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Synthesis of Macrosphelides with a Thiazole Side Chain: New Antitumor Candidates Having Apoptosis-Inducing Property

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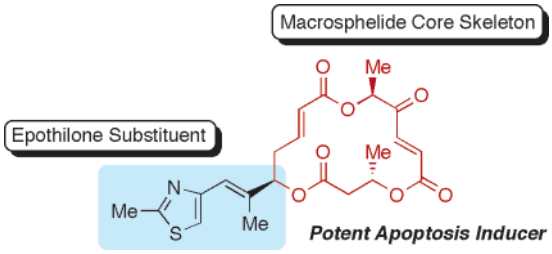
ABSTRACT



Hybrid compounds of macrosphelides and epothilones, both of which are natural macrolides having a 16-membered skeleton, were designed and synthesized using a ring-closing metathesis (RCM) strategy. Some of these hybrids were found to exhibit notable apoptosis-inducing activity against human lymphoma cells with higher potency than parent natural macrosphelides, and to be a promising lead compound for development of a new antitumor agent.

Hybridization of two or more biologically active natural products has been one of the most promising approaches for the development of new lead compounds and drug discovery in the field of medicinal chemistry.[1] In particular, artificial hybrid molecules of partial structures of natural compounds have been, in some cases, demonstrated to exhibit more potent activity than the parent compounds.[1] The concept based on the combination of fragments of natural bioactive compounds seems to have advantages because numerous numbers of such hybrid structures can be designed and accessible in the light of recent advances of molecular biology and synthetic organic chemistry.

During our research project on the synthesis and biological properties of macrosphelides,[2] 16-membered natural macrolide compounds,[3] we have recently noticed that some of natural macrosphelides and analogues (**1**−**5**, Scheme 1) can activate apoptotic program in human lymphoma U937 cells, albeit with rather weak potency. This preliminary result is the first observation on the apoptosis inducing potential of

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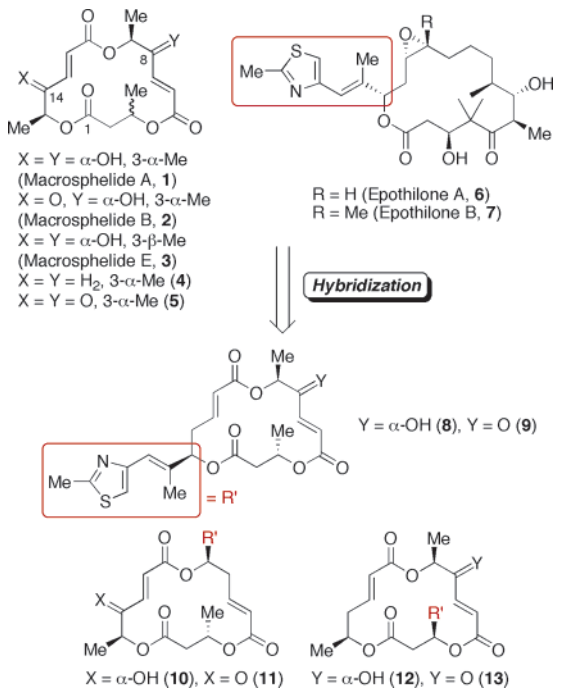
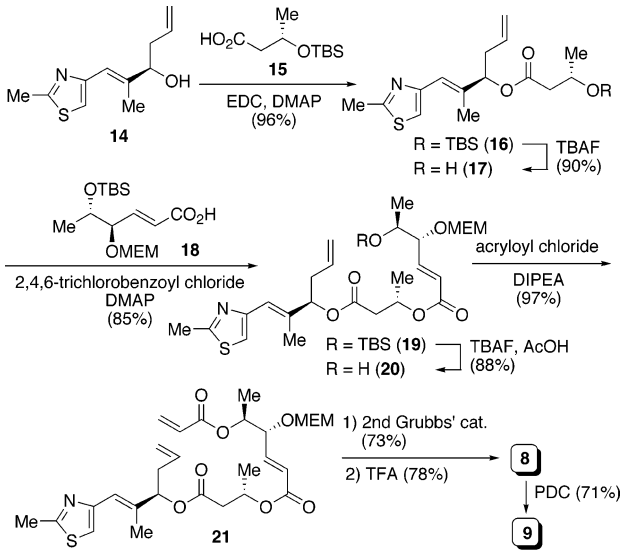
Scheme 1. Hybridization of Natural Macrosphelides and Epothilones



macrosphelides.[4] In conjunction with that, we have noted the same 16-membered natural polyketides, epothilones (Scheme 1),[5] which have been reported to exhibit extraordinarily potent cytotoxicity in a broad range of human cancer cell lines through a paclitaxel (Taxol)-like mechanism of action.[5] In addition, epothilones are known to induce mitotic arrest at the G2/M transition as a result of aberrant spindle formation, leading to apoptotic cell death,[6] which is suggested to have close correlation with the tumor cell growth inhibitory effects. One of the structural features of epothilones is the side-chain containing a thiazole ring, which is supposed to play an important role for their bioactivity.[7] Thus, we designed novel hybrid compounds (**8**−**13**, Scheme 1) composed of the 16-membered trilactone core structure of macrosphelides and the thiazole-containing side chain of epothilones, aiming at improved potential of macrosphelides as a new efficient apoptosis inducing agent. Herein, we wish to report synthesis of the hybrid compounds based on a ring-closing metathesis (RCM) strategy and their potent apoptosis inducing ability.

We have already developed an efficient synthetic strategy of natural macrosphelides A, B, and E using RCM as a key macrocyclization reaction.[8] It is expected that broad applicability and high functional group compatibility of RCM may bring a favorable opportunity for the synthesis of the hybrids containing the thiazole function. For the synthesis of the hybrids **8**−**11**, three chiral blocks **14**,[9] **15**,[2b] and **18**[2b] were prepared according to established methods, and they were combined to RCM substrates having full components (side chains, triester backbone, and requisite chiral centers) of the target compounds. Practically, as shown in Scheme 2, thiazole-containing chiral alcohol **14** was subjected to esterification with TBS-masked hydroxy acid **15**, and then successive desilylation−esterification sequence with **18** and acryloyl chloride provided the compound **21** with high efficiency. RCM of **21** proceeded smoothly in the presence of the second Grubbs' ruthenium catalyst[10] to afford the 16-membered macrocycle with an exclusive stereoselectivity. Subsequent removal of the MEM group and further oxidation of the hydroxyl group gave the hybrids **8** and **9**, respectively. Similarly, the hybrids **10** and **11** were synthesized by simply changing the coupling order of the chiral parts **14**, **15**, and **18** (Scheme 3).

Scheme 2. Synthesis of Hybrid Compounds **8** and **9**



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(4) Unpublished results; manuscript has been submitted for publication.

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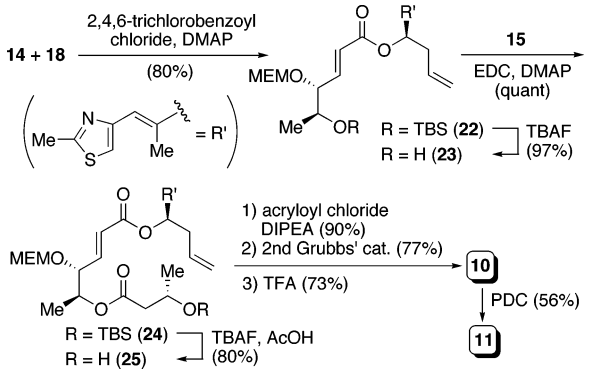
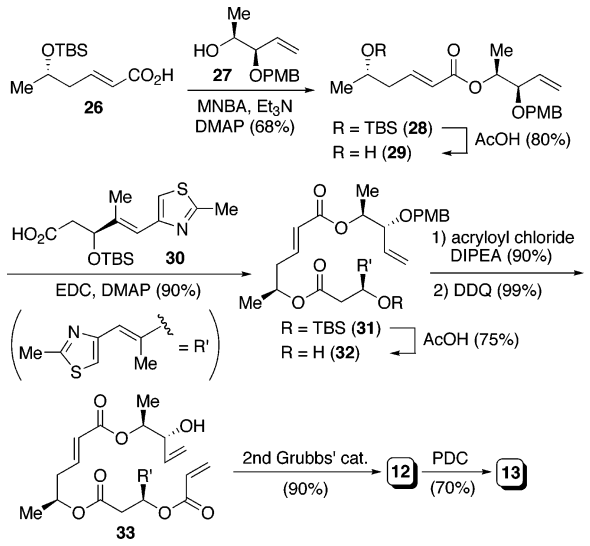
Scheme 3. Synthesis of Hybrid Compounds **10** and **11**



The hybrids **12** and **13**, which have a thiazole side chain at the 3-position, were synthesized according to Scheme 4.

Scheme 4. Synthesis of Hybrid Compounds **12** and **13**



A carboxylic acid **26**[11] was coupled with an alcohol **27**[2b] by dehydration using 2-methyl-6-nitrobenzoic anhydride (MNBA) as a reagent,[12] and the resulting ester **28** was subjected to sequential desilylation−esterification with **30**,[13] analogous to Scheme 2. After acryloylation of **32**, the PMB group was removed by DDQ prior to RCM, considering our previous finding that a steric congestion considerably affected the efficiency of RCM.[2b] Cyclization of **33** by RCM provided the hybrid **12**, which was further transformed to **13** by PDC oxidation.

Examination of the apoptosis-inducing activity of the hybrid compounds synthesized in this study gave results of great interest: that introduction of the thiazole substituent significantly enhanced the activity as compared with the parent natural macrosphelides and analogous compounds (**1**−**5**). The assay was performed using a human lymphoma cell line (U937), and assessment of early apoptosis and secondary necrosis was carried out by flow cytometry of annexin V/FITC and propidium iodide (PI) staining cells.[14] As shown in Figure 1, the hybrid **9** exhibited the most potent activity with negligible secondary necrosis at 1 μM concentration after 6 h incubation, and the hybrids **13** and **11** also induced apoptosis slightly. It is an important note that the parent macrosphelides **1**−**5** did not display any apoptosis-inducing ability at the same concentration even after 12 h incubation. Although detailed mechanism of action remains unclear at present, these results indicate that the thiazole-containing substituent installed at a suitable position can give rise to significant interactions with a living substance controlling an apoptotic program.

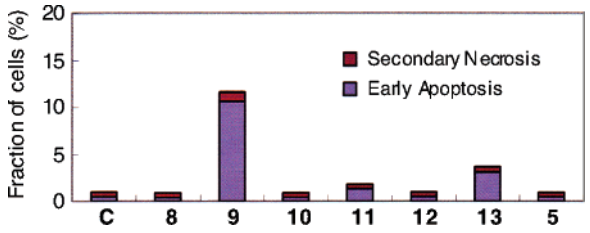


Figure 1. Apoptosis-inducing activities of the hybrid compounds **8**−**13**. U937 cells were treated with 1 μM concentration of each compound and incubated for 6 h. Early apoptosis and secondary necrosis were measured by flow cytometry using annexin V-FITC and PI staining. **C** represents a control experiment without drug treatment. Under the same conditions, fractions of cells (%) treated with the parent compounds **1**−**5** were almost identical with the control. Among them, the most potent derivative **5**, which exhibited the activity at 10 μM concentration, is shown as a positive control for comparison.

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(13) This carboxylic acid was prepared by $NaClO_2$ oxidation of the corresponding known aldehyde; see ref 9b.

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In this paper, we designed and synthesized novel macrosphelide−epothilone hybrids based on the RCM strategy and revealed their high potential as a new artificial antitumor agent having an apoptosis inducing ability. Elucidation of functions of the thiazole side chain is currently underway in our laboratory and will be reported in due course.

Supporting Information Available: Experimental procedures, compound characterization data, and assay protocol. This material is available free of charge via the Internet at http://pubs.acs.org.

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