharoentrakul, R. Sangsuwan, S. Chaichana, P. Tuchinda, P. Cleason and V. Reutrakul, *Cancer Lett.*, 1997, **112**, 113.
15 Y. Yang, Z. Liu, E. Tolosa, J. Yang and L. Li, *Immunopharmacology*, 1998, **40**, 139.
16 B. J. Chen, *Leuk. Lymphoma*, 2001, **42**, 253.
17 S. Yang, J. Chen, Z. Guo, X. M. Xu, L. Wang, X. F. Pei, J. Yang, C. B. Underhill and L. Zhang, *Mol. Cancer Ther.*, 2003, **2**, 65.
18 T. M. Kiviharju, P. S. Lecane, R. G. Sellers and D. M. Peehl, *Clin. Cancer Res.*, 2002, **8**, 2666.
19 B. Z. Carter, D. H. Mak, W. D. Schober, T. McQueen, D. Harris, Z. Estrov, R. L. Evans and M. Andreeff, *Blood*, 2006, **108**, 630.
20 T. Tengchaisri, R. Chawengkirttikul, N. Rachaphaew, V. Reutrakul, R. Sangsuwan and S. Sirisinha, *Cancer Lett.*, 1998, **133**, 169.
21 W. T. Chang, J. J. Kang, K. Y. Lee, K. Wei, E. Anderson, S. Gotmare, J. A. Ross and G. D. Rosen, *J. Biol. Chem.*, 2001, **276**, 2221.
22 S. Frese, F. Pirnia, D. Miescher, S. Krajewski, M. M. Borner, J. C. Reed and R. A. Schmid, *Oncogene*, 2003, **22**, 5427.
23 D. Dai, H. Yuan and J. H. Musser *U.S. Patent* 6548537, 2003.
24 J. M. Fidler and J. H. Musser *PCT Int.*, *WO* 2005/000291, 2005.
25 B. Zhou, Z. H. Miao, G. Deng, J. Ding, Y. X. Yang, H. J. Feng and Y. C. Li, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 6217.
26 B. Zhou, X. M. Li, H. J. Feng and Y. C. Li, *Tetrahedron*, 2010, **66**, 5396.
27 A. J. Mancuso and D. Swern, *Synthesis*, 1981, **1981**, 165.
28 K. Tamao, N. Ishida, Y. Ito, M. Kumada, V. D. Tran and L. E. Overman, *Org. Synth.*, 1990, **69**, 96.
29 (*a*) A. G. Godfrey and B. Ganem, *Tetrahedron Lett.*, 1990, **31**, 4825; (*b*) W. P. Griffith, J. M. Jolliffe, S. V. Ley, K. F. Springhorn and P. D. Tiffin, *Synth. Commun.*, 1992, **22**, 1967; (*c*) W. D. Paquette and R. E. Taylor, *Org. Lett.*, 2004, **6**, 103.
30 S. S. Yu, D. M. Zhang, D. Q. Yu and X. T. Liang, *Chin. Chem. Lett.*, 1994, **5**, 217.
31 Crystallographic data for compound **18** and **3** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication No. CCDC800757 and No. CCDC800758, respectively..
32 D. Yang, X. Y. Ye and M. Xu, *J. Org. Chem.*, 2000, **65**, 2208.
33 H. Becker, T. Bremholt and E. Adler, *Tetrahedron Lett.*, 1972, **13**, 4205.
34 D. Yang, M. K. Wong and Y. C. Yip, *J. Org. Chem.*, 1995, **60**, 3887.
35 I. S. Marcos, A. Beneitez, R. F. Moro, P. Basabe, D. Diez and J. G. Urones, *Tetrahedron*, 2010, **66**, 8605.
36 M. Padma and A. L. Henry *U.S. Patent* 6384251, 2002.
37 L. V. Rubinstein, R. H. Shoemaker, K. D. Paull, R. M. Simon, S. Tosini, P. Skehan, D. A. Scudiero, A. Monks and M. R. Boyd, *J. Natl. Cancer Inst.*, 1990, **82**, 1113.