Bioorganic & Medicinal Chemistry Letters 21 (2011) 3046–3049

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Semisynthesis of triptolide analogues: Effect of γ-lactone and C-14 substituents on cytotoxic activities

Yutaka Aoyagi [a], Yukio Hitotsuyanagi [a], Tomoyo Hasuda [a], Haruhiko Fukaya [a], Koichi Takeya [a,*], Ritsuo Aiyama [b], Takeshi Matsuzaki [b], Shusuke Hashimoto [b]

[a] School of Pharmacy, Tokyo University of Pharmacy & Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan
[b] Yakult Central Institute for Microbiological Research, 1976 Yaho, Kunitachi, Tokyo 186-8650, Japan

ARTICLE INFO

Article history:
Received 17 February 2011
Revised 8 March 2011
Accepted 9 March 2011
Available online 16 March 2011

Keywords:
Triptolide analogues
Semisynthesis
Cytotoxity
A549 cells
HT29 cells

ABSTRACT

Triptolide γ-lactone and C-14 analogues were prepared and evaluated cytotoxity against human lung adenocarcinoma epithelial A549 cells and human colon adenocarcinoma HT-29 cells. γ-Lactone substructure and C-14 substituents affected the biological activities significantly.

Triptolide (**1**) and triptonide (**2**), isolated from *Tripterygium wilfordii* (Celastaceae) (Fig. 1), and their semisynthetic analogues 14-epitriptolide (**3**) having a unique sequential triepoxide system on the B/C rings, are reported to be cytotoxic.[1,2] In our previous Letters[3,4] on the synthesis of triptolide analogues, we reported that the stereochemistry at C-14 and the presence of 12α,13α-oriented epoxide might be essential factors for the triptolide analogues to show cytotoxic activity, and that 14-fluorotriptolide (**4**) showed a stronger cytotoxity than the natural triptolides against human tumor cells.

In this Letter, we prepared a series of triptolide analogues in which the γ-lactone and C-14 groups were modified, and assayed their cytotoxities against human lung adenocarcinoma epithelial A549 cells and human colon adenocarcinoma HT-29 cells. The structure–activity relationship was also discussed.

Triptolide γ-lactone analogues (**8** and **9**) were prepared as shown in Scheme 1. Thus, the 14-hydroxy group of **1** was protected with *tert*-butyldimethylsilyl (TBS) group, and the product **5** was treated with methylmagnesium bromide to give furanoditerpene **6**. The deprotection of **6** with tetrabutylammonium fluoride (TBAF) gave **8**. When ethylmagnesium bromide was used, the reaction gave **7**, which, on deprotection product **9**.

Then, triptonide (**2**), derived from **1** by Dess–Martin periodinane oxidation,[3] was treated with alkylmagnesium bromide under several different reaction conditions. The results are shown in Table 1. Reaction of **2** with methylmagnesium bromide at 0 °C for 20 min gave two furanoditerpenes **10** and **11**, having different stereochemistry at C-14. The structures of **10** and **11** were determined mainly by 1D and 2D NMR experiments. The NOE correlation between 5-H and 14-Me protons was observed in **11**, but not in **10**. The yield of **10** was much higher than that of **11**, which indicated that the attack by methyl group to the carbonyl group of **2** might be occurred from the convex face to give **10** as a major product. Reaction of **2** with methylmagnesium bromide at −78 °C gave **12**

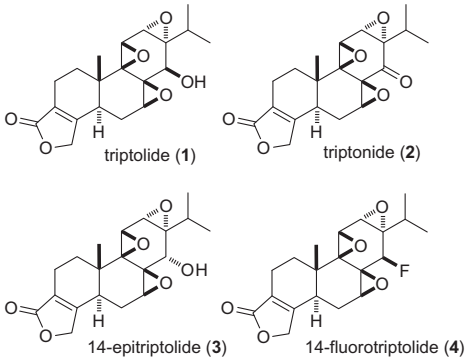

Figure 1. Triptolide (**1**), triptonide (**2**) and its semisynthetic analogues (**3** and **4**).

* Corresponding author. Tel.: +81 426 76 3007; fax: +81 426 77 1436.
E-mail address: takeyak@ps.toyaku.ac.jp (K. Takeya).

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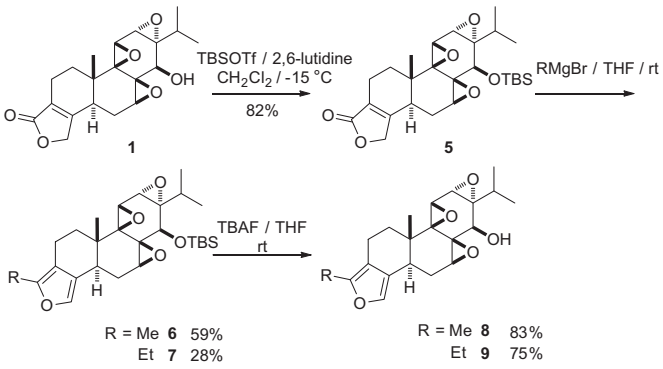
doi:10.1016/j.bmcl.2011.03.025



Scheme 1. Preparation of triptolide γ-lactone analogues **8** and **9**.

as a sole product in 83%. Its structure was established by X-ray crystallographic analysis to be as shown in Figure 2.

By the reaction with ethylmagnesium bromide, **2** gave an alkylated compound **13** in a low yield along with the hydrogenated compounds **1** and its epimer **3**. With sterically bulky reagent, butylmagnesium bromide at −78 °C, **2** did not give the corresponding C-14 alkylated compound but hydrogenated compounds **1** and its epimer **3**.

A triptolide analogue having a restricted angle between CH_2-14 and O-14, that is, 14-epoxytriptolide (**14**) was prepared and its steric effect on cytotoxities was studied.

Namely, as shown in Scheme 2, the reaction of **2** with trimethylsilyldiazomethane at rt gave tetraepoxide **14** in 82%. Li et al. previously reported the synthesis of **14** by the reaction of **2** with a combination of potassium *tert*-butoxide and trimethyl-sulfoxonium iodide.[6] Our present method, a one step reaction under very mild reaction conditions, gave the aimed compound in a very high yield. The structure of product **14** was comfirmed by X-ray crystallographic analysis to be as shown in Figure 3.[5]

The cytotoxicities of triptolide (**1**) and its analogues (**2–3** and **8–14**) were assayed on human lung adenocarcinoma epithelial A549 cells and human colon adenocarcinoma HT-29 cells, and the results are shown in Table 2.

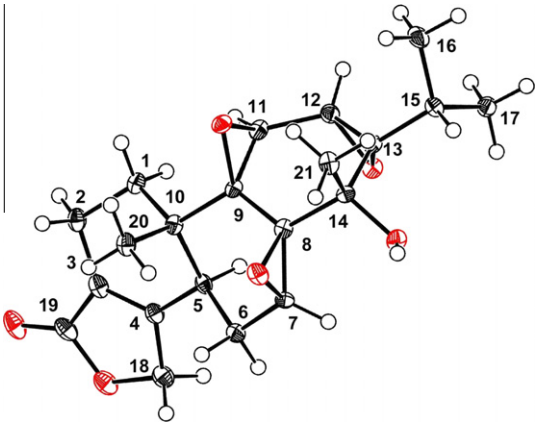
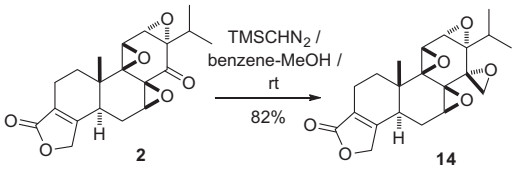


Figure 2. Ortep representation for **12**.



Scheme 2. Preparation of triptolide C14-oxirane analogue **14**.

The furanoditerpenes **8–11**, triptolide analogues having a furan ring in place of γ-lactone, had very or extremely low activities. Thus, the presence of γ-lactone subunit, which is an electron acceptor, is considered to be essential for those compounds to show cytotoxic activity, and that of a furan unit fused with ring A, an electron donor group, on the other hand, to have a negative effect on the activity. The presence of an electron negative group such as hydroxyl group of β-face orientation at C-14 seems to increase the cytotoxic effect of this series of compound, as demonstrated by the activities of **1** and **3**. Another compound having a fluorine atom at C-14 of β-face orientation[4] is reported to be more

Table 1
Reaction of triptonide **(2)** with alkylmagnesium bromide under defferent reaction conditions

Entries	Reaction conditions	Results[a] (%)		
1	MeMgBr (5 equiv)/0 °C/20 min	**10** (47%)	**11** (14%)	
2	MeMgBr (5 equiv)/−78 °C/50 min	**12** (83%)		
3	EtMgBr (5 equiv)/−78 °C/180 min	**13** (29%)	**3** (31%)	**1**(28%)
4	BuMgBr (5 equiv)/−78 °C/60 min	**3** (16%)	**1** (10%)	

[a] Isolated yields.

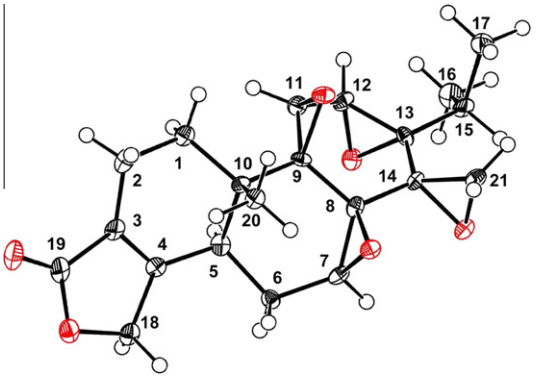


Figure 3. Ortep representation for **14**.

Table 2
Cytotoxity of triptolide (**1**) and its analogues **2–3** and **8–14** on A549 and HT29 cells

Compounds	IC_{50}[a] (μM)	
	A549	HT29
Triptolide (**1**)	0.019	0.0021
2	0.053	0.0084
3	1.69	0.078
8	7.85	2.01
9	>13.4	>13.4
10	>13.4	>13.4
11	>13.4	>13.4
12	0.35	0.022
13	2.83	0.39
14	0.053	0.00065
SN-38[b]	0.047	0.0059
Docetaxel[b]	0.0012	0.00075

[a] IC_{50} values are means of at least two measurements.
[b] Positive controls.

active than **1**, a corresponding natural compound having a hydroxyl group of β-face orientation.[4] The C-14 alkyl groups which are arranged at β-orientation may have a decreasing effect on the activity. The relationship between the 3D-structure and activity was studied by aligning the common A/B/γ-lactone ring system of the X-ray structures of **1**–**3**, **12**, and **14**, all having relatively potent cytotoxicity, by using SYBYL 7.3 software.[7] The superimposition drawings are shown in Figure 4. As shown, the A/B/γ-lactone portions of all the compounds are almost superimposable: Differences in conformation are noted only in ring C involving C-14. Of

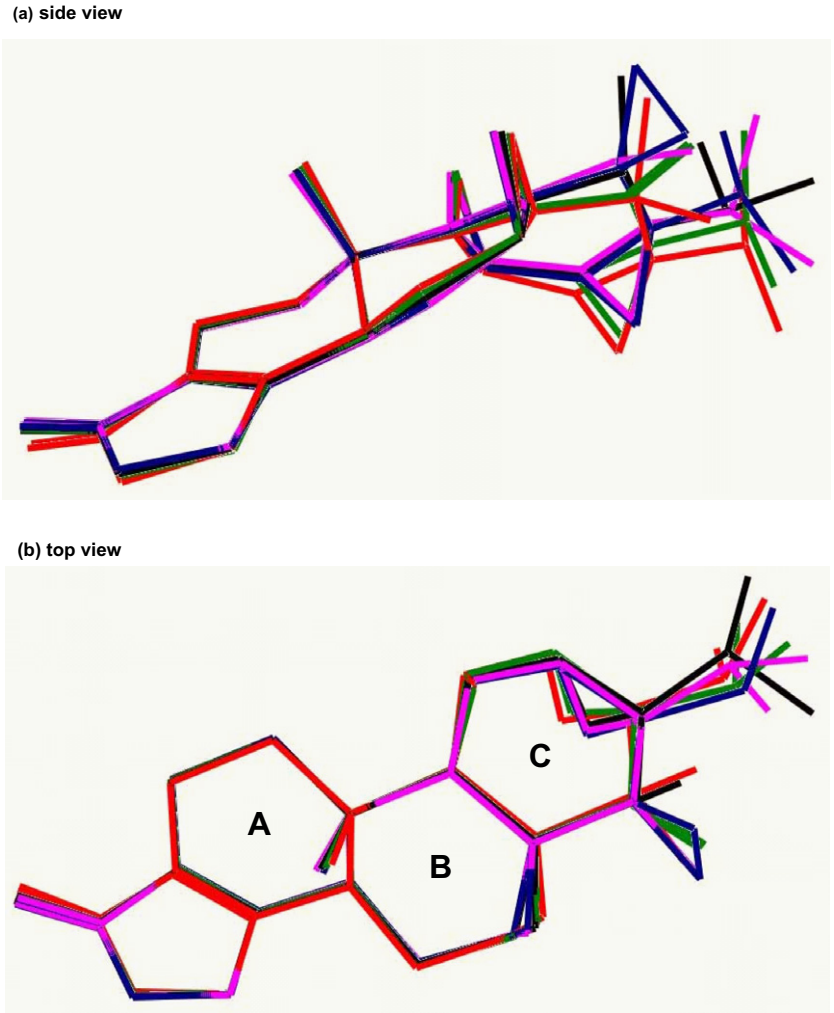


Figure 4. Allign stereodrawing of compounds **1**–**3**, **12** and **14**, which were produced by X-ray crystallographic analysis.

the present triptolide analogues (**12**–**14**) having different groups at C-14, **13** is less active than **12**. The bulkiness of alkyl group may affect the activity. Of the three, **14** is the most active. The C-14 alkyl groups in 14-CH_2 and in **12** CH_3 are not superimposable (see top view in Figure 4), that is, the Me group in **12** is arranged axial and CH_2 in **14** pseudoaxial (the angles CH_3 (or CH_2)–C–14–O are 110° and 59°, respectively). This specific stereochemistry involving C-14 may play a role in making **14** more active than **12**. Thus, the present observations emphasize that two essential features are needed for the triptolide analogues to show potent cytotoxity, which are certain defined 3D-alignment of the molecule, especially of the unit involving C-14, and the presence of an electron negative group such as a hydroxy group or a fluorine atom of β-face orientation at C-14.

Acknowledgment

This work was supported by a Grant-in-aid for Scientific Research (C) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (to Y.A.).

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7. Molecular modeling software SYBYL7.3 (Tripos associates, St. Louis, MI, USA was employed. The X-ray structures obtained were aligned so that A and B rings were superimposed to form a common structure by using the database aligned method.