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Synthesis of the Novel Analogues of Dysidiolide and their Structure–Activity Relationship

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Abstract—The novel analogues of natural cdc25A inhibitor dysidiolide were synthesized. To investigate the structure–activity relationship, the inhibitory activity to enzyme and cell cycle was examined.

Dysidiolide (**1**) is the first natural inhibitor of dual-specificity phosphatase cdc25A,[1] which is expressed in the early G1 phase of the cell cycle and promotes G1/S transition by dephosphorylation of the cyclin/CDK complex.[2] Cdc25A also proved to be oncogenic and overexpressed in a number of tumor cell lines.[3] Therefore, the cdc25A-specific inhibitor is expected as the hopeful candidate for the chemotherapy of human cancers. As dysidiolide has attracted much attention because of its bioactivity and complex structure, four groups have already reported the total syntheses of natural,[4] unnatural[5] and racemic[6] dysidiolide. However, its structure–activity relationship remains unclear. Recently, we have completed the asymmetric total synthesis of dysidiolide.[7] Herein, we report the synthesis of potent dysidiolide analogues and their structure–activity relationship.

Our strategy for structural modification is shown in Figure 1. It is assumed that the γ-hydroxybutenolide

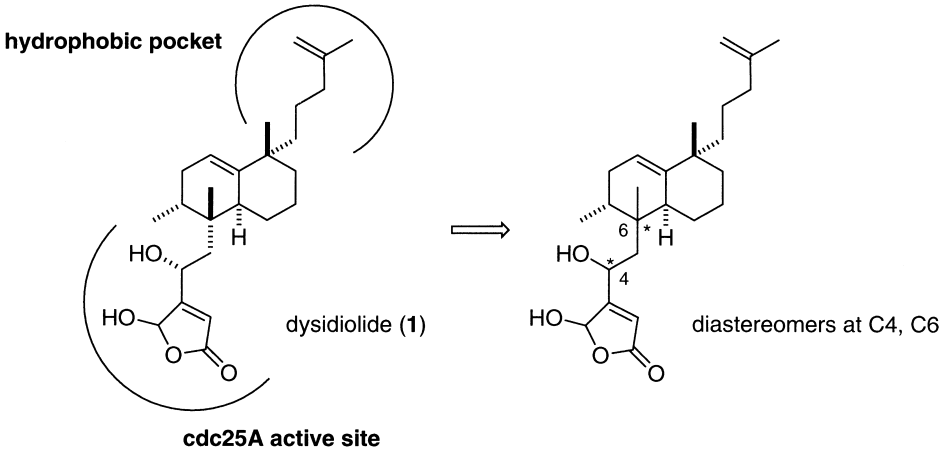

Figure 1. Modification of dysidiolide.

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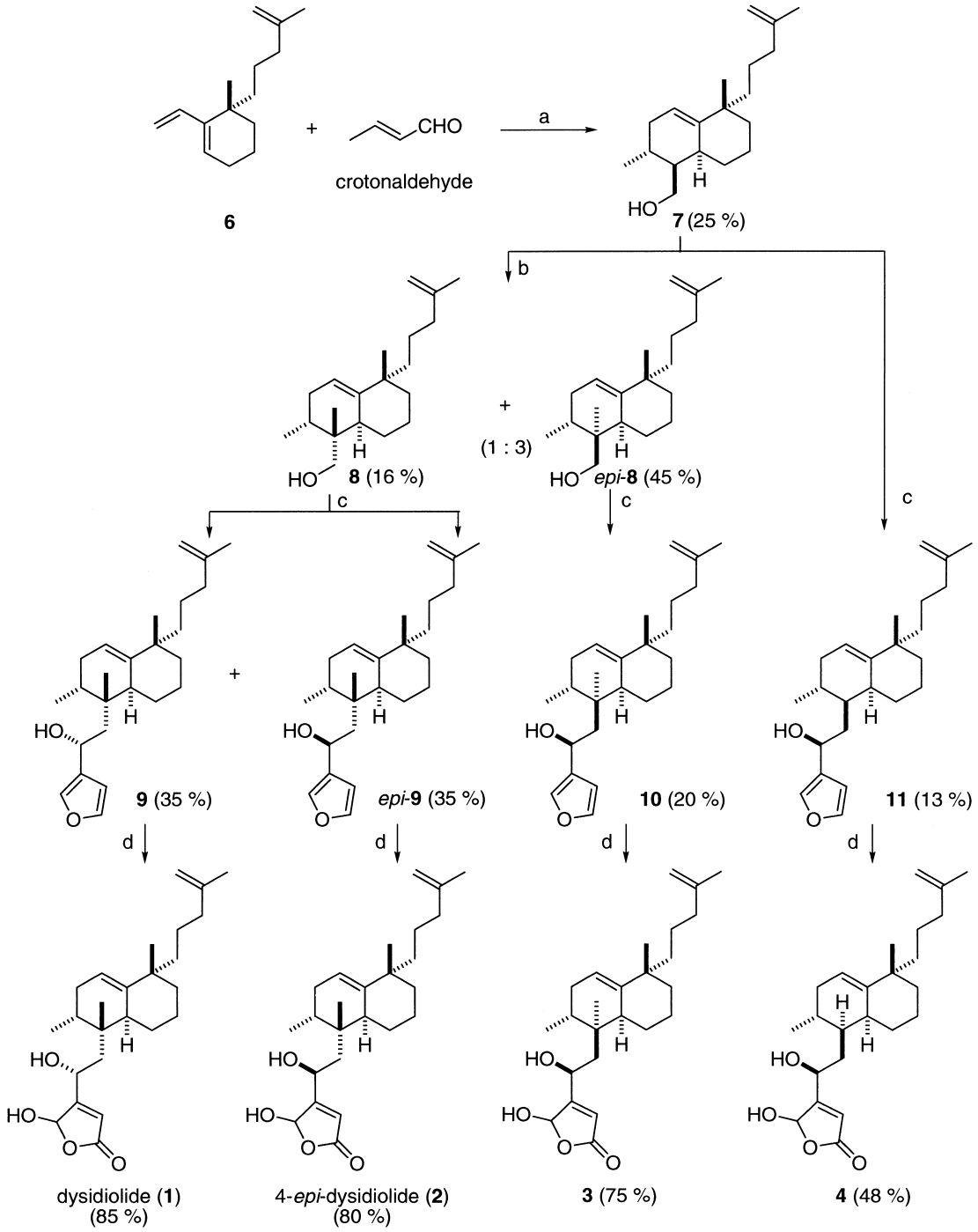
moiety serves as a surrogate phosphate and the long side chain occupies a hydrophobic binding pocket when the molecule is bound to cdc25A. Therefore, we considered that the stereochemistry of the C-6 quaternary carbon center bearing a γ-hydroxybutenolide side chain, which is in the center of the molecule, is significant for the expression of cdc25A inhibition. Furthermore, the interaction between the C-4 hydroxy group and the catalytic domain of cdc25A may be concerned with cdc25A inhibitory activity. In order to prove this hypothesis, a series of dysidiolide analogues such as 4-*epi*-dysidiolide (**2**), **3**, **4** and **5** were synthesized based on our efficient synthesis of natural dysidiolide (Fig. 2). Diastereomers of dysidiolide **2**, **3** and **4** were synthesized according to our developed total synthesis of dysidiolide (Scheme 1).[7] The key steps of this synthesis are the intermolecular Diels–Alder reaction of the triene **6** with crotonaldehyde and construction of a quaternary center at C-6 by methylation of the exocyclic enolate generated from the aldehyde. Synthesis of both 4-*epi*-**3** and 4-*epi*-**4** was not successful because corresponding furylcarbinol intermediates were too labile to isolate. Monocyclic



Scheme 1. Reagents and conditions: (a) i. $EtAlCl_2$·THF (1:1), CH_2Cl_2, −30 °C to 0 °C; ii. AlH_3, THF, 0 °C and separation; (b) i. TPAP, NMO, MS 4 Å, rt; ii. CH_3I, *t*-BuOK, THF, rt; iii. $NaBH_4$, MeOH, 0 °C and separation; (c) i. TsCl, pyridine, 0 °C; ii. NaCN, HMPA, 90 °C; iii. DIBAL-H, CH_2Cl_2, −78 °C; iv. 3-bromofuran, *n*-BuLi, THF, −78 °C; (d) O_2, hv, Rose Bengal, *i*-Pr_2EtN, CH_2Cl_2, −78 °C.

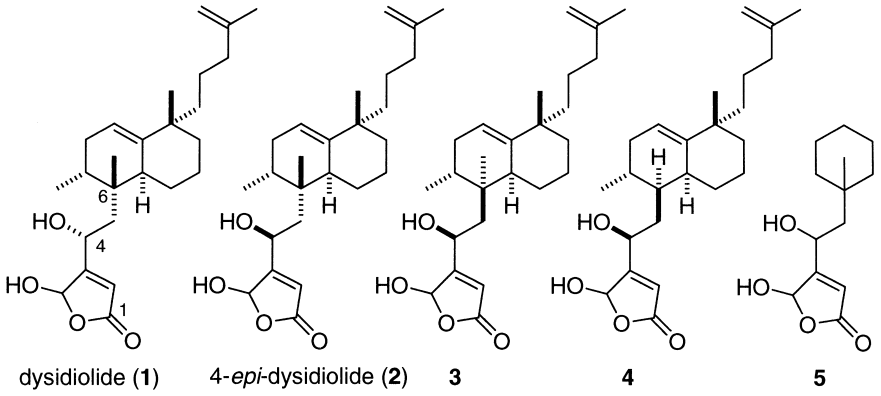


Figure 2. Structure of dysidiolide and its derivatives.

analogue (**5**) was prepared in racemic form from 1-methyl-1-cyclohexanecarboxylic acid (Scheme 2). The structures of these compounds were characterized by NMR and MS.[8] The C-4 carbinol absolute configurations of **10** and **11** were determined by modified Mosher's method using α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) esters.[9]

These compounds were tested for the inhibitory activity of cdc25A, its isoform cdc25B[10] and in vitro antiproliferative activity.[11] As shown in Table 1, **1** showed cdc25A specific inhibition with moderate activity. Monocyclic analogue **5** did not inhibit both cdc25A and B at all. The bicyclic structure and/or the alkenyl side chain of dysidiolide may be necessary to inhibit cdc25A. Although C-4 epimer **2** showed higher inhibitory activity toward both cdc25A and cdc25B, its inhibition of proliferation of tumor cells was relatively weak. The absolute configuration of the C-4-hydroxy group may be quite concerned with cdc25A inhibitory activity and enzyme specificity. Unexpectedly, both **3** and **4** had stronger activity against cdc25A and B than **1**, and **3** also exhibited much stronger antitumor activity. Interestingly, analogue **4**, a C-6-desmethyl analogue of **3**, showed moderate specificity to cdc25A than **2** and **3**. These results suggested that the stereochemistry of the C-6 quaternary carbon center is not critical and the original orientation of the two side chains is not necessary for the expression of cdc25A inhibition.

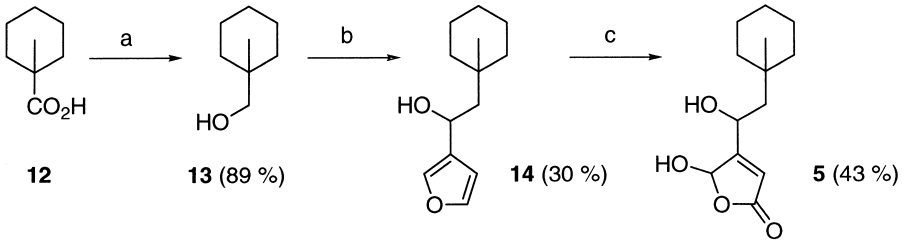
Table 1. Inhibition of cdc25A, B and proliferation of tumor cells by dysidiolide analogues

Compound	IC_{50} (μM)			
	cdc25A	cdc25B	SBC-5[a]	HL60[b]
Dysidiolide (**1**)	35	87	5.4	7.1
4-*epi*-Dysidiolide (**2**)	15	19	16	13.1
3	13	18	1.3	1.0
4	15	27	4.4	–
5	>300	>300	12	–

[a]Human lung cancer cell line.
[b]Human leukemia cell line.

To confirm the effect of these compounds on cell cycle progression, cell cycle analysis was performed.[12] After 20 h treatment with compounds, cell cycle distribution of HL60 (human leukemia cell line) cells was analyzed by flow cytometry. The percentage of cells in each phase of the cell cycle is shown in Figure 3. Simultaneous increase of G1 phase population and decrease of S phase population indicates G1 arrest, an inhibition of G1/S progression. Dysidiolide (**1**) and its derivatives **2** and **3** induced G1 arrest in accordance with the cell growth inhibition. Especially, compound **3**, which showed stronger activity than **1** in both enzyme inhibition and cell growth inhibition, induced G1 arrest at very low concentration (0.5 μM).

Thus, we could synthesize the potent dysidiolide analogues with unnatural configurations having higher activity of cdc25A inhibition than natural dysidiolide. These findings on the structure–activity relationship should provide crucial information for the future design of cdc25A inhibitor. Synthesis of advanced analogues and further studies are in progress.



Scheme 2. Reagents and conditions: (a) $LiAlH_4$, THF, 0 °C; (b) i. TsCl, pyridine, 0 °C; ii. NaCN, HMPA, 160 °C; iii. DIBAL-H, CH_2Cl_2, −78 °C; iv. 3-bromofuran, *n*-BuLi, THF, −78 °C; (c) O_2, hν, Rose Bengal, *i*-Pr_2EtN, CH_2Cl_2, −78 °C.

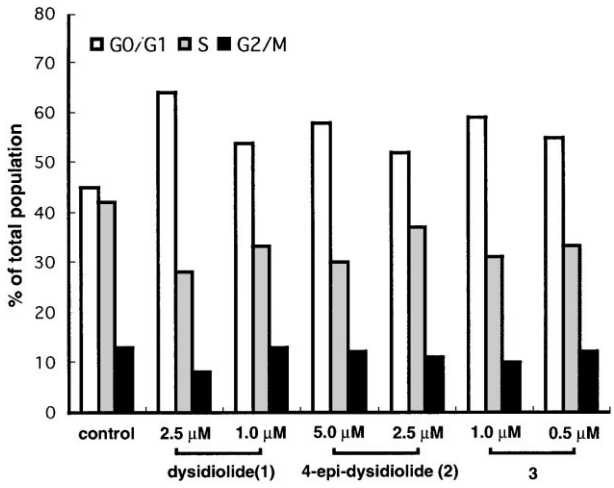


Figure 3. Effect on the cell cycle of HL60 cells.

Acknowledgements

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8. ^{1}H NMR (500 MHz, $CDCl_3$) data are as follows: **2** (clear oil, d_6-DMSO): δ 0.77 (3H, d, J = 7.0 Hz), 0.93 (3H, s), 1.08 (3H, s), 1.69 (3H, s), 1.80 (1H, m), 1.90–2.10 (3H, m), 2.70 (1H, m), 4.50 (1H, brs), 4.60 (2H, s), 5.24 (1H, brs), 5.92 (1H, brs), 6.10 (1H, brs), 7.86 (1H, s). **3** (clear oil, $CDCl_3$): δ 0.81 (3H, d, J = 6.5 Hz), 0.96 (3H, s), 0.98 (3H, s), 1.08–1.34 (4H, m), 1.69 (3H, s), 1.94–2.00 (3H, m), 2.10 (2H, m), 4.67 (2H, s), 4.80 (1H, m), 5.29 (1H, s), 6.06 (1H, s), 6.22 (1H, s). **4** (clear oil, $CDCl_3$): δ 0.93 (3H, d, J = 6.5 Hz), 0.97 (3H, s), 1.69 (3H, s), 1.85 (1H, m), 1.96 (2H, m), 2.09–2.17 (2H, m), 4.67 (2H, s), 4.70 (1H, m), 5.30 (1H, s), 6.05 (1H, s), 6.19 (1H, s). **5** (clear oil, $CDCl_3$): δ 1.00 (3H, s), 1.56–1.67 (2H, m), 4.75 (1H, d, J = 9.0 Hz), 6.00 (1H, brs), 6.14 (1H, brs).
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10. Cdc25A phosphatase assay: Catalytic domain protein of human cdc25A (283-523 aa) was produced from *Escherichia coli* strain DH5α using pGEX-2T glutathione-S-transferase (GST)-fusion protein expression vector (Pharmacia) according to the instructions provided by the manufacturer. Phosphatase activity of cdc25A was assayed in 100 μL of buffer containg 10 mM HEPES (pH 8.0), 50 mM NaCl, and 1 mM dithiothreitol (DTT), with 10 mM *p*-nitrophenol phosphate (Sigma) as a substrate, using 96-well microtiter plates. Cdc25B phosphatase assay was performed in a similar manner as above.
11. In vitro antitumor activity evalution: The cells (SBC-5 and HL60) were incubated with the test compound at various concentrations for 72 h. The IC_{50} value was defined as the drug concentration needed to cause 50% inhibition of cell growth with respect to the control.
12. Flow cytometric analysis: HL60 cells were treated with compounds for 20 h at various concentrations. Cell cycle distributions were determined using a Becton Dickinson fluorescence-activated cell analyzer. Data were interpreted using the ModiFit LT software provided by the manufacturer.