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# Design and synthesis of dysidiolide analogs from vitamin $D_3$: novel class of Cdc25A inhibitors

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**Abstract**—Potent dysidiolide analogs were synthesized by structural hybridization of dysidiolide and vitamin $D_3$. These analogs exhibited strong inhibitory activity toward dual-specificity phosphatase Cdc25A ($IC_{50} = 0.44$–$0.89$ μM).


Dysidiolide (**1**) was the first natural inhibitor of dual-specificity phosphatase Cdc25A ($IC_{50} = 9.4$ μM),[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 to be overexpressed in a number of tumor cell lines.[3] Consequently, inhibitors of Cdc25A are possible for new therapeutic agents to treat human cancers.

Total synthesis of **1** was accomplished by us and other groups.[4–9] After completion of the asymmetric total synthesis of **1**, we also synthesized its unnatural diastereomers and investigated their structure–activity relationship. Through biochemical evaluation of these dysidiolide analogs, we found that some unnatural diastereomers of **1** were more potent inhibitors of Cdc25A than **1**.[10,11] These findings were applied to the combinatorial development of Cdc25C inhibitors by Waldmann and co-workers.[12] Thus, the skeletal framework of unnatural dysidiolide analogs was valuable in the development of new synthetic inhibitors. However, access to various target molecules is still limited due to the multistep synthetic processes involved.

It has been suggested that the γ-hydroxybutenolide residue (hydrophilic substructure) of **1** serves as a surrogate phosphate, and that the octahydronaphthalene framework and side chain (hydrophobic substructure) occupy a hydrophobic binding pocket when the molecule binds Cdc25A (Fig. 1).[1] In a previous report, we also demonstrated that perhydroindan framework, which is readily available from vitamin $D_3$ (**2**) via

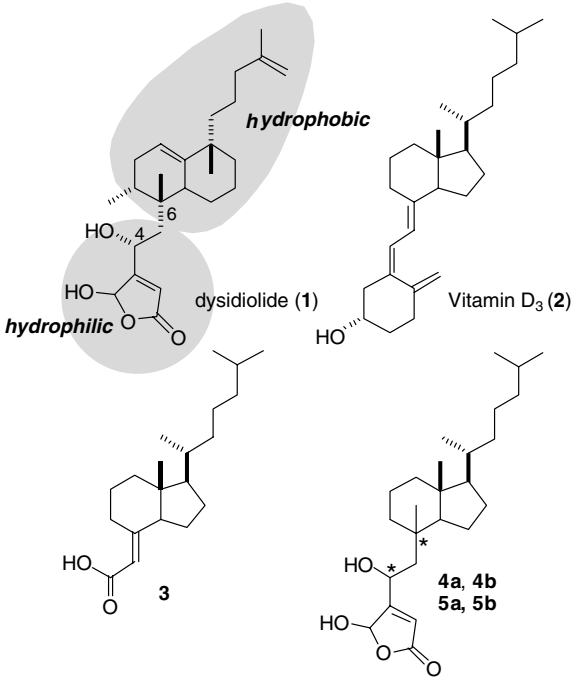


**Figure 1.**

*Keywords*: Dysidiolide; Cdc25A; Dual-specificity phosphatase; Enzyme inhibitor; Vitamin $D_3$.
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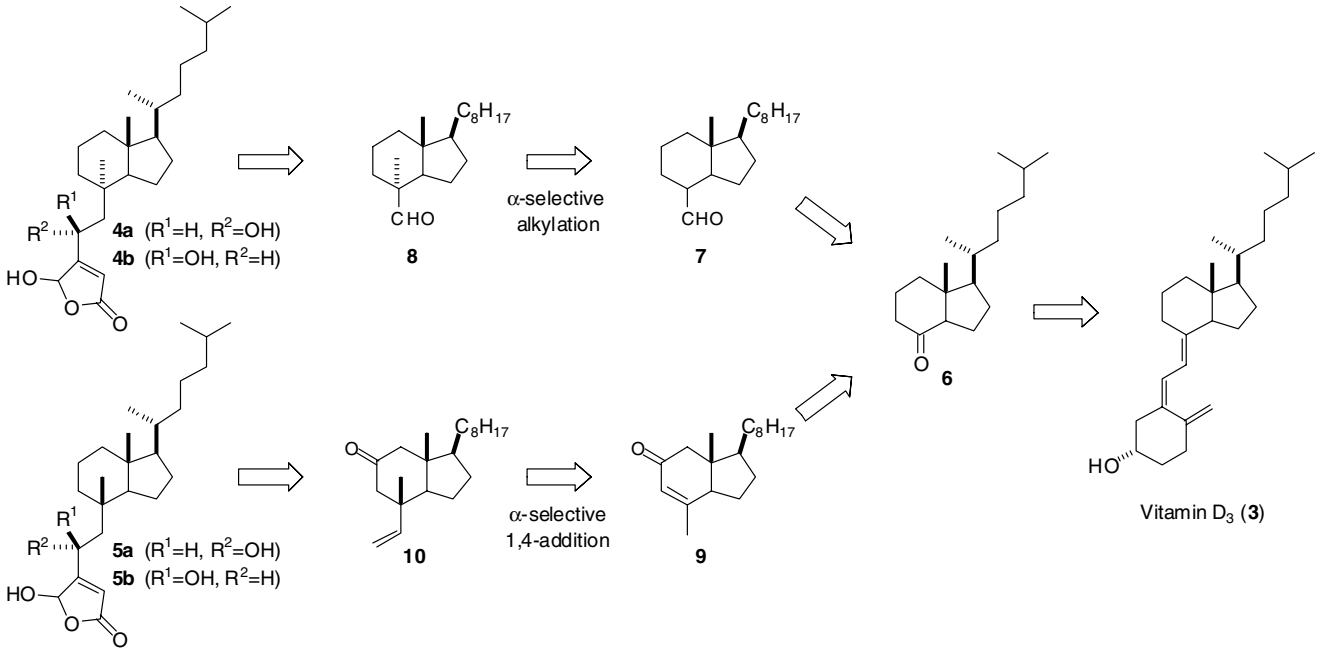
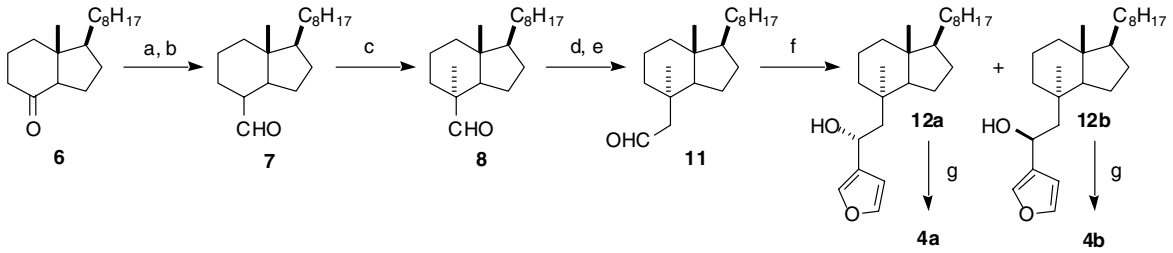
Grundmann's ketone, is useful to construct a hydrophobic substructure through the evaluation of the carboxylic acid **3** and its analogs.[10] Furthermore, the structure–activity relationship of dysidiolide analogs implies that the original stereochemistry of the carbinol center and C-6 quaternary center is not critical for Cdc25A-inhibitory activity. Therefore, substitution of the whole hydrophobic structure of dysidiolide with certain carbon frameworks, such as those in steroids or vitamin $D_3$, may afford new classes of potent inhibitors.[11,13] Here, we report the design, synthesis and biological activities of dysidiolide analogs having steroid framework, representing a novel class of potent inhibitors of dual-specificity phosphatase Cdc25A.

The synthesis of these compounds involved two different synthetic tactics, and the retrosynthetic analysis starting from a common intermediate, Grundmann's ketone **6**,[14] is shown in Scheme 1. The perhydroindan framework, the hydrophobic substructure of neodysidiolide, could be derived from vitamin $D_3$ via **6**. In order to synthesize all four compounds, the α-methyl derivatives (**4a**,**b**) were synthesized via α-selective methylation of the exocyclic enolate generated from the aldehyde **7**, and the β-methyl derivatives (**5a**,**b**) were prepared via conjugate addition of the organometallic reagent to the β-methylenone **9**. The γ-hydroxybutenolide moiety could be introduced by 1,2-addition of 3-furyllithium to the aldehyde **8**, followed by photo-sensitized $^1O_2$ oxidation of the furan.[15,16]

First, the synthesis of α-methyl derivatives (**4a**,**b**) was performed as shown in Scheme 2. The starting Grundmann's ketone **6** was obtained by complete ozonolysis of vitamin $D_3$ (**2**).[14] The aldehyde **7**, which was obtained by Magnus carbonyl homologation[17] of **6**, was methylated with *t*-BuOK–MeI in the presence of HMPA to give the α-methyl compound **8** in 79% yield as the sole diastereomer accompanied with a small amount of the *O*-methylated product. The absolute configuration of **8** was confirmed by observation of NOE in the $^1H$ NMR. Then, the aldehyde **11**, obtained by homologation of the




**Scheme 1.** Retrosynthetic analysis of neodysidiolides.




**Scheme 2.** Reagents and conditions: (a) $Me_3SiCH_2OMe$, *s*-BuLi, THF, −78 °C, 86%; (b) TFA/$H_2O$/$CHCl_3$, 1/1/8, rt, 87%; (c) (i) *t*-BuOK, HMPA, THF, rt; (ii) MeI, 0 °C, 79%; (d) (i) $Me_3SiCH_2OMe$, *s*-BuLi, THF, −78 °C; (ii) KH, THF, rt, 50%, $E/Z = 9/16$; (e) TFA/$H_2O$/$CHCl_3$, 1/1/8, rt, 84%; (f) 3-bromofuran, *n*-BuLi, THF, −78 °C, **12a** (54%) and **12b** (40%); (g) $O_2$, h$\nu$, Rose Bengal, *i*-$Pr_2EtN$, $CH_2Cl_2$, −78 °C, **4a** (58% from **12a**), **4b** (66% from **12b**).

aldehyde **8**, was allowed to react with excess 3-furyllithium[15] to produce a mixture of **12a** and **12b**, which were separated by silica gel chromatography. The absolute configurations of **12a** and **12b** were determined by a modified Mosher's method. Finally, photo-sensitized $^1O_2$ oxidation[16] of **12a** and **12b** afforded **4a** and **4b**, respectively.

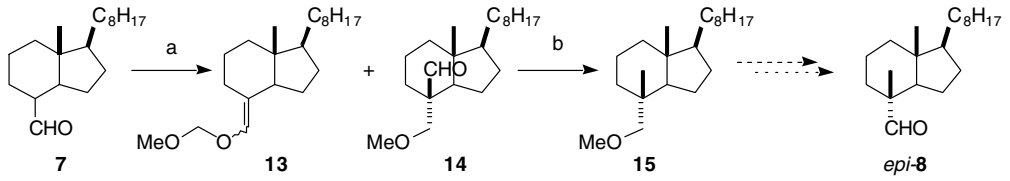
As the α-methyl dysidiolide analogs (**4a**,**b**) had been successfully synthesized, we started the diastereoselective synthesis of the β-methyl derivatives (**5a**,**b**). Initially, α-selective alkylation of the exocyclic enolate generated from **7** with methoxymethyl chloride was attempted. However, the *O*-methoxymethylated product (**13**) was obtained predominantly (Scheme 3). Therefore, we abandoned the alkyation approach and decided to take an alternative route via β-face-selective conjugate addition of an organometallic reagent to the β-methylenone (**9**) (Scheme 4).

Kinetic deprotonation of **6** with LDA in the presence of TMSCl provided unstable trimethylsilyl enol ether, which was successively oxidized to the enone **16** in good yield. The 1,2-addition of methylcerium reagent, prepared from methylmagnesium bromide and $CeCl_3$, took place smoothly to give the allyl alcohol **17**, which was oxidized with PCC to generate the β-methylenone **9**. The 1,4-addition of vinylmagnesium bromide, catalyzed by cuprous iodide–TMEDA, gave the desired β-methyl product **10** as the sole diastereomer and its absolute configuration was confirmed by NOE. Reductive removal of the carbonyl group with a modified Wolff–Kishner protocol provided the deoxygenated product,[18] which was converted to the alcohol **18** by hydroboration–oxidation. Swern oxidation of **18** gave the aldehyde **19**, which was reacted with 3-furyllithium[15] to give the carbionol **20a** and **20b** as a mixture. Separation of this mixture was successfully performed by preparative HPLC (ODS, acetonitrile–water) of the corresponding 2-naphthoates. After hydrolysis of each 2-naphthoate, the absolute configurations of **20a** and **20b** were determined by a modified Mosher's method, respectively. Finally, photo-sensitized $^1O_2$ oxidation[16] of **20a** and **20b** afforded **5a** and **5b**, respectively.

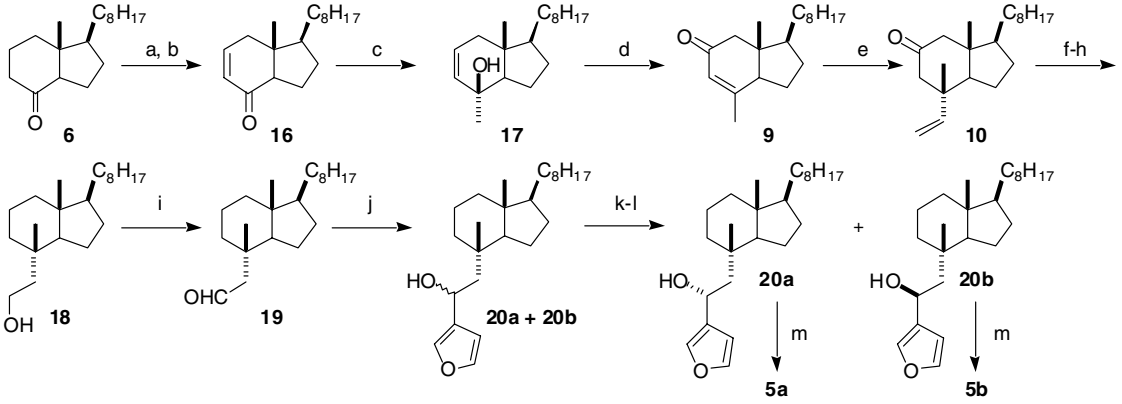
Next, the synthesized dysidiolide analogs **4a**, **4b**, **5a**, and **5b** were tested for Cdc25A and Cdc25B-inhibitory activities (Table 1).[19] The carboxylic acid derivative **3**, a potent Cdc25A inhibitor, was used as the reference

**Table 1.** Inhibition of Cdc25A and B by **3** and dysidiolide analogs

| Compound | $IC_{50}$ (μm) | |
|---|---|---|
| | Cdc25A | Cdc25B |
| **3** | 5.50 | 8.00 |
| **4a** | 0.48 | 1.44 |
| **4b** | 0.89 | 1.62 |
| **5a** | 0.86 | 1.88 |
| **5b** | 0.44 | 1.90 |




**Scheme 3.** Reagents and conditions: (a) (i) *t*-BuOK, HMPA, THF, rt; (ii) MOMCl, 0 °C, **13** (52%) and **14** (34%); (b) KOH, $NH_2NH_2$, diethylene glycol, reflux, **14** (65% from **14**).




**Scheme 4.** Reagents and conditions: (a) LDA, TMSCl, THF, −78 °C; (b) $Pd(OAc)_2$, 1,4-benzoquinone, $CH_3CN$, rt, 69% from **6**; (c) MeMgBr, $CeCl_3$, THF, 0 °C, 95%; (d) PCC, $CH_3CO_2Na$, $CH_2Cl_2$, rt, 65%; (e) vinylmagnesium bromide, CuI, TMEDA, THF, −78 °C, 87%; (f) $TsNHNH_2$, EtOH, reflux; (g) $NaBH_3CN$, $ZnCl_2$, MeOH, reflux; (h) $BH_3$–THF, $H_2O_2$, NaOH, EtOH, 30% from **10**; (i) $(COCl)_2$, DMSO, $Et_3N$, $CH_2Cl_2$; (j) 3-bromofuran, *n*-BuLi, THF, −78 °C, 74% from **18**; (k) 2-naphthoyl chloride, pyridine, rt, 74%; (k) HPLC (COSMOSIL 5C18-MS-II, $CH_3CN/H_2O = 95/5$); (l) $K_2CO_3$, MeOH, reflux, **20a** (80% from 2-naphthoate of **20a**), **20b** (86% from 2-naphthoate of **20b**); (m) $O_2$, h*v*, Rose Bengal, *i*-$Pr_2EtN$, $CH_2Cl_2$, −78 °C, **5a** (96% from **20a**), **5b** (94% from **20b**).

compound.[10] The dysidiolide analogs **4a**, **4b**, **5a**, and **5b** displayed strong inhibitory activity toward both Cdc25A and Cdc25B. The results demonstrate that the perhydroindan framework serves effectively as a hydrophobic substructure of dysidiolide. All of the tested compounds showed strong inhibitory activity, so it remains unclear how the stereochemistry of the carbinol center and quaternary carbon center affects Cdc25A-inhibitory activity. All the dysidiolide analogs showed moderate specificity for inhibition of Cdc25A as compared with Cdc25B. It is suggested that dysidiolide and its potent analogs, including neodysidiolide presented here, can differentiate different types of phosphatases, including the three Cdc25 family members.[12]

In conclusion, we synthesized potent dysidiolide analogs having high Cdc25A-inhibitory activity. These findings on the structure–activity relationship should prove very helpful for the design of novel Cdc25A inhibitors. Design and synthesis of further analogs as candidate potent inhibitors of Cdc25 family members are in progress.

## Acknowledgements

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19. Cdc25A/Cdc25B phosphatase assay: Catalytic domain proteins of human Cdc25A and Cdc25B were purchased from Sigma (Product Number C7484 and C7609, respectively). Phosphatase activity of Cdc25A/Cdc25B was assayed in 100 μL of buffer containing 10 mM HEPES (pH 8.0), 50 mM NaCl, and 1 mM dithiothreitol (DDT), with 40 μM *O*-methylfluorescein monophosphate as the substrate, using 96-well microtiter plates.