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# Design and synthesis of novel Cdc25A-inhibitors having phosphate group as a hydrophilic residue

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**Abstract**—Compounds (**6a**–**e**) were synthesized by phosphorylation of hydrophobic perhydroindan derivatives derived from vitamin $D_3$, and were found to show strong inhibitory activity towards dual-specificity phosphatase Cdc25A ($IC_{50} = 0.7$–$24.5\,\mu M$).


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

Through biochemical evaluation of synthetic dysidiolide and its analogs, we found that some unnatural diastereomers were more potent inhibitors of Cdc25A than dysidiolide itself (Fig. 1).[4–7] However, access to various target molecules is still limited due to the multistep synthetic processes involved. We demonstrated that the perhydroindan framework, easily available from vitamin $D_3$ via Grundmann's ketone, is useful to construct a hydrophobic substructure of novel Cdc25A-inhibitors (**3**, **4**).[6,8] It has been suggested that the γ-hydroxybutenolide residue (hydrophilic substructure) of dysidiolide serves as a surrogate phosphate, and the octahydronaphthalene framework and side chain (hydrophobic substructure) occupy a hydrophobic binding pocket

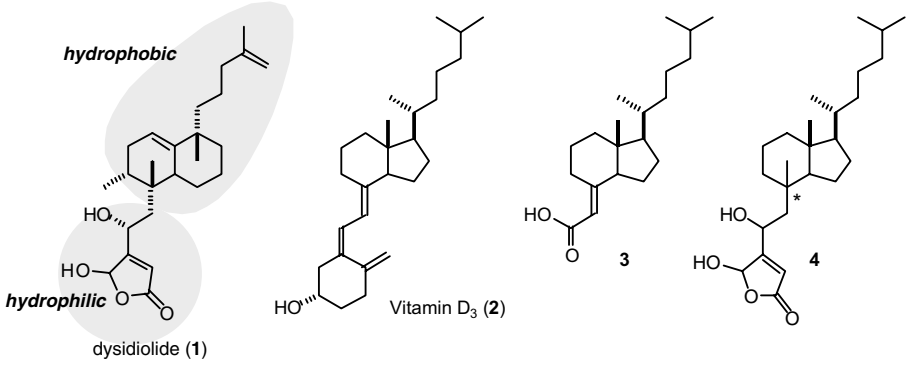


**Figure 1.**

*Keywords*: Dysidiolide; Dual-specificity phosphatase; Cdc25A; Cdc25B; Enzyme inhibitor.
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when the molecule is bound to Cdc25A (Fig. 1).[1] Therefore, introduction of a hydrophilic phosphate residue into carbon frameworks such as those found in steroids or vitamin $D_3$, might generate a new class of potent inhibitors.[7–9]

Here, we report the design, synthesis and biological activities of novel inhibitors of dual-specificity phosphatase Cdc25A. These inhibitors contain both a phosphate group as a hydrophilic residue and a perhydroindan framework, derived from vitamin $D_3$, as the hydrophobic structure. In order to evaluate the effect of the stereochemistry of the quaternary carbon centre and the length of the spacer between the perhydroindan framework and phosphate, five compounds (**6a**–**e**) were designed and synthesized (Fig. 2).

The synthesis of **6a**, having no spacer between the perhydroindan framework and phosphate, was performed as shown in Scheme 1. The alcohol **5a** was obtained by complete ozonolysis of vitamin $D_3$ followed by reductive work-up with $NaBH_4$. Phosphorylation of **5** by the amidite method gave the dibenzyl phosphate **7a**, which was deprotected by hydrogenolysis using Pd-black to give the phosphate **6a**.

The α-methyl derivatives (**6b**,**d**) and β-methyl derivatives (**6c**,**e**) were synthesized via the aldehyde **8** and the vinyl derivative **10**, both of which were the key intermediates of dysidiolide analogs in previous report, respectively.[8] The α-methyl alcohol **5b**, having one-methylene as a spacer, was obtained by reduction of the aldehyde **8**. The α-methyl alcohol **5d**, having two-methylene as a spacer, was synthesized from the aldehyde **9**, which was obtained by Magnus homologation of the aldehyde **8**. Finally, phosphorylation–deprotection of **5b** and **5d**, as described for the synthesis of **6a**, afforded **6b** and **6d**, respectively (Scheme 2).

The synthesis of β-methyl derivatives (**6c**,**e**) were started from the β-methyl vinyl compound **10**[8] as shown in Scheme 3. Reductive removal of the carbonyl group by means of Wolff–Kishner protocol provided the deoxygenated product **11**, which was converted to the alcohol **5c** by ozonolysis followed by $NaBH_4$ reduction, and to the alcohol **5e** by hydroboration–oxidation transformation. Introduction of phosphate residue was performed as described above to give **6c** and **6e**, respectively.

Next, the synthesized compounds **6a**–**e**[10] were tested for Cdc25A and Cdc25B-inhibitory activities in an assay system utilizing dephosphorylation of *O*-methylfluorescein monophosphate (Table 1).[11] As the phosphate analogs might be substrates of Cdc25A and Cdc25B to generate dephosphorylated carbinol products (**5a**–**e**), the inhibitory activity of these compounds was also examined. The carboxylic acid derivative **3**, a potent Cdc25A-inhibitor, was employed as a positive reference compound.[6]

The phosphate analogs **6a**–**e** displayed moderate to strong inhibitory activity towards both Cdc25A and Cdc25B. The strength of the Cdc25A/Cdc25B-inhibitory activity depended on the length of the carbon chain between the hydrophilic substructure and the hydrophobic substructure. Both of the derivatives **6b** and **6c** having a one-methylene spacer showed higher Cdc25A/Cdc25B-inhibitory activity than the other analogs. However, the analogs **6d** and **6e** having a two-methylene spacer showed contrasting results. The β-methyl derivative **6e** showed higher inhibitory activity while the α-methyl derivative **6d** displayed only moderate activity. Interestingly, **6b** and **6c** were Cdc25A-specific, while **6e** was Cdc25B-specific. Although the observed specificity was moderate, these results demonstrated that the phosphate analogs can differentiate the active site of

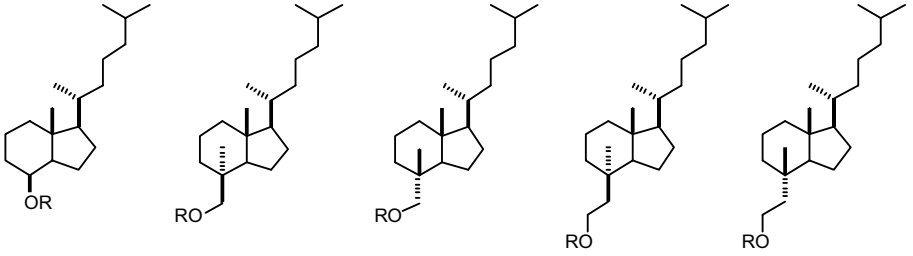
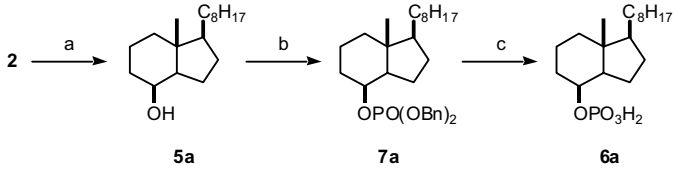
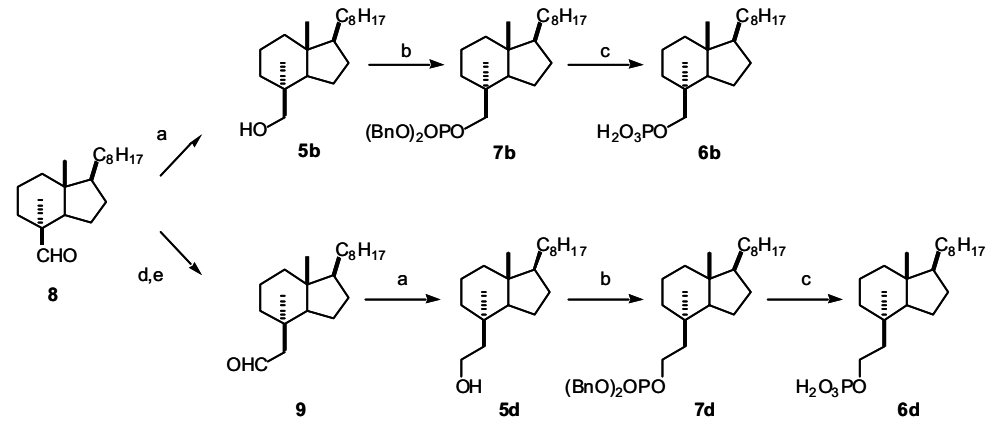


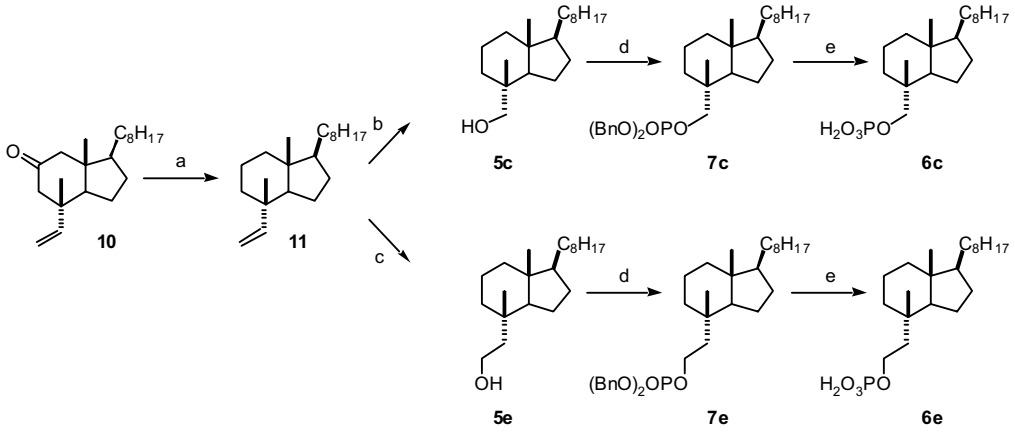

**Figure 2.**




**Scheme 1.** Reagents and conditions: (a) i. $O_3$, MeOH, pyridine, −78 °C, ii. $NaBH_4$, −78 °C to rt, 89%; (b) i. $(BnO)_2PNEt_2$, 1*H*-tetrazole, $CH_2Cl_2$, rt, ii. *m*CPBA, $H_2O$, −78 °C to rt, 8%; (c) Pd-black, EtOH, 87%.




**Scheme 2.** Reagents and conditions: (a) $NaBH_4$, MeOH, 0 °C, **5b**: 85%, **5d**: 97%; (b) i. $(BnO)_2PNEt_2$, 1*H*-tetrazole, $CH_2Cl_2$, rt, ii. *m*CPBA, $H_2O$, −78 °C to rt, **7b**: 73%, **7d**: 84%; (c) Pd-black, EtOH, **6b**: 88%, **6d**: 71%; (d) i. $Me_3SiCH_2OMe$, *s*-BuLi, THF, −25 to −78 °C, ii. KH, THF, rt, 54% *E*/*Z* = 1/2; (e) 90% $HCO_2H$, reflux, 96%.




**Scheme 3.** Reagents and conditions: (a) $N_2H_4{\cdot}H_2O$, KOH, DEGL; (b) i. $O_3$, MeOH, pyridine, −78 °C, ii. $NaBH_4$, −78 °C to rt, 29% from **10**; (c) i. $BH_3{\cdot}THF$, 0 °C to rt, ii. NaOH, $H_2O_2$, reflux, 20% from **10**; (d) i. $(BnO)_2PNEt_2$, 1*H*-tetrazole, $CH_2Cl_2$, rt, ii. *m*CPBA, $H_2O$, −78 °C to rt, **7c**: 88%, **7e**: 53%; (d) Pd-black, EtOH, **6c**: quant., **6e**: quant.

**Table 1.** Inhibition of Cdc25A and B by phosphate and carbinol analogs

| Compound | $IC_{50}$ (μM) | |
|---|---|---|
| | Cdc25A | Cdc25B |
| **3** | 17.2 | 5.9 |
| **5a** | 3.9 | 5.8 |
| **5b** | 22.7 | 4.5 |
| **5c** | 8.5 | 3.3 |
| **5d** | 6.9 | 1.3 |
| **5e** | 5.5 | 1.7 |
| **6a** | 8.2 | 5.0 |
| **6b** | 0.7 | 3.7 |
| **6c** | 0.9 | 3.0 |
| **6d** | 24.5 | 6.8 |
| **6e** | 5.5 | 1.2 |

Cdc25A and Cdc25B. As prolonged preincubation of **6c** with Cdc25A prior to the addition of *O*-methylfluorescein monophosphate did not decrease the activity of **6c** itself, it was demonstrated that **6c** acts as an excellent inhibitor of Cdc25A, rather than a substrate of dephosphorylation.[12]

In conclusion, we synthesized potent phosphate dysidiolide analogs, having high Cdc25A-inhibitory activity. The structure–activity data should be helpful for the design of novel Cdc25A/Cdc25B-inhibitors. Design and synthesis of further analogs as candidate inhibitors of Cdc25 family members are in progress.

## Acknowledgement

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10. The $^{31}P$ NMR data of **6a**–**e** (161 MHz in $CDCl_3$, external references: $H_3PO_4 + (PhO)_3P$): **6a** ($\delta$: 1.72); **6b** ($\delta$: 2.10); **6c** ($\delta$: 2.30); **6d** ($\delta$: 0.39); **6e** ($\delta$: 0.93).
11. Cdc25A/Cdc25B phosphatase assay: catalytic domain protein 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 a substrate, using 96-well microtiter plates.
12. Compound **6c** (5 μM, $IC_{50} = 0.9$ μM) was preincubated with Cdc25A in the absence of *O*-methylfluorescein monophosphate for 30 min. After that period, *O*-methyl fluorescein monophosphate was added and the phosphatase activity of Cdc25A was assayed as usual.[11] The preincubated **6c** (5 μM) showed 89.8% inhibition of Cdc25A, while the inhibitory activity of **6c** (5 μM) without preincubation was 74.9%.