ChemPubSoc
Europe

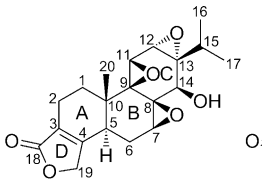
DOI: 10.1002/cmdc.201300409

Design, Synthesis and Structure–Activity Relationships Studies on the D Ring of the Natural Product Triptolide

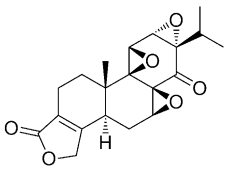
Hongtao Xu, Huanyu Tang, Huijin Feng, and Yuanchao Li*[a]

Triptolide is a diterpene triepoxide natural product isolated from *Tripterygium wilfordii* Hook F, a traditional Chinese medicinal herb. Triptolide has previously been shown to possess antitumor, anti-inflammatory, immunosuppressive, and antifertility activities. Earlier reports suggested that the five-membered unsaturated lactone ring (D ring) is essential for potent cytotoxicity, however, to the best of our knowledge, systematic structure–activity relationship studies have not yet been reported. Here, four types of D ring-modified triptolide analogues were designed, synthesized and evaluated against human ovarian (SKOV-3) and prostate (PC-3) carcinoma cell lines. The results suggest that the D ring is essential to potency, however it can be modified, for example to C18 hydrogen bond acceptor and/or donor furan ring analogues, without complete loss of cytotoxic activity. Interestingly, evaluation of the key series of C19 analogues showed that this site is exquisitely sensitive to polarity. Together, these results will guide further optimization of this natural product lead compound for the development of potent and potentially clinically useful triptolide analogues.

Triptolide (**1**),[1] a diterpene triepoxide isolated from the Chinese medicinal herb *Tripterygium wilfordii* Hook F (TWHF),[2–4] commonly known as *Lei Gong Teng* or Thunder God Vine, whose extracts have been used to treat autoimmune and inflammatory diseases such as rheumatoid arthritis for centuries. Right after its isolation, it was shown to possess potent antitumor, anti-inflammatory, immunosuppressive, and antifertility activities.[1,5–23] At the cellular level, triptolide (**1**) shows potent antiproliferative activity and inhibits the proliferation of all 60 cancer cell lines of the US National Cancer Institute, with IC_{50} values in a low nanomolar range (average $IC_{50} = 12$ nM). Triptolide (**1**) also inhibits the growth of xenograft models of different solid tumor cell types.[11] Meanwhile, it interferes with a number of transcription factors including NF-κB, Hsp 70, p53, NF-AT and HSF-1 at the molecular level.[11,20,23] Compared with some conventional chemotherapeutic drugs, triptolide has similar or even superior anticancer activity, especially over p53-mutated or multidrug-resistant cells.[14]



Triptolide (**1**)



Triptonide (**2**)

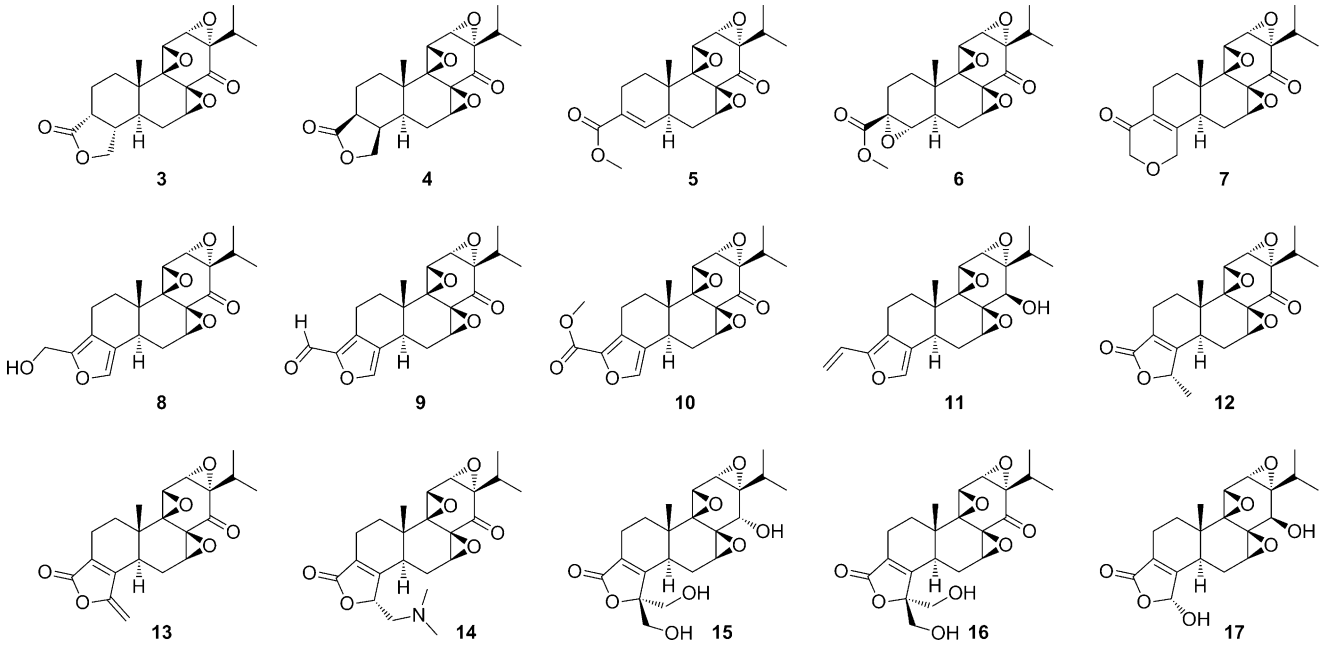
All of the studies mentioned above greatly support the potential development of triptolide (**1**) as an antineoplastic agent. Structural modifications at C14 or on the C ring previously emerged as promising approaches for improving the bioavailability of this compound class, and these studies led to the discovery of clinically important anticancer or anti-inflammatory compounds.[24] Findings described in our previous paper and a report by Takeya et al.[25,26] suggest that the five-membered unsaturated lactone ring (D ring) of triptolide is essential to its potent anticancer activity. However, systematic SAR studies are needed to investigate the role of the C3,C4 double bond, the substituent properties of C19, and the potential replacement of the five-membered unsaturated lactone ring with other ring systems. Herein, as part of our ongoing investigation into the SAR of triptolide and in an attempt to identify triptolide analogues with desirable physicochemical, pharmacokinetic, and pharmacodynamic properties, we describe the design and synthesis of four types of novel triptolide analogues (**3**–**17**).

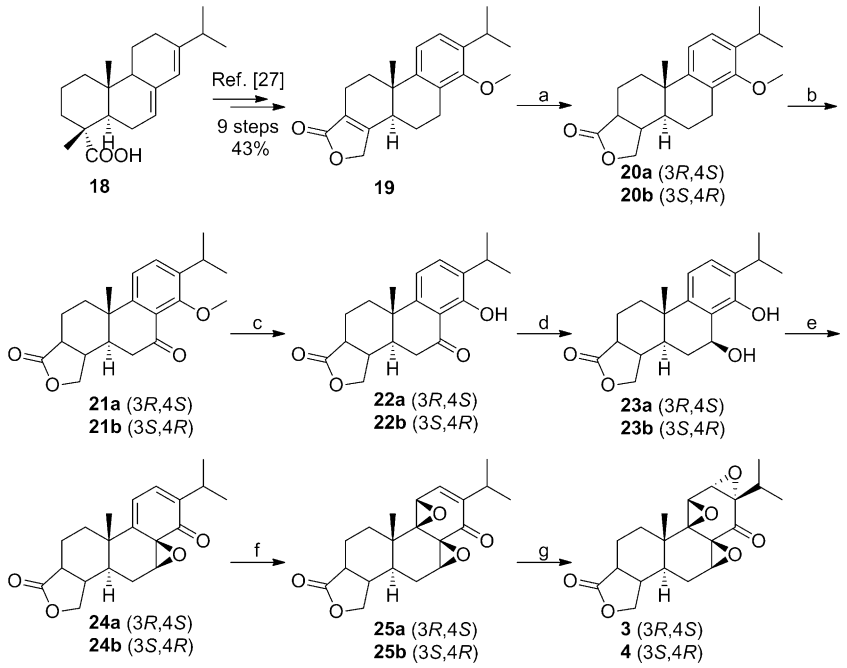
First, to investigate the role of the C3,C4 double bond, compounds with saturated lactone rings (**3** and **4**) were synthesized. Second, to further explore the role of the C3 carbonyl group, compounds in which the C3 carbonyl group conformation is flexible (**5** and **6**) and in which the conformation is fixed by a 3-pyrone D ring (**7**) were synthesized. Then, furan-ring-containing compounds with hydrogen-bond acceptors and/or donor groups at C18 (**8**–**11**) were synthesized to study whether hydrogen bonding interactions between the target and the C18 hydrogen accepter are crucial to the cytotoxic activity of the parent compound, triptolide. Finally, a series of C19-substituted compounds (**12**–**17**) were synthesized to probe and define the importance of the C19 substituent. These tripolide analogues were then evaluated in human ovarian (SKOV-3) and prostate (PC-3) carcinoma cell lines.

The synthetic strategy followed for the preparation of saturated D ring triptonide analogues **3** and **4** is depicted in Scheme 1. Abietic acid (**18**), which was converted to triptophenolidemethyl ether **19** via a known procedure,[27] was used as the starting material. After several different conditions were tried, **19** was reduced by Raney nickel/hydrogen gas in ethanol to give a 2:3 mixture of saturated lactone intermediates **20a** and **20b**. Benzylic oxidation of **20a**/**20b** with ammonium ceric

[a] *H. Xu, H. Tang, H. Feng, Prof. Y. Li*
Department of Medicinal Chemistry
Shanghai Institute of Materia Medica
Chinese Academy of Sciences
555 Zu Chong Zhi Road , Zhang Jiang Hi-Tech Park
Pudong, Shanghai 201203 (China)
E-mail: ycli@mail.shcnc.ac.cn

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.201300409.





Scheme 1. Synthesis of compounds **3** and **4**. *Reagents and conditions*: a) Raney Ni, H_2 (1 atm), EtOH, RT, 64 h, **20a** (58.8%), **20b** (39.2%); b) $(NH_4)_2Ce(NO_3)_6$, CH_3CN/H_2O (1:1), RT, 1.5 h, then DMP, CH_2Cl_2, RT, o/n, **21a** (83%), **21b** (80%); c) BBr_3, CH_2Cl_2, −78 °C→RT, 1 h, **22a** (92%), **22b** (90%); d) $NaBH_4$, CH_3OH, 0 °C, 1 h, **23a** (97%), **23b** (96%); e) $NaIO_4$, CH_3OH/H_2O (3:1), 0 °C, 1 h, **24a** (88%), **24b** (91%); f) CF_3COCH_3, oxone, $NaHCO_3$, CH_3CN/aq Na_2(EDTA) (1:1), RT, 3.5 h, **25a** (84%), **25b** (83%); g) H_2O_2, NaOH, MeOH, RT, 2 h, **3** (80%), **4** (83%).

nitrate in water/acetonitrile gave the benzyl alcohol as the intermediate, which was used in the next step without purification. Oxidation of the hydroxy group with 1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1*H*)-one (DMP) delivered C7 ketones **21a**/**21b** in high yield. Subsequent treatment of ketones **21a**/**21b** with boron trifluoride diethyl etherate removed the methyl protecting group to afford phenols **22a**/**22b**. Reduction of phenols **22a**/**22b** with sodium borohydride in methanol gave C7-β-alcohols **23a**/**23b** as the sole isomer. Periodate oxidation of **23a**/**23b** afforded epoxy dienones **24a**/**24b**.[28] Epoxidation of **24a**/**24b** by in situ generated methyl(trifluoromethyl)-dioxirane yielded C9,C11-β-epoxides **25a**/**25b**.[29] Further epoxidation with basic hydrogen peroxide gave the desired compounds **3**/**4** in high yield. X-ray crystallographic analysis of compounds **20a** and **3** confirmed their structure to be as shown in Figure 1.

With both **3** and **4** in hand, focus was then switched to the synthesis of compounds **5** and **6**. The route employed started with the transformation of abietic acid (**18**) to **26** via an eight-step procedure previously reported by our group (Scheme 2).[27] Treatment of **26** with mercuric chloride afforded a mixture of the C3-α and β methyl ester, which, without purification, was further reacted with sodium borohydride and subsequently dehydrated to provide methyl ester **27**. Oxidation of **27** with ammonium ceric nitrate and Jones reagent, delivered ketone **28**. Deprotection of the methyl ether with boron trifluoride diethyl etherate, followed by reduction of the C7 ketone with sodium borohydride gave C7-β-alcohol **30**. Finally, a three-step sequence similar to

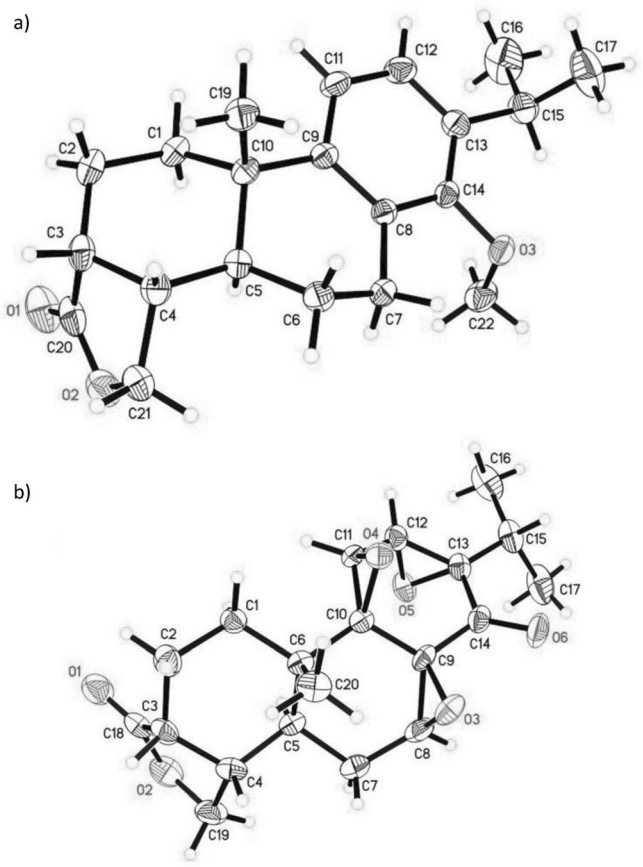


Figure 1. X-ray single-crystal structures of analogues a) **20a** and b) **3**.

that developed previously for construction of the C-ring functionality using sodium periodate, in situ generated methyl(trifluoromethyl)dioxirane, and basic hydrogen peroxide yielded a 1:1 mixture of analogues **5** (33% over three steps) and **6** (32% over three steps).[29] X-ray crystallographic analysis of compound **5** confirmed its structure to be as shown in Figure 2.

The synthesis of analogues **7**–**10** and **12**–**16** used triptonide (**2**), which was obtained through extraction from TWHF of our region, as the starting material (Scheme 3). Treatment of triptonide (**2**) with (isopropoxy-dimethylsilyl)methylmagnesium chloride in tetrahydrofuran at 0 °C and subsequent Tamao oxidation provided a 2:1 mixture of **31** and **32**.[30,31] The C-14 diol of compound **32** was then converted into C14 ketone **8** using sodium periodate. Oxidative Achmatowicz rearrangement of **8**, followed by reduction of the resulting hemiacetal afforded

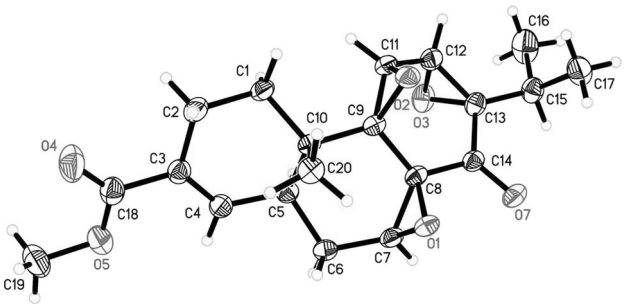
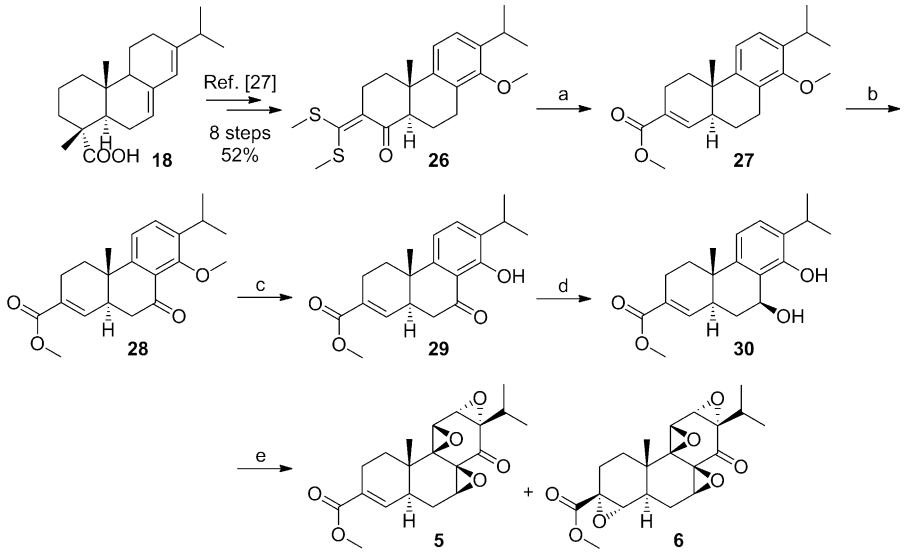
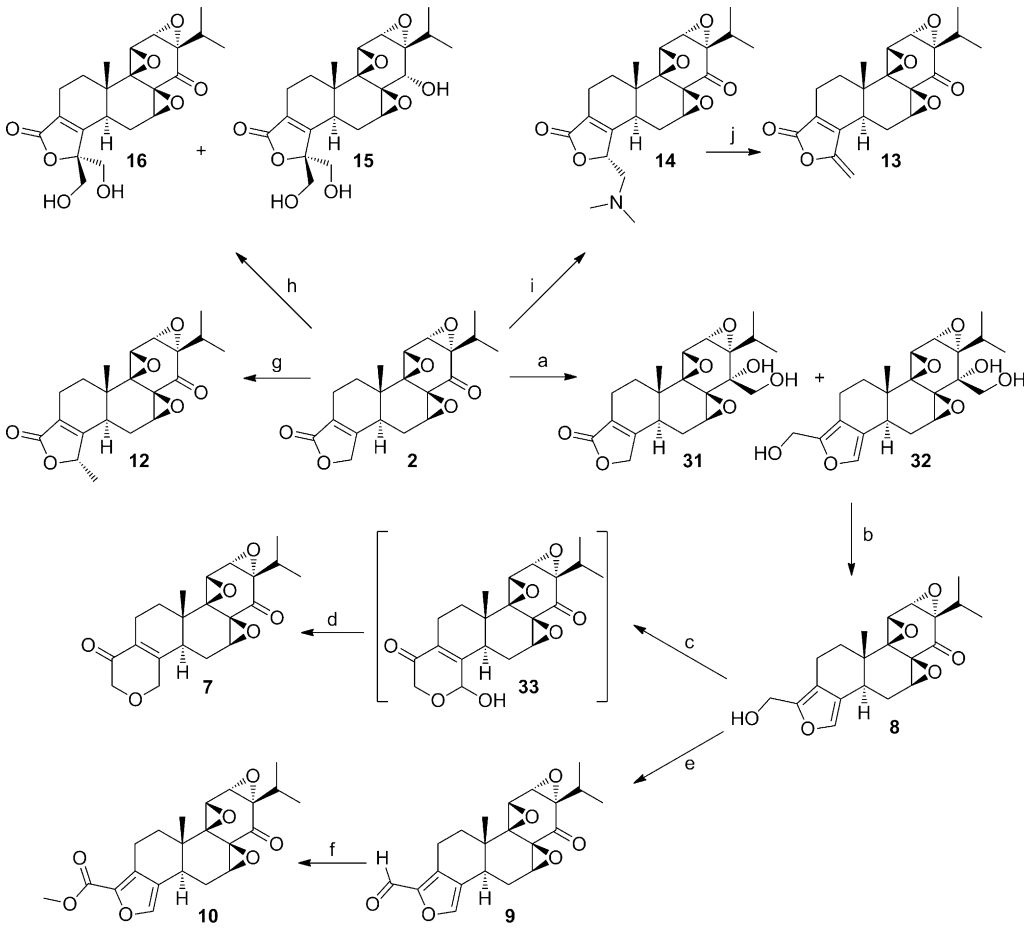


Figure 2. X-ray single-crystal structure of analogue **5**.



Scheme 2. Synthesis of compounds **5** and **6**. *Reagents and conditions*: a) 1. $HgCl_2$, $BF_3{\cdot}Et_2O$, MeOH, RT, 3 h; 2. $NaBH_4$, MeOH, 0 °C, 1.5 h; 3. MsCl, DMAP, pyridine, 0 °C→RT, 2 h, then 60 °C, 4 h, 50% (three steps); b) $(NH4)_2Ce(NO_3)_6$, CH_3CN/H_2O (1:1), RT, 1.2 h, then Jones' reagent, acetone, 0 °C, 15 min, 72% (two steps); c) BBr_3, CH_2Cl_2, −78 °C→RT, 1 h, 91%; d) $NaBH_4$, CH_3OH, 0 °C, 1 h, 93%; e) 1. $NaIO_4$, CH_3OH/H_2O (3:1), 1 h; 2. CF_3COCH_3, oxone, $NaHCO_3$, CH_3CN/aq $Na_2(EDTA)$ (1:1), RT, 4 h; 3. H_2O_2, NaOH, MeOH, RT, 2.5 h, **5** (33%; three steps), **6** (32%; three steps).

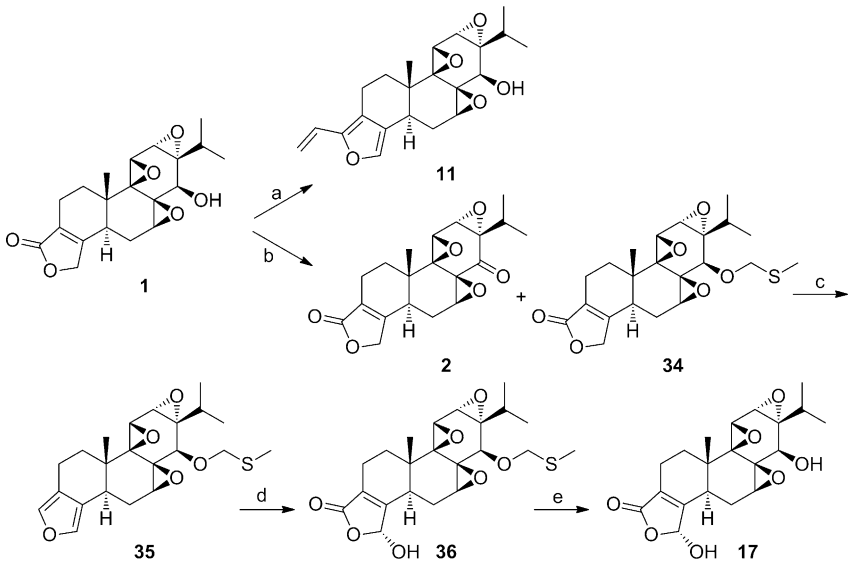
pyrone analogue **7**. Oxidation of **8** with DMP provide aldehyde **9**. Pinnick oxidation of **9** followed by methylation provided methyl ester **10**.[32] Treatment of triptonide **2** with lithium diisopropylamide (LDA) at −78 °C, followed by quenching of the resulting enolate with methyl iodide, thus generated C19-α-methyl substitute analogue **12** as the sole isomer. Similarly, quenching the enolate by addition of paraformaldehyde yielded a 1.5:1 mixture of C19 dimethoxy-substituted triptonide **16** and C19 dimethoxy-substituted epi-triptolide **15**. Treatment of triptonide **2** with lithium bis(trimethylsilyl)-amide (LiHMDS) followed by addition of Eschenmoser's salt generated compound **14**. Finally, oxidative elimination of dimethyl amine **14** provide dien analogue **13**. X-ray crystallographic analysis of compounds **15** and **16**



Scheme 3. Synthesis of analogues **7**–**10** and **12**–**16**. *Reagents and conditions*: a) 1. (isopropoxydimethylsilyl)methyl chloride, Mg, THF; 2. KF, $KHCO_3$, 30% H_2O_2, 0 °C, 2 h, **31** (59%; two steps), **32** (28%; two steps); b) $NaIO_4$, THF/H_2O (1:1), RT, 3 h, 95%; c) *m*-CPBA, CH_2Cl_2, 0 °C, 3 h; d) Et_3SiH, TFA, 0 °C, 2 h, 52%, two steps; e) DMP, $NaHCO_3$, RT, 5 h, 91%; f) $NaClO_2$, $NaH_2PO_4{\cdot}H_2O$, 2-methyl-2-butene, THF/*t*BuOH/H_2O (3:3:1), then 50% KOH, α-nitroso-α-methylurea, Et_2O, 0 °C, 15 min, 83%; g) LDA, CH_3I, −78 °C→RT, 1.5 h, 55%; h) LDA, paraformaldehyde, −78 °C→RT, 2 h, **16** (45%), **15** (30%); (i) LiHMDS, Eschenmoser's salt, −78 °C→RT, 1 h, 62%; (j) *m*-CPBA, $NaHCO_3$, −78 °C→RT, 1.5 h, 90%. Abbreviations: 3-chloroperoxybenzoic acid (*m*-CPBA); trifluoroacetic acid (TFA).

confirmed their structure to be as shown in Figure 3.

The synthesis of analogues **11** and **17** used triptolide (**1**) as the starting material (Scheme 4). Directly treatment of triptolide with vinylmagnesium bromide generated the vinyl-substituted furan ring analogue (**11**). Next, protection of the hydroxy group with dimethyl sulfoxide (DMSO) and acetic anhydride (Ac_2O) in acetic acid (AcOH) gave methylthiomethyl ether **34** in 39% yield, along with a substantial amounts (58%) of oxidation product triptonide (**2**).[25] Reduction of **34** using diisobutylaluminium hydride (DIBAL-H) followed by quenching of the reaction with dilute hydrochloride acid



Scheme 4. Synthesis of analogues **11** and **17**. *Reagents and conditions*: a) $CH_2CHMgBr$, −20 °C→RT, 2 h, 25%; b) DMSO, Ac_2O, AcOH, RT, o/n, **34** (39%), **2** (58%); c) DIBAL-H, CH_2Cl_2, −78 °C, 1 h, then 0.5 N HCl, 15 min, 75%; d) O_2, hν, rose bengal, *i*Pr_2EtN, CH_2Cl_2, −78 °C, 2 h, 75%; e) $HgCl_2$, CH_3CN, H_2O, RT, o/n, 80%.

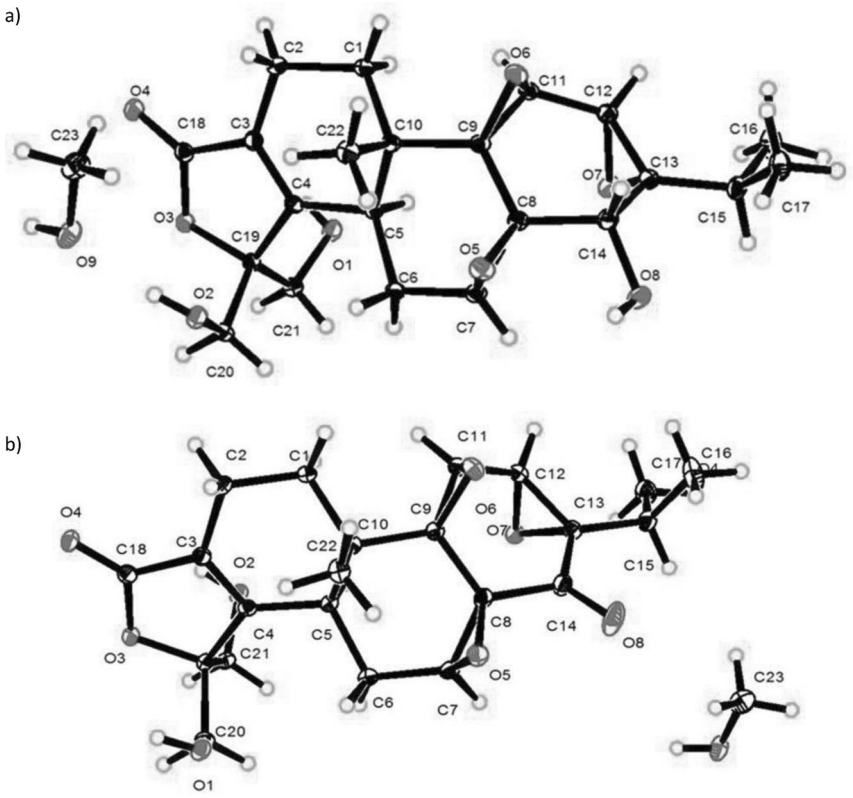


Figure 3. X-ray single-crystal structures of analogues a) **15** and b) **16**.

produced furan ring analogue **35**. Photochemical oxidation of **35** use molecular oxygen and rose bengal afford hemiacetal **36**.[33] Subsequent deprotection of compound **36** with mercury(II) chloride generated compound **17**. X-ray crystallographic analysis of compound **17** confirmed its structure to be as shown in Figure 4.

With target analogues **3**–**17** in hand, the in vitro cytotoxic activity was evaluated against two human tumor cell lines derived from human ovarian (SKOV-3) and prostate (PC-3) carcinoma cells using sulforhodamine B (SRB) assays (Table 1).[34] The results revealed that saturated five-membered lactone ring analogues **3**/**4**, C3 carbonyl group conformation-flexible analogues **5**/**6**, and carbonyl group conformation-locked analogue **7** were shown to be only weakly cytotoxic or inactive relative to the parent compound against both PC-3 and SKOV-3 cell lines. However, with the exception of **10**, furan ring analogues **8**, **9**, and **11** exhibited potent cytotoxicity against both cell lines. C19 hydroxy- and dihydroxymethyl-substituted analogues **15**, **16** and **17** were shown to be only weakly cytotoxic or inactive against both the PC-3 and SKOV-3 cell lines, while analogues **12**, **13** and **14** with methyl, methylene, and dimethylaminomethyl substituents, respectively, were found to be equipotent against the two cell lines as the natural product (Table 1). Based on the above results, we surmise that the five-membered unsaturated lactone ring (D ring) of triptolide is essential for potent cytotoxicity, and it might play a crucial role in defining the three dimensional shape and the electron properties of the whole molecule. Meanwhile, the furan ring analogues with different hydrogen bond acceptor and/or donor groups at the C18 position still exhibit moderate to potent cytotoxicity (**8** > **9** > **11**), and this apparently challenges the classical structure–activity relationship of triptolide that considers the five-membered unsaturated lactone ring as unmodifiable. The evaluation of the key series of C19 analogues revealed that this site is exquisitely sensitive to polarity (**1**, **12**, **13** > **17** > **16**).

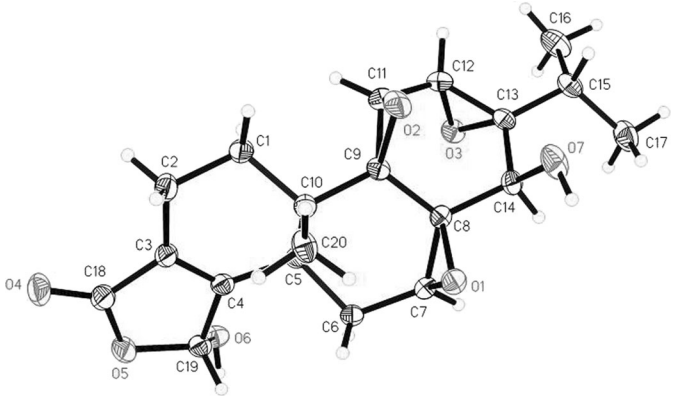


Figure 4. X-ray single-crystal structures of analogue **17**.

In summary, a systematic structure–activity relationship study on the D ring of triptolide was carried out. Four types of novel D ring-modified triptolide analogues were synthesized and tested for their cytotoxicity against two human tumor cell lines. The results of the current investigation suggest that the five-membered unsaturated lactone ring (D ring) of triptolide is essential to its potent cytotoxic activity; this moiety could play a crucial role in defining the three dimensional shape and electron properties of the whole molecule. That said, some modifications are tolerated, since the C18 hydrogen bond acceptor and/or donor furan ring analogues retain cytotoxic activity. In addition, evaluation of the key series of C19-substituted analogues showed that this site is exquisitely sensitive to polarity. These findings, while preliminary, further guide the development of potent and potentially clinically useful triptolide analogues, and confirm the importance of the D ring for cytotoxic activity. Further studies targeting some potent analogues are still in progress and will be disclosed in due course.

Table 1. The in vitro cytotoxic activity of the triptolide analogues in SKOV-3 and PC-3 cell lines.

Compd	IC_{50} [μM][a]		Compd	IC_{50} [μM][a]	
	SKOV-3	PC-3		SKOV-3	PC-3
1	0.006 ± 0.001	0.020 ± 0.003	**10**	1.100 ± 0.064	> 10
2	0.017 ± 0.002	0.075 ± 0.09	**11**	0.414 ± 0.051	0.633 ± 0.039
3	> 10	> 10	**12**	0.171 ± 0.025	1.803 ± 0.135
4	1.756 ± 0.135	9.320 ± 0.444	**13**	0.096 ± 0.016	0.506 ± 0.074
5	> 10	> 10	**14**	0.270 ± 0.022	2.546 ± 0.929
6	1.264 ± 0.101	> 10	**15**	> 10	> 10
7	0.953 ± 0.057	6.820 ± 0.925	**16**	> 10	> 10
8	0.097 ± 0.009	0.397 ± 0.047	**17**	1.246 ± 0.178	> 10
9	0.154 ± 0.013	0.492 ± 0.055			

[a] IC_{50}: the drug concentration required to inhibit cell proliferation by 50%; the maximum concentration tested was 100 μM; data are the mean ± standard deviation (SD) of at least two independent experiments; nine compound concentrations were used to determine the IC_{50} values.

Experimental Section

For experimental procedures, see the Supporting Information.

CCDC reference numbers 959971, 960042, 960043, 960044, 960045, and 960046 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Acknowledgements

This work was supported by the Shanghai Science and Technology Commission (China) "Biomedicine Technology Support" project (no. 13431900403) and the National Natural Science Foundation of China (NSFC) (no. 81373479).

Keywords: antitumor agents · cytotoxicity · natural products · structure–activity relationships · triptolide

[1] M. Kupchan, W. A. Court, R. G. Dailey, Jr., C. J. Gilmore, R. F. Bryan, *J. Am. Chem. Soc.* **1972**, *94*, 7194.
[2] S. Huang, *The Saint Peasant's Scripture of Materia Medica (18357)*, The Publishing House for Chinese Medicine Classics, Beijing, republished in **1982**, pp. 309 – 310.
[3] X. X. Wang, R. Matta, G. Shen, L. D. Nelin, D. H. Pei, Y. S. Liu, *J. Mol. Med.* **2006**, *84*, 405.
[4] G. S. Ding, *Clin. Ther.* **1987**, *9*, 345.
[5] S. M. Kupchan, R. M. Schubert, *Science* **1974**, *185*, 791.
[6] K. Y. Lee, W.-T. Chang, D.-M. Qiu, P. N. Kao, G. D. Rosen, *J. Biol. Chem.* **1999**, *274*, 13451.
[7] Y. L. Zheng, J. F. Lin, C. C. Lin, Y. Xu, *Acta Pharm. Sin.* **1994**, *15*, 540.
[8] J. R. Zheng, K. X. Gu, L. F. Xu, J. W. Gao, Y. H. Yu, M. Y. Tang, *Acta Acad. Med. Sin.* **1991**, *13*, 391.
[9] W. Z. Gu, R. Chen, S. Brandwein, J. McAlpine, N. Burres, *Int. J. Immunopharmacol.* **1995**, *17*, 351.
[10] S.-X. Yang, H. L. Gao, S. S. Xie, W. R. Zhang, 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, P. N. Kao, *J. Biol. Chem.* **1999**, *274*, 13443.
[12] Q. S. Zhen, X. Ye, 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, H. Z. Sun, *Acta. Acad. Med. Sin.* **1987**, *9*, 323.
[14] S. Yang, J. Chen, Z. Guo, X.-M. Xu, L. Wang, X.-F. Pei, J. Yang, C. B. Underhill, L. Zhang, *Mol. Cancer Ther.* **2003**, *2*, 65.
[15] Y. Yang, Z. Liu, E. Tolosa, J. Yang, L. Li, *Immunopharmacology* **1998**, *40*, 139.
[16] B. J. Chen, *Leuk. Lymphoma* **2001**, *42*, 253.
[17] L. A. Shamon, J. M. Pezzuto, J. M. Graves, R. R. Mehta, S. Wangcharoentrakul, R. Sangsuwan, S. Chaichana, P. Tuchinda, P. Cleason, V. Reutrakul, *Cancer Lett.* **1997**, *112*, 113.
[18] T. M. Kiviharju, P. S. Lecane, R. G. Sellers, 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, M. Andreeff, *Blood* **2006**, *108*, 630.
[20] T. Tengchaisri, R. Chawengkirttikul, N. Rachaphaew, V. Reutrakul, R. Sangsuwan, 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, G. D. Rosen, *J. Biol. Chem.* **2001**, *276*, 2221.
[22] S. Frese, F. Pirnia, D. Miescher, S. Krajewski, M. M. Borner, J. C. Reed, R. A. Schmid, *Oncogene* **2003**, *22*, 5427.
[23] S. D. Westerheide, T. L. Kawahara, K. Orton, R. I. Morimoto, *J. Biol. Chem.* **2006**, *281*, 9616.
[24] J. M. Fidler, K. Li, C. Chung, K. Wei, J. A. Ross, M. Gao, G. D. Rosen, *Mol. Cancer Ther.* **2003**, *2*, 855.
[25] B. Zhou, X. M. Li, H. T. Tang, Z. H. Miao, H. J. Feng, Y. C. Li, *Org. Biomol. Chem.* **2011**, *9*, 3176.
[26] Y. Aoyagi, Y. Hitotsuyanagi, T. Hasuda, H. Fukaya, K. Takeya, R. Aiyama, T. Matsuzaki, S. Hashimoto, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3046.
[27] B. Zhou, X. M. Li, H. J. Feng, Y. C. Li, *Tetrahedron* **2010**, *66*, 5396.
[28] H. Becker, T. Bremholt, E. Adler, *Tetrahedron Lett.* **1972**, *13*, 4205.
[29] D. Yang, X. Y. Ye, M. Xu, *J. Org. Chem.* **2000**, *65*, 2208.
[30] K. Tamao, N. Ishida, Y. Ito, M. Kumada, *Org. Synth.* **1990**, *69*, 96.
[31] Z. Li, Z. L. Zhou, Z. H. Miao, L. P. Lin, H. J. Feng, L. J. Tong, J. Ding, Y. C. Li, *J. Med. Chem.* **2009**, *52*, 5115.
[32] B. S. Bal, W. E. Childers, Jr., H. W. Pinnick, *Tetrahedron* **1981**, *37*, 2091.
[33] M. R. Kernan, D. J. Faulkner, *J. Org. Chem.* **1988**, *53*, 2773.
[34] L. V. Rubinstein, R. H. Shoemaker, K. D. Paull, R. M. Simon, S. Tosini, P. Skehan, D. A. Scudiero, A. Monks, M. R. Boyd, *J. Natl. Cancer Inst.* **1990**, *82*, 1113.

Received: October 16, 2013
Published online on December 11, 2013