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Synthesis of the naphthalene-derived inhibitors against Cdc25A dual-specificity protein phosphatase and their biological activity

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Abstract—The novel naphthalene-type analogues **14** and **18** and the naphthoquinone-type analogues, **8**, **9**, **15**, **16**, **19**, **21**, **22**, and **23**–**28** have been synthesized, and their in vitro Cdc25A phosphatase-inhibitory activity was examined. In assessment of the inhibitory activity, it was revealed that the naphthoquinone core is contributed to the activity, rather than the alkyl side chain.

1. Introduction

Cell cycle progression is controlled by the cyclin-dependent kinases (CDKs), which are positively regulated by association with cyclins and negatively regulated by binding to inhibitory subunits.[1] CDKs are also inactivated by phosphorylation with the protein kinases *wee1*[2] and activated by dephosphorylation with the Cdc25 phosphatases. Mammalian cells contain three Cdc25 genes, named Cdc25A, Cdc25B, and Cdc25C.[3] Cdc25A, a dual-specificity protein phosphatase, activates cyclin-dependent kinase 2 (CDK2), promoting entry into the S phase of the cell cycle.[4] Overexpression of Cdc25A has been shown previously to have oncogenic potential,[5] making Cdc25A inhibitors candidates of new lead molecule for anticancer chemotherapies.[6]

There are several inhibitors of Cdc25A protein phosphatase in such natural products, as dysidiolide,[7] glucolipsin A,[8] indolyldihydroxyquinone,[9] and menadione.[10] Among them, dysidiolide is the most famous substance exhibiting the Cdc25A inhibitory activity.[7] Although there are a number of inhibitors exhibiting potent activity, their detailed structure–activity relationships has been remained unclear, and no active substance carrying both arrest of cells in G_1/S phase and selectivity for Cdc25A, has been reported. Against such backgrounds, we envisaged a new approach to synthetic lead molecules of Cdc25A inhibitors for anticancer chemotherapy, and initiated synthetic studies on the novel pyranonaphthoquinone derivatives **1**–**4**,[11] isolated from *Streptomyces* sp. Although they exhibited no remarkable inhibitory activity in comparison with other Cdc25A inhibitors, the family shares the structurally unique characteristics that the benzoquinone core attached with the methylamino group is located at the side of the tricyclic molecule (Fig. 1).

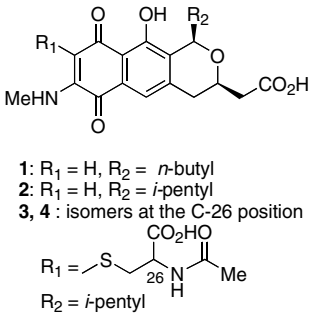

Figure 1. Structure of Cdc25A phosphatase inhibitor possessing the pyranonaphthoquinone core.

Keywords: Cell cycle regulation; Dual-specificity phosphatase; Cdc25A phosphatase inhibitor; Naphthoquinone; Biological activity.
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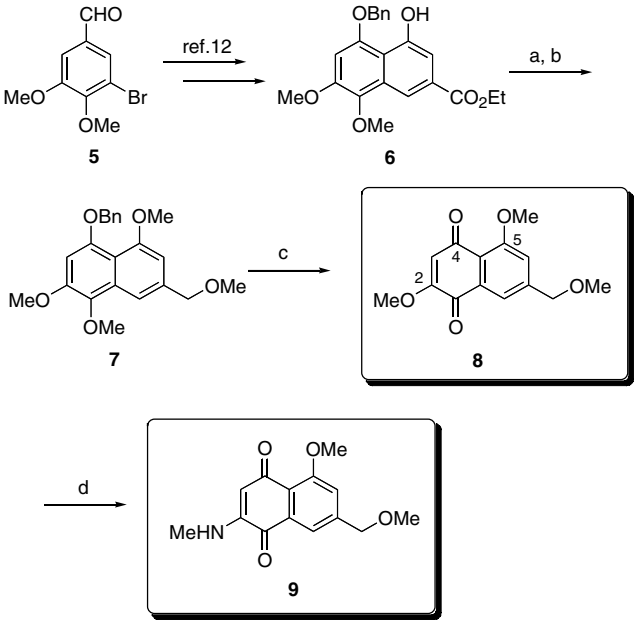
In a previous investigation,[12a,b] we succeeded total synthesis of (±)-**1**, carrying the fundamental framework of the family. It was observed that the naphthoquinone derivatives, produced as synthetic intermediates of **1**, exhibits more effective inhibitory activity against the Cdc25A phosphatase than those of the natural pyranonaphthoquinones.[12b] In this context, our attention was mainly focused on biological activity of other naphthoquinone derivatives. We describe herein synthesis of new naphthoquinone analogues and their structure–activity relationship, as part of our extensive investigation of inhibitors against Cdc25A phosphatase.

2. Synthesis of the benzyl methyl ether analogues 8 and 9

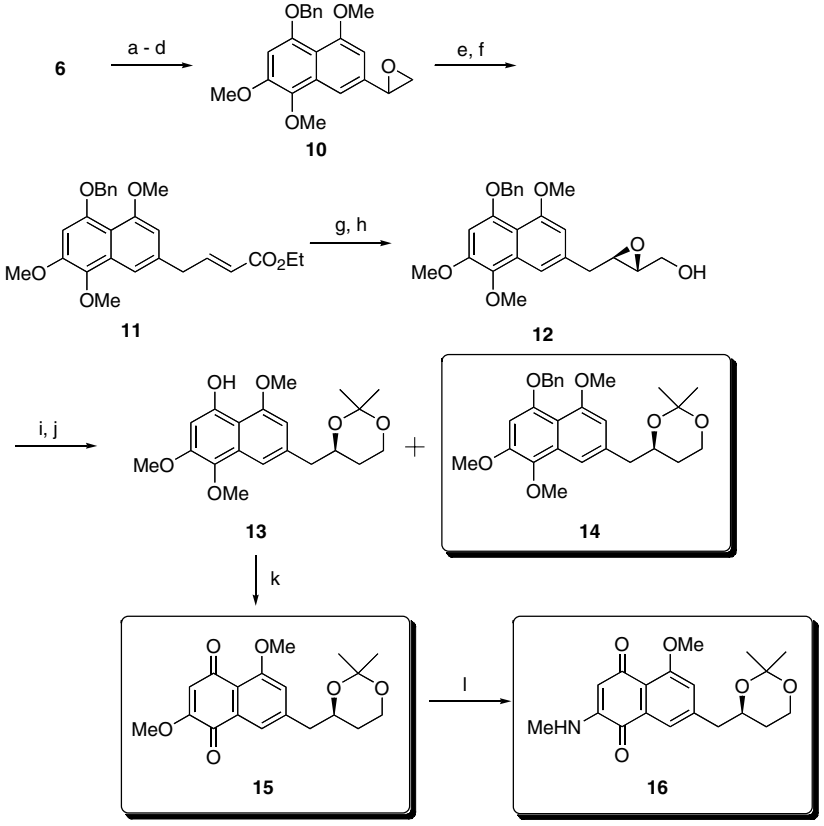
As can be seen in Scheme 1, naphthol **6** was easily obtained from 5-bromoveratraldehyde **5**.[12] Sequential manipulation involving protection, reduction, and further methylation afforded the benzyl methyl ether **7**. Oxidation under DDQ conditions provided the benzyl ether **8**. Subsequent introduction of a methylamino group gave **9** in 89% yield.[13]



Scheme 1. Reagents and conditions: (a) $LiAlH_4$, THF, rt, 61%; (b) MeI, NaH, DMF, rt, 65%; (c) DDQ, *t*-BuOH, H_2O–CH_2Cl_2, rt, 84%; (d) $MeNH_2$, THF, 0 °C, 89%.

3. Synthesis of the 1,3-diol analogues 14, 15, and 16

Oxirane **10** was obtained in good yield from **6** by Me_3SI (Scheme 2). Isomerization and the Horner–Wadsworth–Emmons reaction provided the α,β-unsaturated ester **11** in 57% yield, which was exposed to DIBAL to afford an



Scheme 2. Reagents and conditions: (a) MeI, K_2CO_3, DMF, rt, 92%; (b) $LiAlH_4$, THF, rt, 80%; (c) SO_3·Py, TEA, DMSO, CH_2Cl_2, rt, 100%; (d) Me_3SI, NaH, DMSO, THF, 0 °C, quant.; (e) $ZnBr_2$, PhH, reflux; (f) $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF, −78 °C, 57% in two steps; (g) DIBAL, THF, −78 °C, 88%; (h) L-(+)-DIPT, $Ti(O\textit{i}\text{-}Pr)_4$, TBHP, MS4A, CH_2Cl_2, −20 °C, 85%, 95% ee; (i) Red-Al®, THF, −78 °C; (j) 2,2-dimethoxypropane, CSA, 0 °C, 71% (**13**) and 25% (**14**) in two steps; (k) DDQ, H_2O-1,4-dioxane, rt, 89%; (l) $MeNH_2$, MeOH, 0 °C, 38%.

allyl alcohol. Sharpless asymmetric epoxidation of the allyl alcohol was carried out with L-(+)-DIPT to give the epoxy alcohol **12** in 85% yield (95% *ee*). Reductive cleavage of the epoxide ring with Red-Al®, and acetal-protection of the 1,3-diol gave **13** and **14**. Immediate oxidation of **13** afforded quinone **15** in 63% yield from **12**. Finally, introduction of a methylamino group provided the methylamine **16** in 38% yield.

4. Synthesis of the naphthalene 18 and the naphthoquinones 19, 21, 22

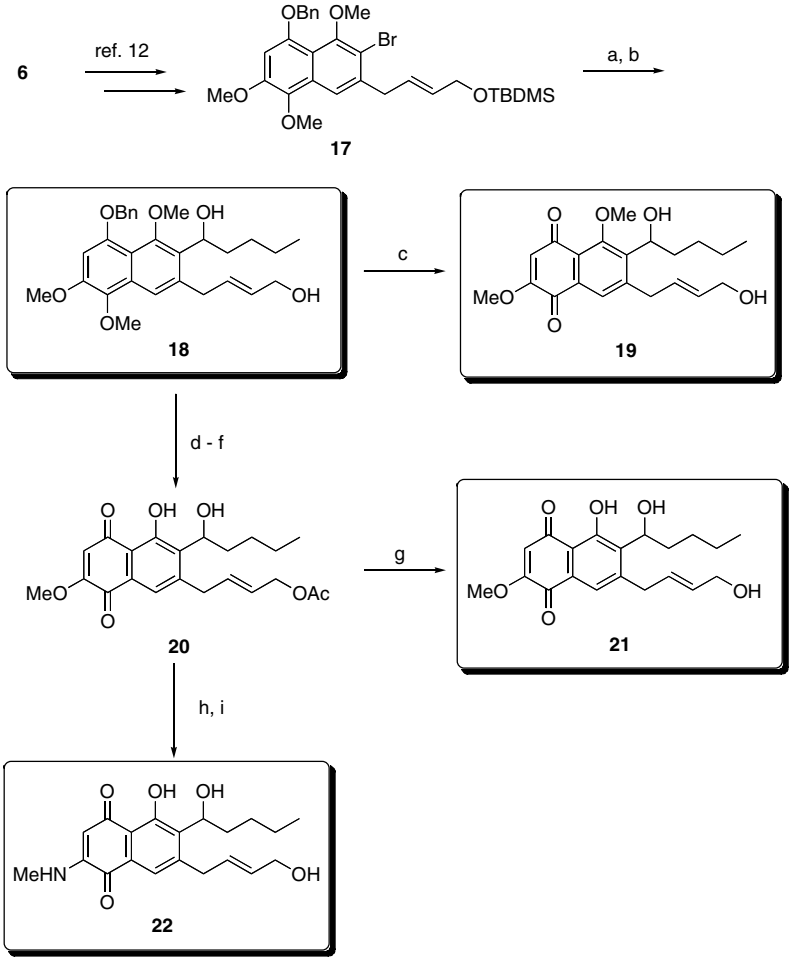
Compound **6** was converted into the silyl ether **17**, according to the previous synthesis of the natural product **1**[12] (Scheme 3). A lithiated derivative of **17** was reacted with valeraldehyde and successive desilylation, afforded the allyl alcohol **18** in 80% yield. Compound **18** was oxidized under DDQ conditions to give the naphthoquinone **19** in 79% yield. Naphthoquinones **21** and **22** were obtained from the allyl alcohol **18**, as follows. Selective acetylation, oxidation to the quinone, and selective demethylation at the C-6 position gave **20** in 39% yield from **18**. Compound **20** was reacted with K_2CO_3 in MeOH afforded **21**. Introduction of a methylamino group and deacetylation provided **22** in 74% yield from **20**.

5. Synthesis of the naphthoquinone derivatives 23–28

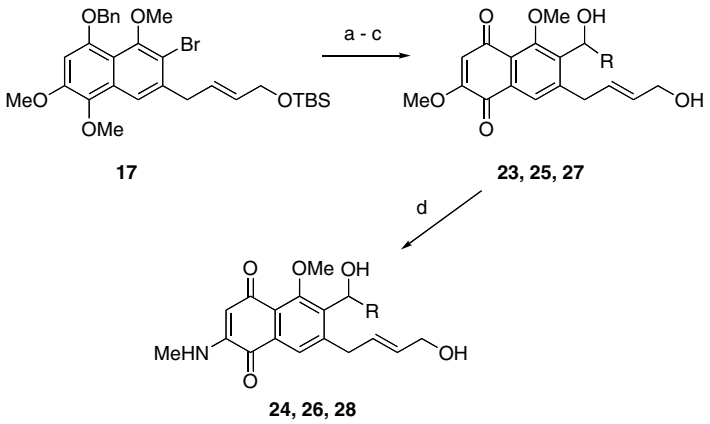
To obtain **23**–**28**, the synthetic route to **19**–**22** was slightly modified (Scheme 4). Upon using the same method as described in Scheme 3, deacetylation was unsuccessful. Accordingly, the order of oxidation and desilylation was reversed. Thus, the silyl ether **17** was reacted with the corresponding aldehyde, and oxidized to naphthoquinones, which were successfully converted into the allyl alcohols, **23**, **25**, and **27**. Treatment of **23**, **25**, and **27** with $MeNH_2$ gave the corresponding methylamines **24**, **26**, and **28**.

6. Biological activity

The naphthalene and naphthoquinone derivatives were submitted to assessment for in vitro inhibition of Cdc25A phosphatase using 4-nitrophenyl phosphatase



Scheme 3. Reagents and conditions: (a) valeraldehyde, *n*-BuLi, THF, −78 °C; (b) TBAF, THF, 0 °C, 80% in two steps; (c) DDQ, *t*-BuOH, H_2O–CH_2Cl_2, rt, 79%; (d) Ac_2O, Py, CH_2Cl_2, rt, 85%; (e) DDQ, *t*-BuOH, H_2O–CH_2Cl_2, 0 °C, 87%; (f) BBr_3, CH_2Cl_2, −78 °C, 53%; (g) K_2CO_3, MeOH, rt, quant.; (h) $MeNH_2$, THF, 0 °C, 88%; (i) K_2CO_3, MeOH, rt, 84%.



aldehyde	R	a → c yield	d yield
propionaldehyde	Et	**23** 24%	**24** 61%
hexaldehyde	*n*-pentyl	**25** 47%	**26** 54%
benzaldehyde	Ph	**27** 16%	**28** 75%

Scheme 4. Reagents and conditions: (a) aldehyde, *n*-BuLi, THF, −78 °C; (b) DDQ, *t*-BuOH, H_2O–CH_2Cl_2, 0 °C; (c) TBAF, AcOH, THF, rt; (d) $MeNH_2$, THF, 0 °C.

(*p*NPP) as a substrate. As shown in Table 1, the low activities of naphthalenes **14** and **18** indicated the naphthoquinone structure has a positive effect on inhibition of the Cdc25A phosphatase. The naphthoquinones possessing OMe groups at the C-2 position (**8**, **15**, **19**, **21**, **23**, **25**, and **27**) showed stronger Cdc25A inhibitory activity than the methylamino derivatives (**9**, **16**, **22**, **24**, **26**, and **28**) as well as (±)-**1**. In preceding investigations of Cdc25A phosphatase inhibition, hydrophilic subunits, such as a carboxylic acid, were reported to serve as a surrogate phosphatase. A hydrophobic substructure, such as a long side chain, might occupy a hydrophobic binding pocket, when the molecule is bound to Cdc25A.[14] However, our compounds without a carboxylic acid and a long alkyl side chain (**8**, **15**), showed effective Cdc25A phosphatase-inhibitory activity.[15] Furthermore, length of alkyl side chain (**19**, **23**, **25**), and conversion of alkyl side chain into phenyl group (**27**) provided no serious effect on the inhibitory activity. In addition, protection at C-6 as a methyl ether exhibited no serious activity difference (**19**, **21**), which indicated little contribution of the hydrogen bond between the C-4 carbonyl oxygen and the C-6 phenol group.

Table 1. Inhibitory activity against Cdc25A protein phosphatase (*p*NPP)[a]

Compound	IC_{50} (μg/mL)	Compound	IC_{50} (μg/mL)
8	0.4	**22**	>10
9	>10	**23**	1.86
14	>10	**24**	>10
15	0.4	**25**	1.6
16	>10	**26**	>10
18	>10	**27**	2.74
19	1.77	**28**	>10
21	1.51	(±)-**1**	>10
Na_3VO_4[b]	0.005		

[a] Cdc25A assay in vitro: The activity of the GST-cdc25A was measured in a 96-well microtiter plate using *p*NPP (sigma) as a substrate. Approximately 30 μg of purified GST-cdc25A was preincubated at 37 °C for 15 min in reaction buffer containing of 50 mM Tris–HCl (pH 7.5) and various concentrations of inhibitors in 1% MeOH, and then reaction was started by adding of 5 mM *p*NPP and incubated at 37 °C for 60 min. Absorbance at 405 nm was measured.

[b] Positive control.

In conclusion, the naphthalene and naphthoquinone derivatives were synthesized and their inhibitory activity against Cdc25A phosphatase was evaluated. It was detected that the naphthoquinone core with a methoxy group, instead of a methylamino group as in natural pyranonaphthoquinones **1**–**4**, is an important factor to show high activity of Cdc25A phosphatase inhibition, and the long alkyl side chain might not be required to inhibit the phosphatase, contrary to the previously reported data.

Further investigation of the structure–activity relationship and selectivity for Cdc25A is in progress.

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