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Synthesis and biological evaluation of novel triptolide analogues for anticancer activity

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ABSTRACT

Three types of novel triptolide analogues with 9,11-olefin (**3**–**5**), five-membered unsaturated lactam ring (**6**–**7**) or A/B cis ring junction (**8**–**14**) were synthesized. Although with 9,11-olefin instead of 9,11-β-epoxide, compound **4a** was much more active than the parent natural triptolide (**1**) with the lowest IC_{50} value of 0.05 nM for SKOV-3 cells, clearly challenging the traditional viewpoint on the necessity of 9,11-β-epoxide group of triptolide. In addition, structure–activity relationships for three classes of compounds were studied.

Triptolide (**1**) and triptonide (**2**) (Fig. 1), the major components for the clinical properties of *Tripterygium wilfordii* Hook. f. (TWHF)[1–3], were 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, triptolide (**1**) and triptonide (**2**) were shown to possess potent antitumor, antiinflammatory, immunosuppressive, and antifertile activities.[4–22] Compared with some conventional chemotherapeutic drugs, triptolide has similar or even superior anticancer activity, especially over p53 mutated or multi-drug resistant cells.[17] All these 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.

Previous studies on the structure–activity relationship of triptolide indicated that the activation of the 9,11-β-epoxide group by the hydrogen bonding between the 14β-hydroxyl group hydrogen atom and the 9,11-β-epoxide oxygen atom may account for its antitumor effect and the 9,11-β-epoxide is faithfully critical to its anticancer activity.[5] Base on the principle, for a long time no 9,11-β-epoxide modification was reported and 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.[23,24] However, some medicinal chemists have found recently that substituting C14-hydroxyl with other groups such as fluoride while keeping β orientation of the substituents

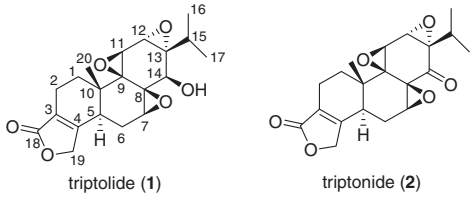

Figure 1. Triptolide and triptonide from *Tripterygium wilfordii* Hook. f.

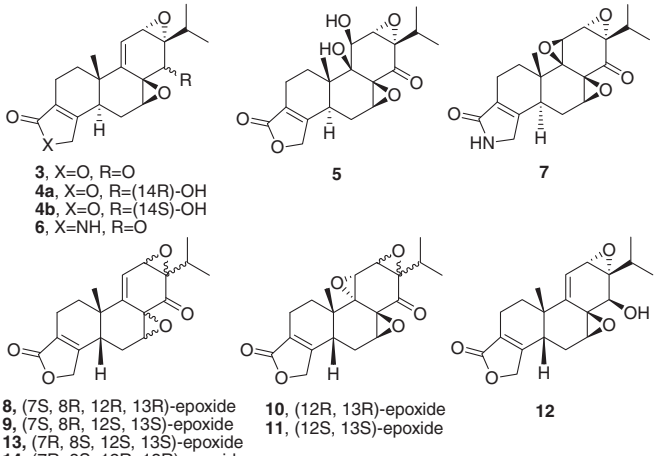

Figure 2. Triptolide analogues **3**–**14**.

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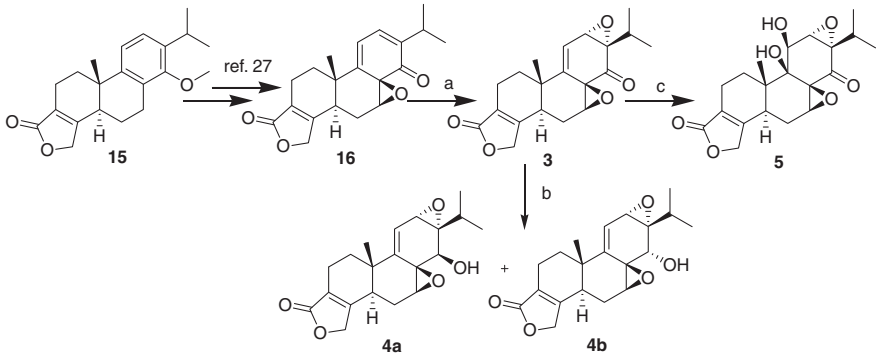
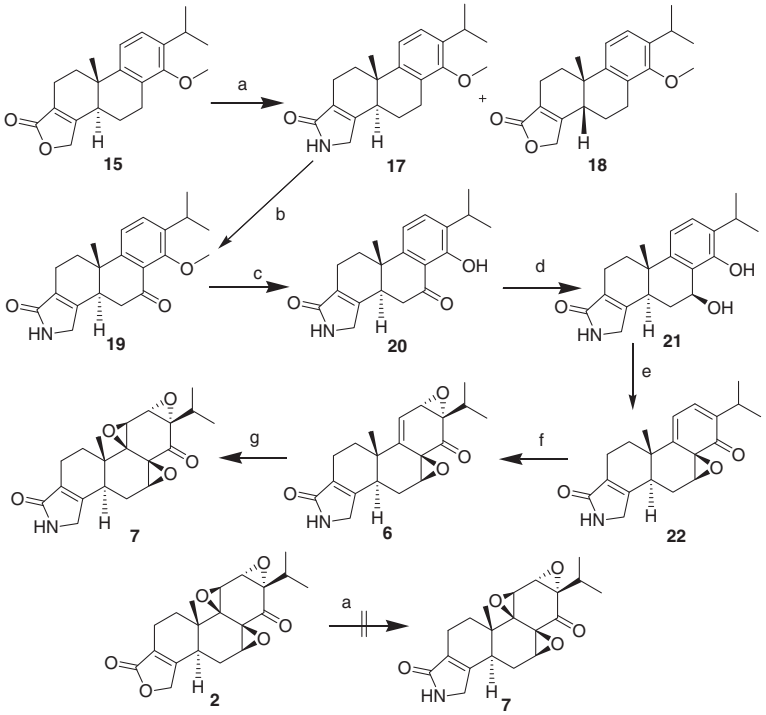
or with a chiral epoxy group having a different oxygen direction at C14 position could retain the cytotoxicity of triptolide,[25,26] and the anticancer activity of triptolide analogues may not be explained so simply as before.[5]

In the present study, we designed three types of analogues of triptolide **1**. To begin with, to explore whether the 9,11-β-epoxide of triptolide is faithfully critical to its anticancer activity, 9,11-β-epoxide modified analogues (compounds **3**–**5**) were synthesized (Fig. 2). Then, analogues of triptonide were sought in which five-membered unsaturated lactone ring was replaced with the five-membered unsaturated lactam ring (compounds **6** and **7**) as amide often behaves as ester isostere and the water-solubility of amide is greater than that of corresponding ester. This would have its practical significance because good aqueous solubility of products applied is invaluable and the clinical development of triptolide have been challenged by water-insolubility and severe toxicity. Finally, a series of analogues (compounds **8**–**14**) with A/B cis ring junction were designed for more comprehensive SAR studies. The SAR studies of these tripolide analogues were performed by using ovary (SK-OV-3) and prostate (PC-3) tumor cells.

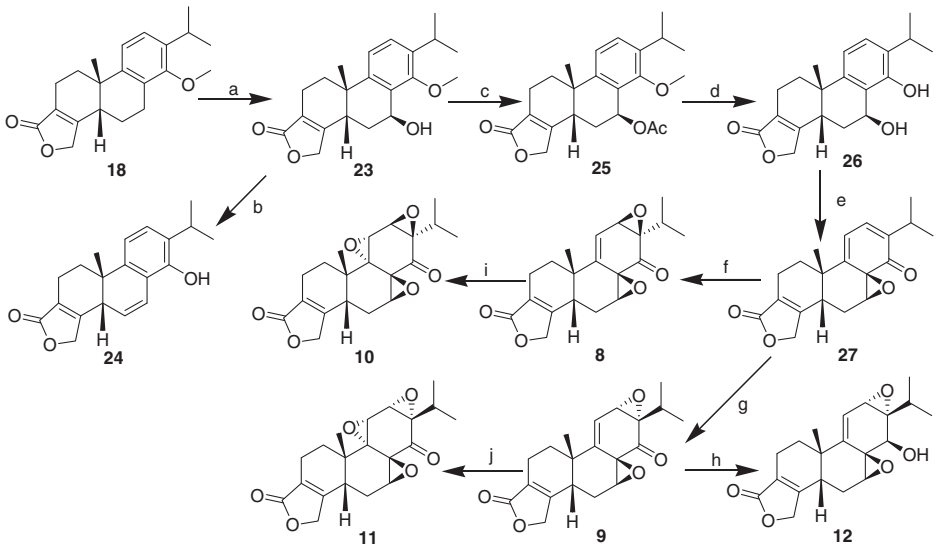
The synthetic strategy followed for the preparation of the triptonide analogues **3**–**14** is depicted in Schemes 1–3. Considering that direct modification of 9,11-β-epoxide on triptonide **2** was difficult owing to the presence of 12,13-α-epoxide which was very sensitive to nucleophilic attack, we chose the triptophenolide methyl ether **15** as the starting material which was prepared from abietic acid according to the reported procedure[27] and compound **16** was also prepared by the procedure described (Scheme 1).[27] Treatment of **16** with basic H_2O_2 provided compound **3** in 56% yield, along with the 12,13-β-epoxy isomer.[28] Subsequent reduction of **3** with $NaBH_4$ in MeOH furnished **4a** (23%) together with its α-hydroxyl epimer **4b** (72%) and a modification of Sharpless'



Scheme 1. Synthesis of analogues **3**, **4a**, **4b** and **5**. Reagents and conditions: (a) H_2O_2, NaOH, MeOH, 25 °C, 2 h, 56%; (b) $NaBH_4$, CH_3OH, rt, 0.5 h, 23% of **4a** and 72% of **4b**; (c) $K_2OsO_4{\cdot}(H_2O)_2$, NMO, pyridine, acetone/water = 5:1, reflux, 2 days, 65%.



Scheme 2. Synthesis of analogues **6** and **7**. Reagents and conditions: (a) $NH_3{\cdot}H_2O$–NH_4Cl buffer solution (pH 9), H_2O, reflux, 2 days, 57% of **17** and 32% of **18**; (b) CrO_3, HOAc (aq), rt, 4 h, 35%; (c) BBr_3, CH_2Cl_2, −78 °C to rt, 92%; (d) $NaBH_4$, CH_3OH, 0 °C, 0.5 h, 91%; (e) $NaIO_4$, CH_3OH/H_2O = 3:1, 0 to 25 °C, 0.5 h, 85%; (f) H_2O_2, K_2CO_3, MeOH, 25 °C, 2 h, 65%; (g) mCPBA, Na_2CO_3, CH_2Cl_2/H_2O = 4:3, 10 h, 45%.



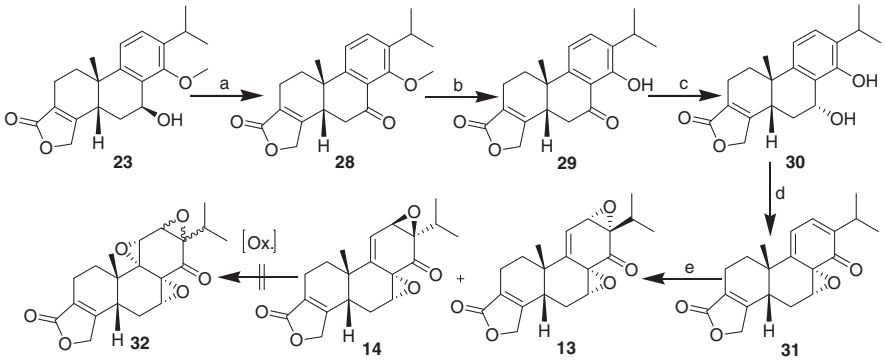
Scheme 3. Synthesis of analogues **8**–**12**. Reagents and conditions: (a) $(NH_4)_2Ce(NO_3)_6$, CH_3CN/H_2O = 1:1, rt, 2 h, 80%; (b) BBr_3, CH_2Cl_2, −78 °C to rt, 2 h, 90%; (c) acetic anhydride, pyridine, 50 °C, 3 h, 98%; (d) BBr_3, CH_2Cl_2, −78 °C to rt, 2 h, 60%; (e) $NaIO_4$, CH_3OH/H_2O = 3:1, 0 to 25 °C, 0.5 h, 96%; (f) H_2O_2, K_2CO_3, MeOH, 25 °C, 2 h, 62%; (g) *t*-BuOOH, $(CH_3)_4NOH$, benzene, 45 °C, 5 h, 60%; (h) $NaBH_4$, CH_3OH, 0 °C, 0.5 h, 95%; (i) mCPBA, Na_2CO_3, CH_2Cl_2/H_2O = 4:3, 10 h, 62%; (j) mCPBA, Na_2CO_3, CH_2Cl_2/H_2O = 4:3, 10 h, 56%.

original procedure[29] using potassium osmate, pyridine and NMO (*N*-methylmorpholine-*N*-oxide) in aqueous acetone afforded 9,11-β-diol **5** in 65% yield by dihydroxylation of **3**.

Then, we switched to the construction of the five-membered unsaturated lactam ring triptonide's analogues. Treatment of **15** with $NH_3{\cdot}H_2O$–NH_4Cl buffer solution provided lactam **17** and compound **18** with the desired A/B cis ring junction (Scheme 2). Unfortunately, attempts to direct ammonolysis of **2** with $NH_3{\cdot}H_2O$–NH_4Cl buffer solution led to decomposition. Benzylic oxidation of **17** with CrO_3 in $AcOH/H_2O$[28] provided the ketone **19** that was demethylated to afford phenol **20**. Reduction of **20** with $NaBH_4$ gave exclusively the C-7β alcohol **21**.[28] The prototypic oxidation course described by Adler et al.[30] was applied in the conversion of **21** to epoxy dienone **22**, which was treated with basic H_2O_2, giving compound **6** in 65% yield, along with the 12,13-β-epoxy isomer.[28] Exposure of **6** to mCPBA underwent selective β-face epoxidation to afford compound **7** having triepoxides as the sole epoxidation product in 45% yield.

Treatment of **18** with ammonium ceric nitrate in H_2O/CH_3CN gave, as shown in Scheme 3, exclusively the benzyl alcohol **23**. Direct demethylation of **23** with BBr_3 generated the dehydration product **24**, so protection of hydroxyl with acetic anhydride provided compound **25** which was treated with BBr_3 in CH_2Cl_2, affording **26** in 59% yield for the two steps. Periodate oxidation of **26** afforded epoxy dienone **27** in 96% yield which upon treatment with H_2O_2–K_2CO_3 in MeOH furnished 12,13-β-epoxide **8** as a single diastereomer. In addition, epoxidation of **27** with *tert*-butyl hydroperoxide in benzene in the presence of tetramethylammonium hydroxide[31] gave the 12,13-α-epoxy isomer **9** in 60% yield, along with **8** in 5% yield. Borohydride reduction of **9** afforded exclusively C14-β alcohol **12** in 95% yield. Further epoxidation of **8** with mCPBA afforded triepoxides **10** as a single diastereomer in 62% yield. Likewise, treatment of **9** with mCPBA gave **11** in 56% yield.

In order to get the 7,8-α-epoxide analogues, **23** was first treated with Collins reagent to afford ketone **28** which was demethylated to give **29** that upon treatment with $NaBH_4$ in MeOH furnished exclusively C7-α alcohol **30** as a single diastereomer with an overall yield of 70% (Scheme 4). Periodate oxidation of **30** provided compound **31** in 90% yield which upon treatment with H_2O_2–K_2CO_3 in MeOH gave **13** and **14** in 15% and 60% yields, respectively. The X-ray crystallographic analysis of compounds **13** and **14** confirmed their structures to be as shown in Figures 3 and 4.[32] Direct epoxidation of compounds **13** and **14** with some oxidizing agents, such as dimethyldioxorane, mCPBA, or hydrogen peroxide–sodium hydroxide, failed to give a triepoxy compound **32**.



Scheme 4. Synthesis of analogues **13** and **14**. Reagents and conditions: (a) Collins reagent, CH_2Cl_2, rt, 3 h, 89%; (b) BBr_3, CH_2Cl_2, −78 °C to rt, 2 h, 96%; (c) $NaBH_4$, CH_3OH, 0 °C, 0.5 h, 82%; (d) $NaIO_4$, CH_3OH/H_2O = 3:1, 0 to 25 °C, 0.5 h, 90%; (e) H_2O_2, K_2CO_3, MeOH, 25 °C, 2 h, 15% of **13** and 60% of **14**.

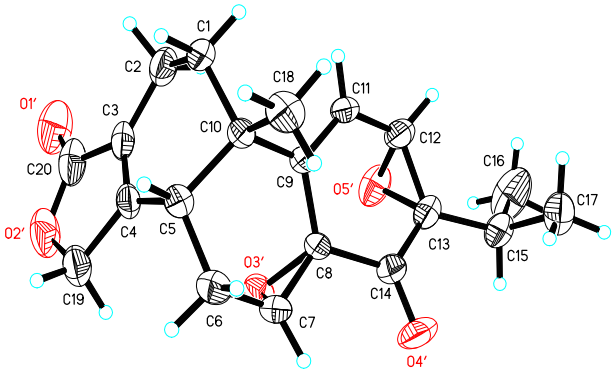


Figure 3. X-ray single crystal structure of compound **13**.

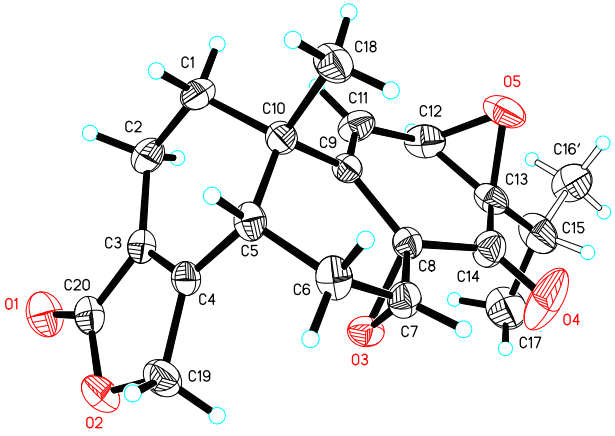


Figure 4. X-ray single crystal structure of compound **14**.

As shown above, we obtained three types of novel triptolide analogues with 9,11-olefin, five-membered unsaturated lactam ring or A/B cis ring junction and we evaluated the in vitro anticancer effects of those target compounds (**3**–**14**) against two human tumor cell lines derived from ovary (SK-OV-3) and prostate (PC-3) using sulforhodamine B (SRB) assays.[33] The result revealed that although there is no 9,11-β-epoxide group in our new analogues, the series of 9,11-olefin analogues (**3**, **4a**, **4b** and **6**) were all effective against those two cell lines, with IC_{50} values ranging from 0.05 nM to 29 μM (Table 1). Among them, compounds **3** and **4a** exhibited the highest potency, with the lowest IC_{50} value (3 nM and 0.05 nM for SKOV-3 cells). However, compound **5** with the 9,11-β-dihydroxyl was shown to be only weakly cytotoxic. The data indicates that the 9,11-β-epoxide group of triptolide is not unchangeable even in order to generate analogues of potent anticancer activity. Our result apparently challenges the classical structure-activity relationship of triptolide that considers 9,11-β-epoxide group to be essential for its anticancer activity[5] and the present result indicates that the activity of triptolide (**1**) series seems to owe more to the C-ring three-dimensional structure which may easily and inevitably be affected by the introduction of new groups or by changes of the groups attached to ring C or by the changes of C-ring structure itself as compound **1**–**4a**, having 9,11-β-epoxide or 9,11-olefin, are significantly cytotoxic while the cytotoxicity of compound **5**, having 9,11-β-dihydroxyl, reduced greatly against the two cell lines. On the other hand, the series of analogues (**6** and **7**) with five-membered unsaturated lactam ring were found to be still effective against the two cell lines with the very low IC_{50} value (0.02 μM for SKOV-3 cells) and more soluble in water than triptonide (**2**). Besides, the series of analogues (**8**–**14**) with A/B cis ring junction nearly completely lost their cytotoxicity against PC-3, and only compound **13** retained weak potency against SKOV-3 line. Based on the above results, we presumed that the trans A/B-ring junction is important for retaining cytotoxic activity.

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

Compound	IC_{50}[a] (μM)	
	SK-OV-3	PC-3
1	0.006	0.02
2	0.008	0.6
3	0.003	2.7
4a	0.00005	0.01
4b	0.5	29
5	>100	>100
6	0.02	0.9
7	0.02	1.4
8	95	>100
9	66	29
10	>100	>100
11	>100	>100
12	>100	>100
13	4.6	>100
14	56	>100

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

In summary, three types of novel triptolide analogues (**3**–**14**) were prepared. The results suggested the possible effect of the C-ring three-dimensional structure, the plane structure of five-membered unsaturated D-ring and the trans A/B-ring junction on the cytotoxic activity of the compounds of these series. Compound **4a** was much more active than the parent natural triptolide (**1**) with the lowest IC_{50} value of 0.05 nM for SKOV-3 cells, which suggested that the 9,11-β-epoxide group of triptolide is not unchangeable even in order to generate analogues of potent anticancer activity.

Acknowledgements

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Supplementary data

Supplementary data (synthesis, specific rotation, ^{1}H NMR, ^{13}C NMR, MS, HRMS, bioassay method[34] and HPLC analyses of final compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.106.

References and notes

1. Huang, S. *The Saint Peasant's Scripture of Materia Medica (18357)*; Publishing House for Chinese Medine Classics: Beijing, 1982. pp 309–310, Republished.
2. Wang, X. X.; Matta, R.; Shen, G.; Nelin, L. D.; Pei, D. H.; Liu, Y. S. *J. Mol. Med.* **2006**, *84*, 405.
3. Ding, G. S. *Clin. Ther.* **1987**, *9*, 345.
4. Kupchan, S. M.; Court, W. A.; Dailey, R. G., Jr.; Gilmore, C. J.; Bryan, R. F. *J. Am. Chem. Soc.* **1972**, *94*, 7194.
5. Kupchan, S. M.; Schubert, R. M. *Science* **1974**, *185*, 791.
6. Lee, K. Y.; Chang, W.-T.; Qiu, D.-M.; Kao, P. N.; Rosen, G. D. *J. Biol. Chem.* **1999**, *274*, 13451.
7. Zheng, Y. L.; Lin, J. F.; Lin, C. C.; Xu, Y. *Acta Pharm. Sin.* **1994**, *15*, 540.
8. Zheng, J. R.; Gu, K. X.; Xu, L. F.; Gao, J. W.; Yu, Y. H.; Tang, M. Y. *Acta Acad. Med. Sin.* **1991**, *13*, 391.
9. Gu, W. Z.; Chen, R.; Brandwein, S.; McAlpine, J.; Burres, N. *Int. J. Immunopharmacol.* **1995**, *17*, 351.
10. Yang, S. X.; Gao, H. L.; Xie, S. S.; Zhang, W. R.; Long, Z.-Z. *Int. J. Immunopharmacol.* **1992**, *14*, 963.

11. Qiu, D. M.; Zhao, G. H.; Aoki, Y.; Shi, L. F.; Uyei, A.; Nazarian, S.; Ng, J. C.-H.; Kao, P. N. *J. Biol. Chem.* **1999**, *274*, 13443.
12. Zhen, Q. S.; Ye, X.; Wei, Z. J. *Contraception* **1995**, *51*, 121.
13. Zheng, J. R.; Fang, J. L.; Gu, K. X.; Yin, Y. Q.; Xu, L. F.; Gao, J. W.; Guo, H. Z.; Yu, Y. H.; Sun, H. Z. *Chung Kuo I. Hsueh Ko Hsueh Yuan Hsueh Pao* **1987**, *9*, 323.
14. Shamon, L. A.; Pezzuto, J. M.; Graves, J. M.; Mehta, R. R.; Wangcharoentrakul, S.; Sangsuwan, R.; Chaichana, S.; Tuchinda, P.; Cleason, P.; Reutrakul, V. *Cancer Lett.* **1997**, *112*, 113.
15. Yang, Y.; Liu, Z.; Tolosa, E.; Yang, J.; Li, L. *Immunopharmacology* **1998**, *40*, 139.
16. Chen, B. J. *Leuk. Lymphoma* **2001**, *42*, 253.
17. Yang, S.; Chen, J.; Guo, Z.; Xu, X. M.; Wang, L.; Pei, X. F.; Yang, J.; Underhill, C. B.; Zhang, L. *Mol. Cancer Ther.* **2003**, *2*, 65.
18. Kiviharju, T. M.; Lecane, P. S.; Sellers, R. G.; Peehl, D. M. *Clin. Cancer Res.* **2002**, *8*, 2666.
19. Carter, B. Z.; Mak, D. H.; Schober, W. D.; McQueen, T.; Harris, D.; Estrov, Z.; Evans, R. L.; Andreeff, M. *Blood* **2006**, *108*, 630.
20. Tengchaisri, T.; Chawengkirttikul, R.; Rachaphaew, N.; Reutrakul, V.; Sangsuwan, R.; Sirisinha, S. *Cancer Lett.* **1998**, *133*, 169.
21. Chang, W. T.; Kang, J. J.; Lee, K. Y.; Wei, K.; Anderson, E.; Gotmare, S.; Ross, J. A.; Rosen, G. D. *J. Biol. Chem.* **2001**, *276*, 2221.
22. Frese, S.; Pirnia, F.; Miescher, D.; Krajewski, S.; Borner, M. M.; Reed, J. C.; Schmid, R. A. *Oncogene* **2003**, *22*, 5427.
23. Qi, Y. M.; Musser, J. H. U.S. Patent 56,63,335, 1997.
24. Dai, D.; Fidler, J. M.; Musser, J. H. U.S. Patent 65,69,893, 2003.
25. Yutaka, A.; Yukio, H.; Tomoyo, H.; Saki, M.; Haruhiko, F.; Koichi, T.; Ritsuo, A.; Takeshi, M.; Shusuke, H. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2459.
26. Li, Z.; Zhou, Z. L.; Miao, Z. H.; Lin, L. P.; Feng, H. J.; Tong, L. J.; Ding, J.; Li, Y. C. *J. Med. Chem.* **2009**, *52*, 5115.
27. Zhou, B.; Li, X. M.; Feng, H. J.; Li, Y. C. *Tetrahedron* **2010**, *66*, 5396.
28. Van Tamelen, E. E.; Demers, J. P.; Taylor, E. G.; Koller, K. *J. Am. Chem. Soc.* **1980**, *102*, 5424.
29. Jacobsen, E. N.; Marko, I.; Mungall, W. S.; Schroder, G.; Sharpless, K. B. *J. Am. Chem. Soc.* **1988**, *110*, 1968.
30. Becker, H.; Bremholt, T.; Adler, E. *Tetrahedron Lett.* **1972**, *41*, 4205.
31. Frieze, D. M.; Berchtold, G. A. *Tetrahedron Lett.* **1978**, *47*, 4607.
32. Crystallographic data for compound **13** and **14** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication No. CCDC779894 and No. CCDC779893, respectively. Copies of the data can be obtained, free of charge, on application to the Director, CCDC (e-mail: deposit@ccdc.cam.ac.uk).
33. Rubinstein, L. V.; Shoemaker, R. H.; Paull, K. D.; Simon, R. M.; Tosini, S.; Skehan, P.; Scudiero, D. A.; Monks, A.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1113.
34. Li, W.; Shao, Y.; Hu, L.; Zhang, X.; Chen, Y.; Tong, L.; Li, C.; Shen, X.; Ding, J. *Cancer Biol. Ther.* **2007**, *6*, 787.