

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

Akiko Shimbashi,^a Ayako Tsuchiya,^b Masaya Imoto^b and Shigeru Nishiyama^{a,*}

^aDepartment of Chemistry, Faculty of Science and Technology, Keio University, Hiyoshi 3-14-1, Kohoku-ku, Yokohama 223-8522, Japan

^bDepartment of Biosciences and Informatics, Faculty of Science and Technology, Keio University, Hiyoshi 3-14-1, Kohoku-ku, Yokohama 223-8522, Japan

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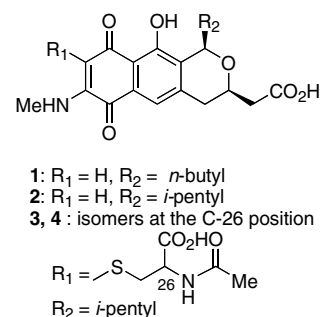
Abstract—The novel naphthalene-type analogues **14** and **18** and the naphthoquinone-type analogues, **8**, **9**, **15**, **16**, **19**, **21**, **22**, and **23–28** have been synthesized, and their in vitro Cdc25A phosphatase-inhibitory activity was examined. In assessment of the inhibitory activity,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

ity, their detailed structure–activity relationships has been remained unclear, and no active substance carrying both arrest of cells in G₁/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).



Keywords: Cell cycle regulation; Dual-specificity phosphatase; Cdc25A phosphatase inhibitor; Naphthoquinone; Biological activity.

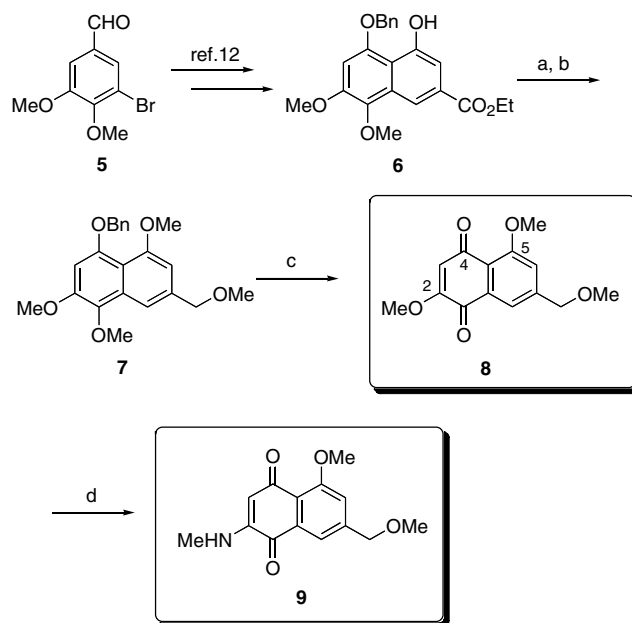
*Corresponding author. Tel./fax: +81 45 566 1717; e-mail: nisiyama@chem.keio.ac.jp

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

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

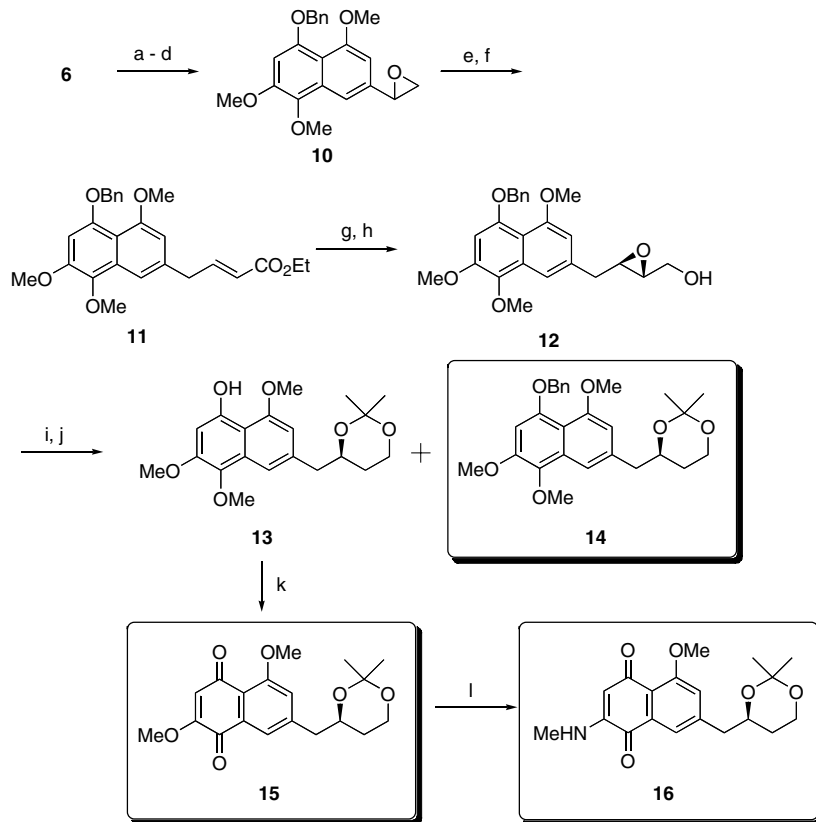


Scheme 1. Reagents and conditions: (a) LiAlH₄, THF, rt, 61%; (b) MeI, NaH, DMF, rt, 65%; (c) DDQ, *t*-BuOH, H₂O–CH₂Cl₂, rt, 84%; (d) MeNH₂, THF, 0 °C, 89%.

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

Oxirane **10** was obtained in good yield from **6** by Me₃SI (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, K₂CO₃, DMF, rt, 92%; (b) LiAlH₄, THF, rt, 80%; (c) SO₃Py, TEA, DMSO, CH₂Cl₂, rt, 100%; (d) Me₃SI, NaH, DMSO, THF, 0 °C, quant.; (e) ZnBr₂, PhH, reflux; (f) (EtO)₂P(O)CH₂CO₂Et, NaH, THF, –78 °C, 57% in two steps; (g) DIBAL, THF, –78 °C, 88%; (h) L-(+)-DIPT, Ti(O-*i*-Pr)₄, TBHP, MS4A, CH₂Cl₂, –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, H₂O–1,4-dioxane, rt, 89%; (l) MeNH₂, 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

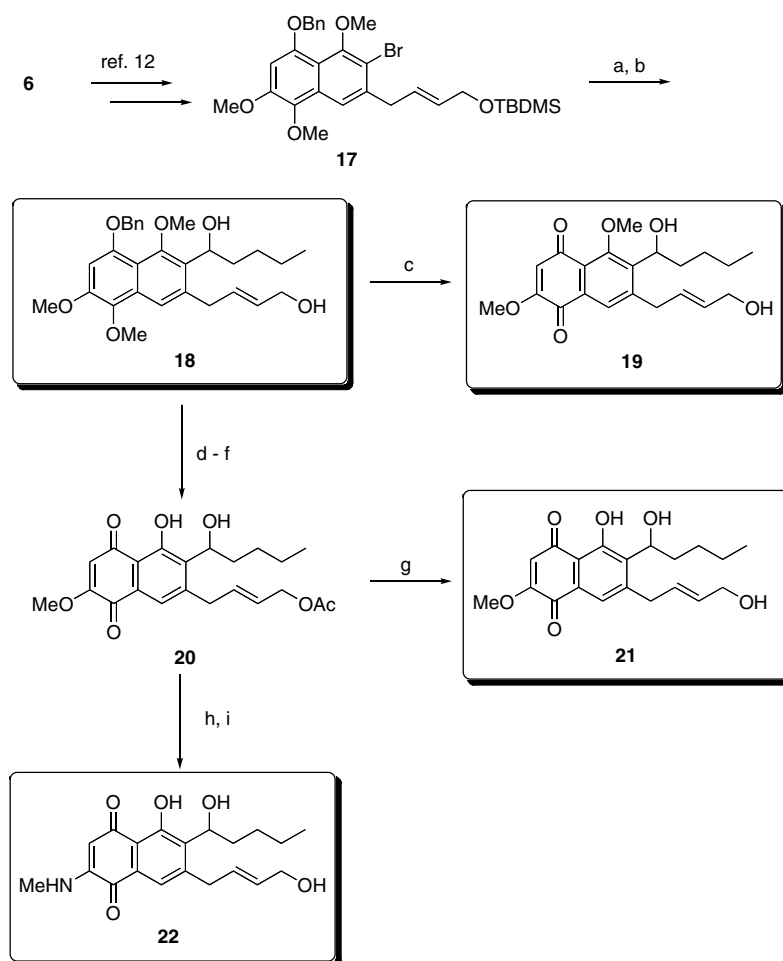
K₂CO₃ in MeOH afforded **21**. Introduction of a methylamino group and deacetylation provided **22** in 74% yield from **20**.

5. Synthesis of the naphthoquinone derivatives **23–28**

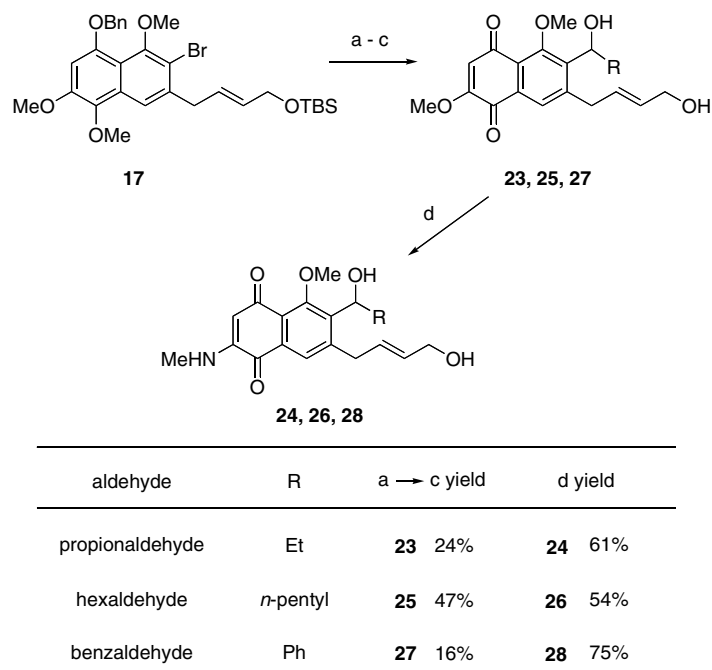
To obtain **23–28**, the synthetic route to **19–22** was slightly modified (Scheme 4). Upon using the same method as described in Scheme 3, deacetylation was unsuccessful. Accordingly, the order of oxidation and desilylation was reversed. Thus, the silyl ether **17** was reacted with the corresponding aldehyde, and oxidized to naphthoquinones, which were successfully converted into the allyl alcohols, **23**, **25**, and **27**. Treatment of **23**, **25**, and **27** with MeNH₂ 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, H₂O–CH₂Cl₂, rt, 79%; (d) Ac₂O, Py, CH₂Cl₂, rt, 85%; (e) DDQ, *t*-BuOH, H₂O–CH₂Cl₂, 0 °C, 87%; (f) BBr₃, CH₂Cl₂, −78 °C, 53%; (g) K₂CO₃, MeOH, rt, quant.; (h) MeNH₂, THF, 0 °C, 88%; (i) K₂CO₃, MeOH, rt, 84%.



Scheme 4. Reagents and conditions: (a) aldehyde, *n*-BuLi, THF, -78°C ; (b) DDQ, *t*-BuOH, $\text{H}_2\text{O}-\text{CH}_2\text{Cl}_2$, 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 ($\pm$)-**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.

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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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	($\pm$)- 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.

References and notes

- (a) Strausfeld, U.; Labbe, J. C.; Fesquet, D.; Cavadore, J. C.; Picard, A.; Sadhu, K.; Russell, P.; Doree, M. *Nature* **1999**, *351*, 242–245; (b) Gautier, J.; Solomon, M. J.; Booher, R. N.; Bazan, J. F.; Kirschner, M. W. *Cell* **1999**, *67*, 197–211; (c) Millar, J. B. A.; McGowan, C. H.; Lenaers, G.; Jones, R.; Russell, P. *EMBO J.* **1991**, *10*, 4301–4309; (d) Nilsson, I.; Hoffmann, I. *Prog. Cell Cycle Res.* **2000**, *4*, 107–114.
- (a) Russell, P.; Nurse, P. *Cell* **1987**, *49*, 559–567; (b) Parker, L.; Atheron-Fessler, S.; Piwnica-Worms, H. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 2917–2921.
- (a) Sadhu, K.; Reed, S. I.; Richardson, H.; Russell, P. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 5130–5143; (b) Galaktionov, K.; Beach, D. *Cell* **1991**, *67*, 1181–1194; (c) Jinno, S.; Suto, K.; Nagata, A.; Igarashi, M.; Kanaoka, Y.; Nojima, H.; Okayama, H. *EMBO J.* **1994**, *13*, 1549–1556.
- (a) Ref. 3c; (b) Hoffmann, I.; Draetta, G.; Karsenti, E. *EMBO J.* **1994**, *13*, 4302–4310.
- (a) Galaktionov, K.; Lee, A. K.; Eckstein, J.; Draetta, G.; Meckler, J.; Loda, M.; Beach, D. *Science* **1995**, *269*, 1575–1577; (b) Galaktionov, K.; Chen, X.; Beach, D. *Nature* **1996**, *382*, 511–517; (c) Gasparotto, D.; Maestro, R.; Piccinin, S.; Vukosavljevic, T.; Barzan, L.; Sulfaro, S.; Boiocchi, M. *Cancer Res.* **1997**, *57*, 2366–2368; (d) Wu, W. G.; Fan, Y. H.; Kemp, B. L.; Walsh, G.; Mao, L. *Cancer Res.* **1998**, *58*, 4082–4085; (e) Dixon, D.; Moyana, T.; King, M. J. *Exp. Cell Res.* **1998**, *240*, 236–243; (f) Cangi, M. G.; Cukor, B.; Soung, P.; Signoretti, S.; Moreira, G.; Ranasinghe, M.; Cady, B.; Pagano, M.; Loda, M. *J. Clin. Invest.* **2000**, *106*, 753–761.
- (a) Draetta, G.; Eckstein, J. *Biochem. Biophys. Acta* **1997**, *1332*, M53; (b) Carnero, A. *Br. J. Cancer* **2002**, *87*, 129–133; (c) Lyon, M. A.; Ducruet, A. P.; Wipf, P.; Lazo, J. S. *Nat. Rev. Drug Discovery* **2002**, *1*, 961–976.
- Study on structure–activity relationship of dysidiolide: Takahashi, M.; Dodo, K.; Sugimoto, Y.; Aoyagi, Y.; Yamada, Y.; Hashimoto, Y.; Shirai, R. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2571–2574.
- Fürstner, A.; Ruiz-Caro, J.; Prinz, H.; Waldmann, H. *J. Org. Chem.* **2004**, *69*, 459–467.
- Sohn, J.; Kiburz, B.; Li, Z.; Deng, L.; Safi, A.; Pirrung, M. C.; Rudolph, J. *J. Med. Chem.* **2003**, *46*, 2580–2588.
- (a) Juan, C.-C.; Wu, F.-Y. H. *Biochem. Biophys. Res. Commun.* **1993**, *190*, 907–911; (b) Ham, S. W.; Park, H. J.; Lim, D. H. *Bioorg. Chem.* **1997**, *25*, 33–36; (c) Nishikawa, Y.; Carr, B. J.; Wang, M.; Kar, S.; Finn, F.; Dowd, P.; Zheng, Z. Z.; Kerns, J.; Naganathan, S. *J. Biol. Chem.* **1995**, *270*, 28304–28310; (d) Ni, R.; Nishikawa, Y.; Carr, B. I. *J. Biol. Chem.* **1998**, *273*, 9906–9911; (e) Ham, S. W.; Park, J.; Lee, S.-J.; Kim, W.; Kang, K.; Choi, K. H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2507–2510.
- Kulanthaivel, P.; Perun, T. J., Jr.; Belvo, M. D.; Strobel, R. J.; Paul, D. C.; Williams, D. C. *J. Antibiot.* **1999**, *52*, 256–262, The activity of 1:15.5% inhibition at 10 μ M concentration.
- (a) Shimbashi, A.; Ishikawa, Y.; Nishiyama, S. *Tetrahedron Lett.* **2004**, *45*, 939–941; (b) Shimbashi, A.; Tsuchiya, A.; Imoto, M.; Nishiyama, S. *Bull. Chem. Soc. Jpn.* **2004**, *77*, 1925–1930; (c) Iinuma, M.; Tanaka, T.; Matsuura, S. *Chem. Pharm. Bull.* **1984**, *32*, 2296–2300.
- Tao, X. L.; Cheng, J.-F.; Nishiyama, S.; Yamamura, S. *Tetrahedron* **1994**, *50*, 2017–2028.
- (a) Baratte, B.; Meijier, L.; Galaktionov, K.; Beach, D. *Anticancer Res.* **1992**, *12*, 873–880; (b) Moran, E. J.; Sarshar, S.; Cargill, J. F.; Shahbaz, M. M.; Lio, A.; Mjalli, A. M. M.; Armstrong, R. W. *J. Am. Chem. Soc.* **1995**, *117*, 10787–10788; (c) Cao, X.; Moran, E. J.; Siev, D.; Lio, A.; Ohashi, C.; Mjalli, A. M. M. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2953–2958; (d) Rice, R. L.; Rusnak, J. M.; Yokokawa, F.; Yokokawa, S.; Messner, D.; Boynton, A. L.; Wipf, P.; Lazo, J. S. *Biochemistry* **1997**, *36*, 15965–15974; (e) Bergnes, G.; Gilliam, C. L.; Boisclair, M. D.; Blanchard, J. L.; Blake, K. V.; Epstein, D. M.; Pal, K. *Bioorg. Med. Chem. Lett.* **1999**, *332*, 19–24; (f) Wipf, P.; Aslan, D. C.; Luci, D. K.; Southwick, E. C. *Biotech. Bioeng.* **2000**, *71*, 58–70.
- Other Cdc25A inhibitors: Indolyldihydroxyquinone analogue was a potent deactivator (IC_{50} = 1.0 μ M, Ref. 9). Dysidiolide derivative effectively arrested cells in G₁/S phase (IC_{50} = 13 μ M, Ref. 7). Glucolipsin A analogue (IC_{50} = 1.6 μ M, Ref. 8) and menadione derivative (IC_{50} = 3.8 μ M, Ref. 10) showed good selectivity for Cdc25A. The IC_{50} values (μ M) of **8** and **15** were 1.5 and 1.2, and their effective selectivity against Cdc25A was determined by comparison with PTP1B (IC_{50} = 20 μ M for **8**, 13 μ M for **15**).