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Design and synthesis of *N*-alkyl oxindolylidene acetic acids as a new class of potent Cdc25A inhibitors

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Abstract—The oxindolylidene acetic acids having long *N*-alkyl chains exhibited strong inhibitory activity toward dual specificity phosphatase Cdc25A.

Cdc25 phosphatases, dual specificity enzymes, which can dephosphorylate both phospho-Ser/Thr and phospho-Tyr residues, are essential regulators by dephosphorylation of Cdk/cyclin complexes. The Cdc25 homologues, Cdc25A, Cdc25B, and Cdc25C, are encoded by the human genome.[1] Cdc25A is responsible for regulating the G1-S cell cycle transition,[2] while Cdc25B and Cdc25C regulate the G2-M cell cycle transition.[3] Cdc25A and B also have oncogenic properties.[4] They are transcriptional targets of the c-Myc oncogene[5] and overexpressed in many human tumors.[6]

Because of their important role in cell cycle regulation and their correlation with a wide variety of cancers, Cdc25A has been one of the attractive targets for drug development.[7] Although great efforts to find effective Cdc25A inhibitors have been reported, most structures developed so far are quinonoid-based compounds,[7] and an efficient strategy to design nonquinone inhibitors is in the process of being developed.[7a,8]

We thought that Cdc25A inhibitors could be created by an appropriate combination of hydrophilic and hydrophobic moieties on the basis of the structure of dysidiolide (**1**), which was the first natural inhibitor of Cdc25A.[9] 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.[9] Through biochemical evaluation of synthetic dysidiolide and its analogs, it was found that some unnatural diastereomers were more potent inhibitors of Cdc25 than dysidiolide itself.[10] Therefore, the introduction of some hydrophilic residues into hydrophobic framework might generate a new class of potent inhibitors. In previous reports, we demonstrated that perhydroindan framework, which is available from vitamin D_3 via Grundmann's ketone, is useful to construct a hydrophobic substructure of novel Cdc25A inhibitors (**2**–**4**).[11]

In this letter, we describe the design, synthesis, and biological activity of *N*-alkyl oxindolylidene acetic acids **5** as Cdc25A inhibitors (Fig. 1). The introduction of *N*-heterocyclic frameworks as a linker module between hydrophobic and hydrophilic substructures may afford a novel class of potent Cdc25A inhibitors.

For initial approach to design a new Cdc25A inhibitor, we introduced a long alkyl chain, dodecanyl group, as a hydrophobic framework. *N*-Dodecanyl substituted derivatives with different hydrophilic motifs were synthesized as shown in Scheme 1.

N-Dodecanyl isatin **7d** was obtained through alkylation of isatin **6** using sodium hydride. The unsaturated ester **8d** was prepared by the Horner–Wadsworth–Emmons reaction.[12] This reaction was completely stereoselective, the formation of the *Z*-isomer consistently not being

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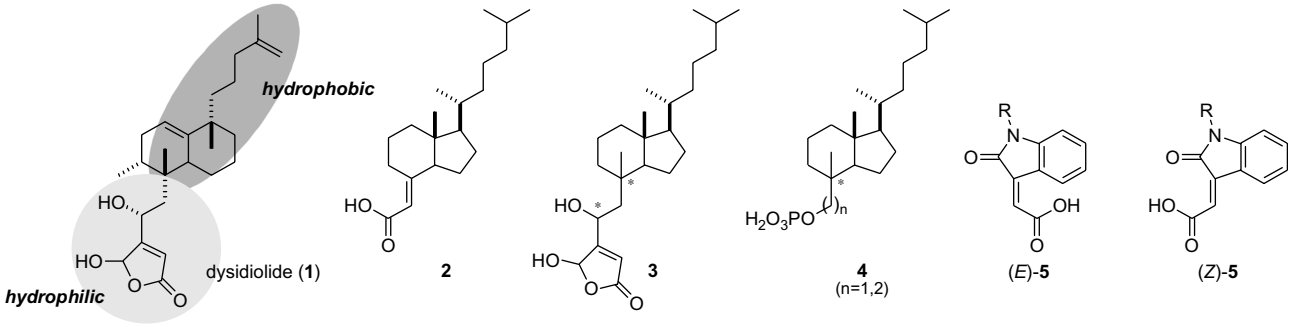
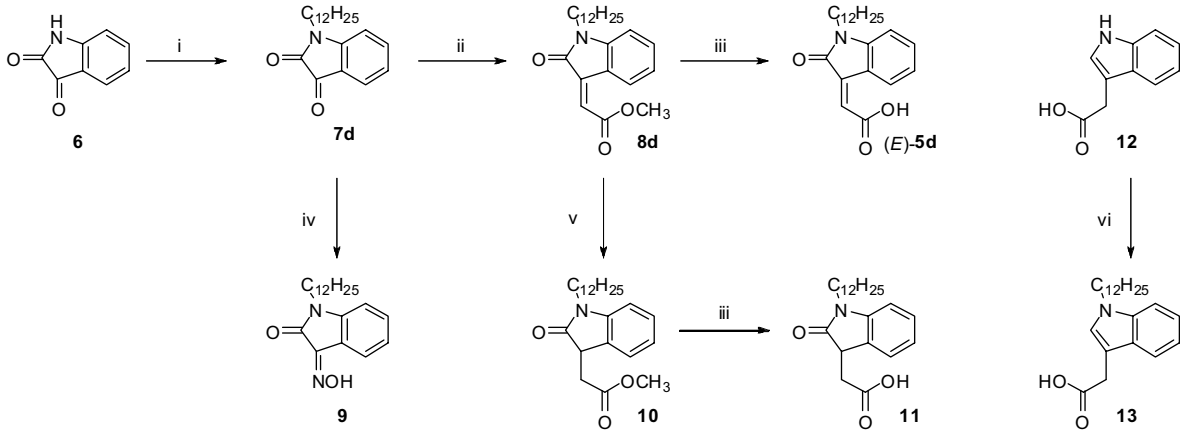


Figure 1.



Scheme 1. Reagents and conditions: (i) $C_{12}H_{25}I$, NaH, DMF, 88%; (ii) $(CH_3O)_2POCH_2CO_2CH_3$, NaH, THF, 78%; (iii) NaOH, MeOH–H_2O or EtOH–H_2O, (*E*)-**5d** (56%), **11** (81%); (iv) $H_2NOH{\cdot}HCl$, CH_3CO_2Na, MeOH, 73%; (v) $NaBH_4$, EtOH, 92%; (vi) $C_{12}H_{25}I$, NaH, DMF, 98%.

observed. The hydrolysis of the unsaturated ester **8d** proceeded quickly to oxindolylidene acetic acid (*E*)-**5d** as the *E*-isomer.[12] Oxime **9** was synthesized by condensation of **7d** with hydroxylamine. The oxindole acetic acid **11** was obtained by the borohydride reduction of **8d** and the hydrolysis of **10**. *N*-Alkylation of **12** gave *N*-dodecanyl indole acetic acid **13**.

The synthesized compounds (*E*)-**5d**, **7d**, **8d**, **9**, **11**, and **13** were tested for Cdc25A-inhibitory activity in an assay system utilizing the dephosphorylation of *O*-methylfluorescein monophosphate (Table 1).[13] The carboxylic acid derivative **2**, a potent Cdc25A inhibitor, was employed as a positive reference compound.[11a] The compound (*E*)-**5d** showed the strongest Cdc25A-inhibitory activity in the investigated compounds, and hence we changed *N*-alkyl group of oxindolylidene acetic acid **5** to investigate the effect of hydrophobic substructures.

Table 1. Cdc25A inhibition assay results for compounds **2**, (*E*)-**5d**, **7d**, **8d**, **9**,**11**, and **13**

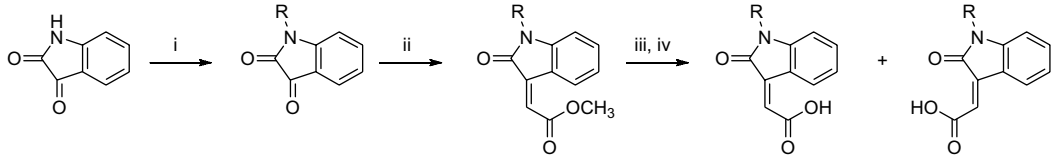
Compound	Cdc25A inhibition IC_{50}, μM (SD)
2	12(±4)
(*E*)-**5d**	2.6(±0.4)
7d	15(±2)
8d	37(±3)
9	33(±2)
11	50(±8)
13	8.0(±0.4)

As shown in Scheme 2, we synthesized compounds (*E*)-**5a**–**h** in the same way as the compound (*E*)-**5d** in Scheme 1. The isomeric acids (*E*)-**5a**–**f** were thermally converted to (*Z*)-**5a**–**f**.[12] Heating the *E*-isomers at 85–120 °C gave a glassy mixture of the *E*-isomers and the *Z*-isomers, which were easily isolated by column chromatography.[14–17]

Cdc25A-inhibitory activity of the compounds (*E*)-**5a**–**h** and (*Z*)-**5a**–**f** is shown in Table 2.

The strength of the inhibitory activity depended on the length of the alkyl chains at the *N* position of an indoline ring. The compounds bearing the longer hydrophobic chains showed the stronger inhibitory activity. The acids having *N*-dodecanyl or above length *N*-alkyl group ((*E*)-**5d**–**f** and (*Z*)-**5d**–**f**) showed higher inhibition than the positive reference compound **2**. Their inhibitory activities were not much different between the *E*-isomers and the *Z*-isomers. The substitution of phenylpropyl ((*E*)-**5g**) or ether ((*E*)-**5h**) group for alkyl groups greatly decreased the inhibition.

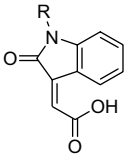
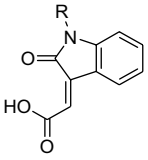
In conclusion, we designed and synthesized novel *N*-alkyl oxindolylidene acetic acids ((*E*)-**5d**–**f** and (*Z*)-**5d**–**f**) having high-Cdc25A-inhibitory activity. These findings



6	7	8	(*E*)-**5**	(*Z*)-**5**
a : R=C_6H_{13}	**7a** (87%)	**8a** (69%)	(*E*)-**5a** (46%)	(*Z*)-**5a** (11%)
b : R=C_8H_{17}	**7b** (93%)	**8b** (70%)	(*E*)-**5b** (8%)	(*Z*)-**5b** (13%)
c : R=$C_{10}H_{21}$	**7c** (99%)	**8c** (83%)	(*E*)-**5c** (14%)	(*Z*)-**5c** (10%)
d : R=$C_{12}H_{25}$	**7d** (88%)	**8d** (78%)	(*E*)-**5d** (14%)	(*Z*)-**5d** (15%)
e : R=$C_{14}H_{29}$	**7e** (87%)	**8e** (67%)	(*E*)-**5e** (25%)	(*Z*)-**5e** (21%)
f : R=$C_{16}H_{33}$	**7f** (89%)	**8f** (83%)	(*E*)-**5f** (15%)	(*Z*)-**5f** (16%)
g : R=$(CH_2)_3Ph$	**7g** (92%)	**8g** (68%)	(*E*)-**5g** (41%)[a]	
h : R=$(CH_2CH_2O)_3CH_2CH_3$	**7h** (78%)	**8h** (60%)	(*E*)-**5h** (36%)[a,b]	

Scheme 2. Reagents and conditions: (i) R–I (**a**–**d**, **f**) or R–Br (**e**, **g**, **h**), NaH, DMF; (ii) $(CH_3O)_2POCH_2CO_2CH_3$, NaH, THF; (iii) NaOH, MeOH–H_2O or EtOH–H_2O; (iv) neat, 85–120 °C; [a]obtained through the reagents and condition (iii) without (iv). [b]*E*/*Z*=4:1.

Table 2. Cdc25A inhibition assay results for compounds (*E*)-**5** and (*Z*)-**5**

Cdc25A inhibition IC_{50}, μM (SD)	Compound	
		
	(*E*)-**5**	(*Z*)-**5**
a: R = C_6H_{13}	>100	>100
b: R = C_8H_{17}	78(±13)	39(±4)
c: R = $C_{10}H_{21}$	13(±0.1)	12(±0.3)
d: R = $C_{12}H_{25}$	2.6(±0.4)	2.9(±0.3)
e: R = $C_{14}H_{29}$	2.3(±0.2)	1.7(±0.0)
f: R = $C_{16}H_{33}$	1.9(±0.1)	1.6(±0.2)
g: R = $(CH_2)_3Ph$	>100	—
h: R = $(CH_2CH_2O)_3$ CH_2CH_3	>100[a]	—

[a] The mixture of *E*- and *Z*-form (*E*/*Z* = 4/1).

on the structure–activity relationship should be helpful for the design of novel Cdc25A inhibitors. We would like to investigate isoform selectivity, because Cdc25B and C inhibitory activities of those compounds have not been tested. Design and synthesis of further isatin analogs as candidate for potent inhibitors of Cdc25 family members are in progress.

Acknowledgments

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References and notes

1. (a) Galaktionov, K.; Beach, D. *Cell* **1991**, *67*, 1181; (b) Nagata, A.; Igarashi, M.; Jinno, S.; Suto, K.; Okayama, H. *New Biol.* **1991**, *3*, 959; (c) Sadhu, K. S.; Reed, I.; Richardson, H.; Russell, P. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 5139.
2. Mailand, N.; Falck, J.; Lukas, C.; Syljuåsen, R. G.; Welcker, M.; Bartek, J.; Lukas, J. *Science* **2000**, *288*, 1425.
3. Bulavin, D. V.; Higashimoto, Y.; Popoff, I. J.; Gaarde, W. A.; Basrur, V.; Potapova, O.; Appell, E.; Fornace, A. J., Jr. *Nature* **2001**, *411*, 102.
4. Galaktionov, K.; Lee, A. K.; Eckstein, J.; Draetta, G.; Meckler, J.; Loda, M.; Beach, D. *Science* **1995**, *269*, 1575.
5. Galaktionov, K.; Chen, X.; Beach, D. *Nature* **1996**, *382*, 511.
6. See the following reviews: (a) Boutros, R.; Dozier, C.; Ducommun, B. *Curr. Opin. Cell Biol.* **2006**, *18*, 185; (b) Kristjansdottir, K.; Rudolph, J. *Chem. Biol.* **2004**, *11*, 1043.
7. See the following reviews of Cdc25s inhibitors and the references therein: (a) Contour-Galcera, M.-O.; Sidhu, A.; Prevost, G.; Bigg, D.; Ducommun, B. *Pharmacol. Ther.* **2007**, *115*, 1; (b) Bialy, L.; Waldmann, H. *Angew. Chem., Int. Ed.* **2005**, *44*, 3814; (c) Lyon, M. A.; Ducruet, A. P.; Wipf, P.; Lazo, J. S. *Nat. Rev. Drug Discov.* **2002**, *1*, 961 ; (d) Carnero, A. *Br. J. Cancer* **2002**, *87*, 129; (e) Eckstein, J. W. *Invest. New Drugs* **2000**, *18*, 149; (f) Pestell, K. E.; Ducruet, A. P.; Wipf, P. *Oncogene* **2000**, *19*, 6607.
8. Docking studies for Cdc25B inhibitors: (a) Lavecchia, A.; Cosconati, S.; Limongelli, V.; Novellino, E. *ChemMedChem* **2006**, *1*, 540; (b) Montes, M.; Braud, E.; Miteva, M. A.; Goddard, M.-L.; Mondesert, O.; Kolb, S.; Brun, M.-P.; Ducommun, B.; Garbay, C.; Villoutreix, B. O. *J. Chem. Inf. Model* **2008**, *48*, 157.
9. Gunesekera, S. P.; McCarthy, P. J.; Kelly-Borges, M.; Lobkovsky, E.; Clardy, J. *J. Am. Chem. Soc.* **1996**, *118*, 8759.
10. (a) Takahashi, M.; Dodo, K.; Sugimoto, Y.; Aoyagi, Y.; Yamada, Y.; Hashimoto, Y.; Shirai, R. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 615; (b) Brohm, D.; Metzger, S.; Bhargava, A.; Muller, O.; Lieb, F.; Waldmann, H. *Angew. Chem., Int. Ed.* **2002**, *41*, 307; (c) Brohm, D.; Philipe, N.; Metzger, S.; Bhargava, A.; Muller, O.; Lieb, F.; Waldmann, H. *J. Am. Chem. Soc.* **2002**, *124*, 13171.
11. (a) Dodo, K.; Takahashi, M.; Yamada, Y.; Sugimoto, Y.; Hashimoto, Y.; Shirai, R. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 615; (b) Shimazawa, R.; Suzuki, T.; Dodo, K.; Shirai, R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3291; (c) Shimazawa, R.; Gochomori, M.; Shirai, R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4339.
12. Autrey, R. L.; Tahk, F. C. *Tetrahedron* **1967**, *23*, 901.

13. *Cdc25A phosphatase assay*. Catalytic domain proteins of human Cdc25A were purchased from BIOMOL (Product Number SE-364). Phosphatase activity of Cdc25A was assayed in 100 μL of buffer containing 50 mM Tris–HCl (pH 8.0), 100 mM NaCl, and 1 mM dithiothreitol, with 40 μM *O*-methylfluorescein monophosphate as the substrate, using 96-well microtiter plate. The IC_{50} values were means of two–five experiments, standard deviation is given in parentheses.
14. *Typical procedure for the synthesis of the oxindolylidene acetic acids* (*E*)-**5d** and (*Z*)-**5d** *from* **8d**. (*E*)-Methyl 2-(1-dodecyl-2-oxoindolin-3-ylidene)acetate **8d** (487 mg) was hydrolyzed at rt in 10 min with 0.4 M NaOH in EtOH–water (3:2). To the solution, 10% HCl, water, and diethyl ether were added. The acidified aqueous layer was extracted with diethyl ether, and the combined organic layers were washed with brine. They were dried over Na_2SO_4 and concentrated to give the crude oxindolylidene acetic acid as an orange solid. The crude acid was heated at 100 °C for 2 h. The obtained glassy mixture was separated by silica gel column chromatography (CH_2Cl_2/AcOEt =20:1–1:1). The *Z*-isomer ((*Z*)-**5d**) and the *E*-isomer ((*E*)-**5d**) were recrystallized from hexane and hexane–AcOEt, respectively. The products (*Z*)-**5d** and (*E*)-**5d** were obtained as yellow needles of mp 92–93 °C (70 mg, 15% from **8d**) and as red needles of mp 111–112 °C (64 mg, 14% from **8d**), respectively.
15. The ether derivatives (*E*)-**5h** were obtained as the mixture (*E*/*Z* = 4:1) through the same hydrolysis condition. Compound (*E*)-**5h** was a red thick oil and could not be isolated by recrystallization.
16. The following is the ^{1}H NMR spectra of the oxindolylidene acetic acids (*E*)-**5d** and (*Z*)-**5d**. (*E*)-**5d** ($CDCl_3$): 0.88 (3H, t, *J* = 7.0 Hz, CH_3), 1.25–1.37 (18H, m, CH_2), 1.68 (2H, pentet, *J* = 7.2 Hz, NCH_2CH_2), 3.72 (2H, t, *J* = 7.3 Hz, NCH_2), 6.81 (1H, d, *J* = 7.8 Hz, H-7), 6.94 (1H, s, =CH), 7.06 (1H, dt, *J* = 0.9, 7.7 Hz, H-5), 7.37 (1H, dt, *J* = 1.2, 7.7 Hz, H-6), 8.51 (1H, d, *J* = 7.8 Hz, H-4); (*Z*)-**5d** ($CDCl_3$): 0.88 (3H, t, *J* = 7.0 Hz, CH_3), 1.26–1.38 (18H, m, CH_2), 1.73 (2H, pentet, *J* = 7.2 Hz, NCH_2CH_2), 3.79 (2H, t, *J* = 7.4 Hz, NCH_2), 6.92 (1H, d, *J* = 7.9 Hz, H-7), 6.95 (1H, s, =CH), 7.16 (1H, dt, *J* = 0.8, 7.6 Hz, H-5), 7.44 (1H, dt, *J* = 1.1, 7.8 Hz, H-6), 7.52 (1H, d *J* = 7.5 Hz, H-4), 14.8 (1H, s, COOH).
17. The structure of oxindolylidene acetic acid *E*-isomer and *Z*-isomer was determined by ^{1}H NMR. The farther downfield shift is observed the shift of the hydrogen at 4-position in the *E*-isomers (e.g., (*E*)-**5d**: 8.51 ppm) than the *Z*-isomers (e.g., (*Z*)-**5d**: 7.52 ppm), because the hydrogen at 4-position in the *E*-isomers is in proximity to a carbonyl group. Additionally, ^{1}H NMR spectrum of the carboxylic proton in the *Z*-isomers shows a sharp singlet peak (e.g., (*Z*)-**5d**: 14.8 ppm) by chelation of the proton in a seven-membered ring. See Ref. 12.