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Semisynthesis of triptolide analogues: Effect of B-ring substituents on cytotoxic activities

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ABSTRACT

A series of B-ring modified analogues of triptolide were synthesized and tested for their cytotoxicity against two human tumor cell lines (U251 and PC-3). From the current investigation, the structure–cytotoxic activity relationships of these analogues suggested that the introduction of hydroxyl, epoxide, halogen or olefinic groups on C5 and/or C6 could still retain the cytotoxicity, albeit a little less potency, and the C7,C8-β-epoxide group of triptolide was essential to its potent cytotoxic activity.

Triptolide (**1**, Fig. 1) is a naturally occurring diterpenoid isolated from extracts of *Tripterygium wilfordii* Hook F. (TWHF),[1] commonly known as *Lei Gong Teng* or Thunder God Vine, a well-known Chinese traditional medicine herbal, whose extracts have been used to treat autoimmune and inflammatory diseases, such as rheumatoid arthritis and systemic lupus erythematosus for centuries.[2] As one of the primary biologically active ingredients of TWHF, triptolide display a wide array of biological activities like antitumor, anti-inflammatory and immunosuppressive.[3] It shows strong antiproliferative activity at the cellular level and inhibits the proliferation of all 60 cancer cell lines of US National Cancer Institute with nanomolar IC_{50} values (average IC_{50} = 12 nM). However, before triptolide can reach its clinical potential, great challenges remain to overcome, such as multi-organ toxicity, narrow therapeutic window and poor water solubility. In order to find ways to enhance its efficacy and reduce toxicity, extensive synthesis and structural modifications of triptolide and its derivatives have been carried out in many research groups worldwide in the past few decades, and already yielded lots of important structure activity relationships information.[4] Triptolide derivatives (e.g., 5*R*-5-hydroxytriptolide, PG490-88 and Minnelide) have already entered human clinical trials for cancer and rheumatoid arthritis.[5]

Previous studies on the structure–cytotoxic activity relationships (SARs) of triptolide were mainly focused on the lactone D-ring, the C-ring epoxide groups and the C14-hydroxy group, and have emerged as the most promising approach for improving bioavailability that led to the discovery and development of clinically important anticancer or antiinflammatory compounds.[4] However, due to the lack of preactivated group, the structure activity relationship of B-ring is still obscure, no systematic SARs studies have been reported.

As an ongoing work of our research on the SARs of triptolide, we think that it is important to execute a systemic SARs studies of B-ring. In addition to further evaluation of the structure–cytotoxic activity relationship of B-ring, for which only limited data are currently available, the ability to access this complex SARs to find new modification site and new triptolide analogues with good pharmacokinetic–pharmacodynamic properties are also what we want.

In the present study, we designed and synthesized a series of B-ring modified analogues of triptolide (Fig. 2), that is, analogues (**4**–**20**) with hydroxyl, epoxide or halogen groups on C5,C6 and

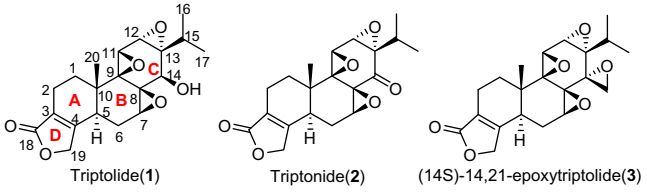

Figure 1. Structure of triptolide, triptonide and (14*S*)-14,21-epoxytriptolide.

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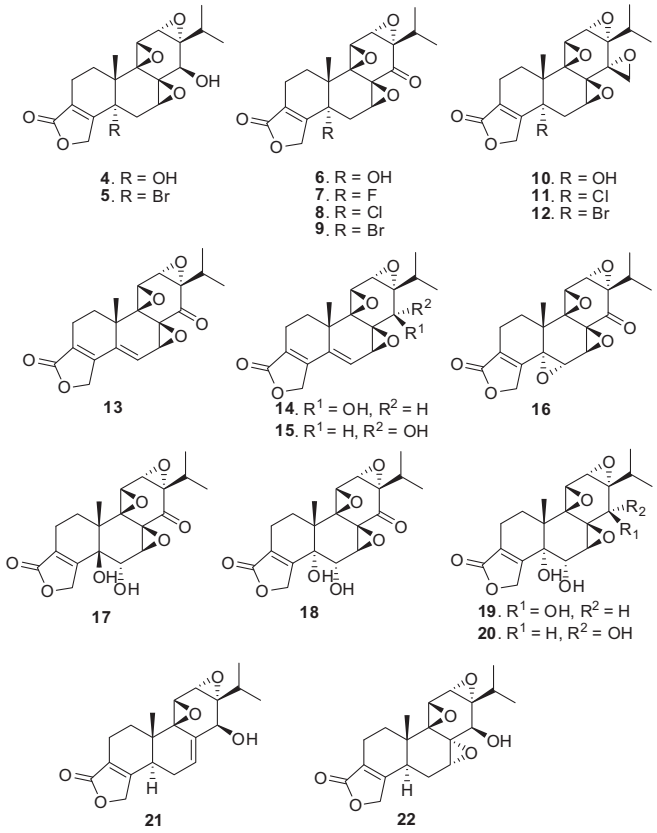


Figure 2. Analogues **4**–**22**.

Table 1
C5 functionalization of triptolide (**1**), triptonide (**2**) and (14*S*)-epoxytriptolide (**3**) under different conditions

Substrate	Reaction conditions (h)	Results (%)[a]
1	SeO_2, 1,4-dioxane, reflux, 24	**4** (5)
2	SeO_2, 1,4-dioxane, reflux, 24	**6** (83)
3	SeO_2, 1,4-dioxane, reflux, 12	**10** (50)
1	NCS, AIBN, DCE, 5	Decomposition
2	NCS, AIBN, DCE, 2	**8** (62)
3	NCS, AIBN, DCE, 2	**11** (71)
1	NBS, AIBN, DCE, 2	**5** (72)
2	NBS, AIBN, DCE, 1.5	**9** (95)
3	NBS, AIBN, DCE, 1.5	**12** (92)

[a] Isolated yield.

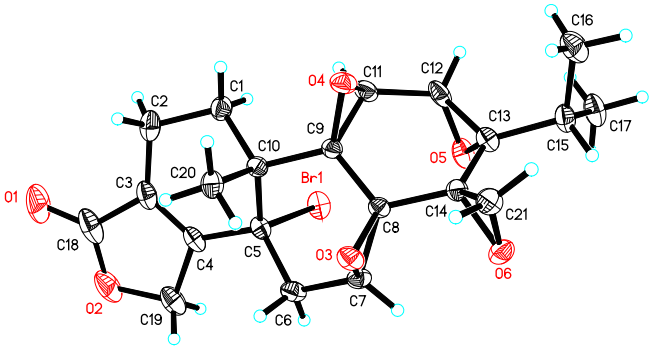
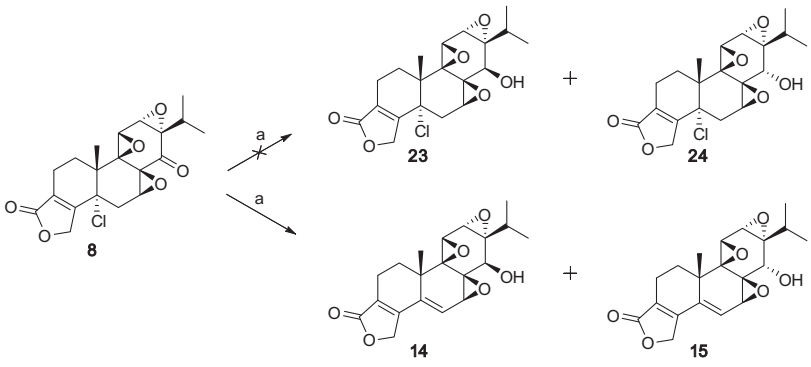


Figure 3. X-ray of analogue **12**.

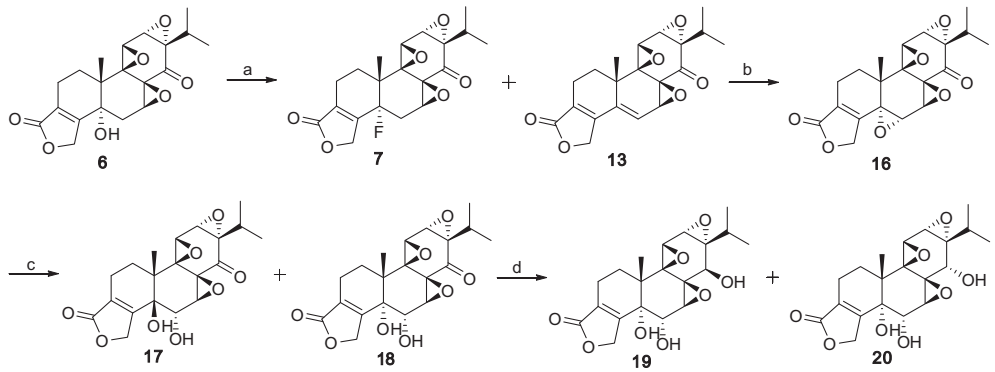
analogues (**21**–**22**) having C7,C8-olefinic or C7,C8-α-epoxide groups instead of the C7,C8-β-epoxide group on triptolide. The SARs studies of these analogues were performed by using human glioma (U251) and human prostate (PC-3) tumor cell lines.

The synthetic strategy followed for the preparation of analogues **4**–**6** and **8**–**12** was depicted in Table 1. Starting material triptolide (**1**) and triptonide (**2**) were extracted from TWHF of our region, (14*S*)-14,21-epoxytriptolide (**3**) was prepared by a three steps procedure that was previously reported by our group.[4d] Allylic oxidation of triptolide (**1**) using selenium dioxide in refluxing 1,4-dioxane gave C5 hydroxyl group substituted analogues **5** only in 5% yield, while under this condition compounds **2** and **3** proceeded efficiently to give the corresponding analogues **6** and **10** in moderate yield. Reaction of compounds **2** and **3** with azodiisobutyronitrile (AIBN) and *N*-chlorosuccinimide (NCS) in refluxing 1,2-dichloroethane produced C5 chloride substituted analogues **8** and **11** in moderate yield. However, compound **1** was quickly decomposition under this condition. We, therefore, turned our attention to the reduction of C5 chloride substituted triptonide **8** to get C5 chloride substituted triptolide analogue **23** (Scheme 1). But, unfortunately, treatment of analogue **8** in methanol with sodium borohydride gave a pair of C5,C6-olefinic substituted epimer **14** an **15** instead of the desired analogue **23**. Under azodiisobutyronitrile (AIBN) and bromosuccinimide (NBS) condition, all of these compounds proceeded efficiently to give the corresponding C5 bromide substituted analogues **5**, **9** and **12** in moderate to high yield. The structure of **12** was confirmed by X-ray crystallographic analysis (Fig. 3).[6] The configurations of the other C5 halogen substituted analogues were assumed to be the same by analogy.

The synthesis of analogues **7**, **13** and **16**–**20** used C5-hydroxytriptonide **6** as the starting material (Scheme 2). Fluorination of **6** with dimethylaminosulfur trifluoride (DAST) gave a 1:1 mixture



Scheme 1. Reagents and conditions: (a) $NaBH_4$, MeOH, 0 °C, 1 h, **14** (42%), **15** (45%).



Scheme 2. Reagents and conditions: (a) DAST, DCM, 0 °C, 1 h, (**7**: 39%, **13**: 44%); (b) CuCl, *m*-CPBA, CH_2Cl_2, reflux, 6 h, 75%; (c) aq 10% H_2SO_4, reflux, 12 h, (**17**: 29%; **18**: 45%). (d) $NaBH_4$, CH_3OH, 0 °C, 1 h, (**19**: 25%; **20**: 62%).

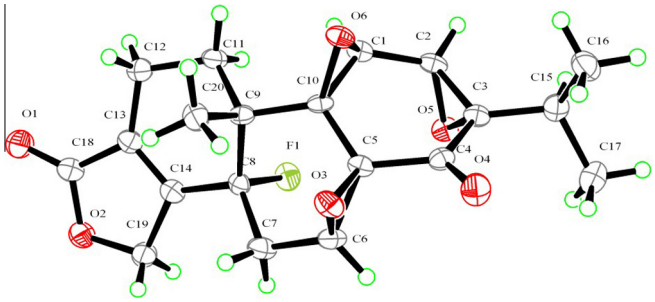


Figure 4. X-ray of analogue **7**.

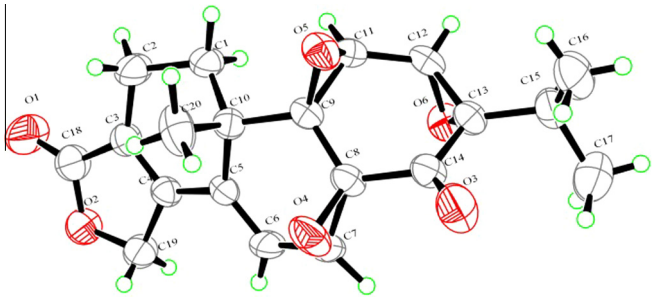


Figure 5. X-ray of analogue **13**.

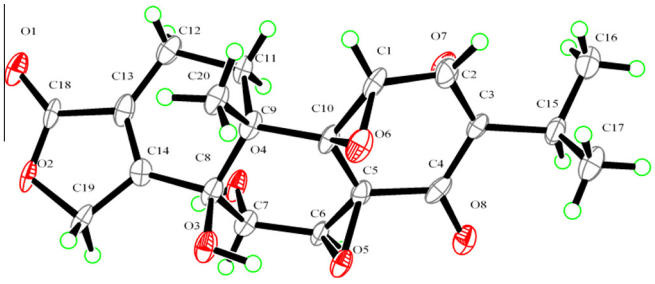


Figure 6. X-ray of analogue **17**.

of C5-α-fluorotriptonide **7** and C5,C6-olefinic analogue **13**. Treatment of **13** with 3-chloroperbenzoic acid and a catalytic amount of copper(I) chloride afforded C5,C6-epoxytriptonide **16** as a single diastereomer in moderate yield. Subsequently, treatment of **16** with dilute sulfuric acid gave a 2:3 mixture of C5,C6-*trans*-dihydroxytriptonide **17** and C5,C6-*cis*-dihydroxytriptonide **18**. Further

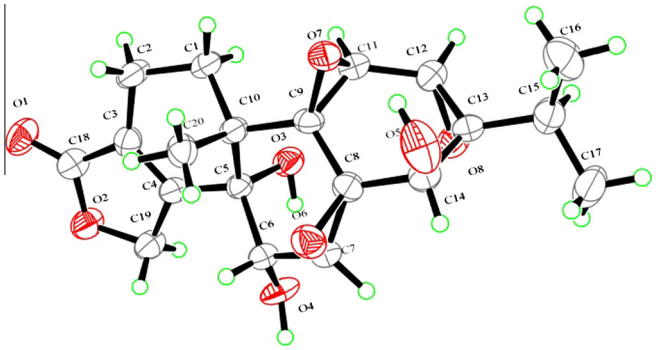
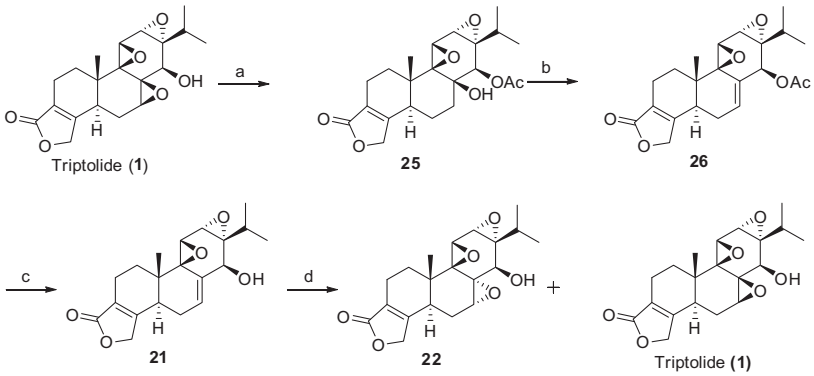


Figure 7. X-ray of analogue **19**.

reduction of **18** with sodium borohydride produced analogues **19** and **20**. The structures of **7**, **13**, **17** and **19** were confirmed by X-ray crystallographic analysis (Figs. 4–7).[6]

The synthetic work for the preparation of analogues **21** and **22** used triptolide (**1**) as the starting material by a known procedure that was previously reported by our group (Scheme 3).[7] Reaction of triptolide **1** with lithium borohydride and boron fluoride ethyl ether result in opining of C7,C8-β-epoxide to give C8,C14-diol intermediate, which was subjected to acetic anhydride and pyridine resulted in selective protection of its C14-hydroxyl group to provide **25**. Dehydration of **25** produced an olefinic intermediate **26**, which was reacted with hydrazine monohydrate in methanol yield alcohol **21**, subsequently, epoxidation of **21** with 3-chloroperbenzoic acid and disodium hydrogen phosphate provided C7,C8-α-epoxide **22** along with a small amount of triptolide **1**.

As shown above, we obtained a series of novel B-ring modified triptolide analogues. To examine whether the substitution affected their cytotoxic activities, we evaluated the cytotoxic effects of these target compounds (**4**-**22**) against two human tumor cell lines derived from human glioma (U251) and prostate (PC-3) using sulforhodamine B (SRB) assays (Table 2).[8] The results revealed that by introduction of hydroxy, epoxide, halogen and/or olefinic groups on C5 and C6, analogue **4**–**16** can still retain the cytotoxicity, albeit a little less potent, among them, C5 chloride substituted analogues **8** showed the highest potency, with the lowest IC_{50} value (85 nM for PC-3 cells). However, analogue **17**–**20** with *trans*- or *cis*-dihydroxy groups on C5,C6 only showed very weak cytotoxicity or lost their cytotoxicity. Substitution of C7,C8-β-epoxide group with an olefinic group or α-epoxide, analogues **21** and **22** all lost their cytotoxicity, suggested that C7,C8-β-epoxide group of triptolide was essential to its potent cytotoxic activity, it might play a crucial role in defining the three dimensional shape of the whole molecule.



Scheme 3. Reagents and conditions: (a) (1) 2M $LiBH_4$ in THF, $BF_3{\cdot}Et_2O$, THF, rt, 4 h; (2) acetic anhydride, pyridine, rt, 24 h, 79% (2 steps); (b) TFAA, MsCl, TEA, pyridine, CH_2Cl_2, rt, 12 h, 61%; (c) 85% $NH_2NH_2{\cdot}H_2O$, CH_3OH, rt, 10 min, 93%; (d) *m*-CPBA, $Na_2HPO_4{\cdot}12H_2O$, CH_2Cl_2, rt, 96 h, (**22**: 40%; **1**: 10%).

Table 2
In vitro cytotoxic activity of triptolide analogues in U251 and PC-3 cells

Compound	IC_{50} (mean ± S.D., μM)[a]	
	U251	PC-3
1	0.033 ± 0.001	0.020 ± 0.003
2	0.031 ± 0.011	0.026 ± 0.006
3	0.657 ± 0.235	0.637 ± 0.218
4	0.488 ± 0.091	0.275 ± 0.028
5	0.796 ± 0.151	0.333 ± 0.079
6	1.031 ± 0.125	0.377 ± 0.077
7	0.429 ± 0.134	0.223 ± 0.023
8	0.170 ± 0.067	0.085 ± 0.007
9	0.558 ± 0.134	0.104 ± 0.022
10	5.466 ± 0.248	2.613 ± 0.357
11	0.925 ± 0.076	0.871 ± 0.058
12	2.037 ± 0.308	0.893 ± 0.026
13	1.632 ± 0.384	0.171 ± 0.045
14	0.718 ± 0.220	0.126 ± 0.024
15	5.044 ± 1.562	3.224 ± 0.929
16	0.197 ± 0.084	4.052 ± 0.069
17	9.855 ± 1.208	5.043 ± 0.109
18	2.966 ± 0.402	2.272 ± 0.366
19	1.585 ± 0.298	0.589 ± 0.029
20	>10	>10
21	>10	>10
22	>10	>10

[a] The drug concentration required for 50% inhibition of cell proliferation, while the maximum concentration used here was 100 μM, each experiment was run at least two independent experiments with nine concentrations, and the results are presented as average values ± S.D.

In summary, a systematic structure–cytotoxic activity relationship study on the B-ring of triptolide has been executed. A series of B-ring modified analogues of triptolide were synthesized and tested for their cytotoxicity against two human tumor cell lines (U251 and PC-3). From the current investigation, the structure–cytotoxic activity relationship of these analogues suggested that the introduction of hydroxy, epoxide, halogen or olefinic groups on C5,C6 could still retain the cytotoxicity, albeit a little less potency and the C7,C8-β-epoxide group of triptolide was essential to its potent cytotoxic activity, it might play a crucial role in defining the three dimensional shape of the molecule. Further studies targeting some potent analogues are still in progress and will be disclosed in due course.

Acknowledgments

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Supplementary data

Supplementary data (synthesis, ^1H NMR, ^{13}C NMR, MS and HRMS) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.10.069.

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