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Communications to the Editor

Novel CDC25A Phosphatase Inhibitors from Pyrolysis of 3-α-Azido-B-homo-6-oxa-4-cholesten-7-one on Silica Gel

Hairuo Peng and Leon H. Zalkow*

School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, Georgia 30332

Robert T. Abraham

Department of Immunology and Pharmacology, Mayo Clinic, Rochester, Minnesota 55905

Garth Powis

Arizona Cancer Center, University of Arizona, 1515 North Campbell Avenue, Tucson, Arizona 85724-5024

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Introduction. Posttranslational modifications of intracellular signaling proteins by addition and removal of phosphate groups provide eukaryotic cells with convenient molecular switches for the control of the normal functions of intracellular signaling pathways. Protein serine/threonine phosphatases (PSTPase) and protein tyrosine phosphatases (PTPase) catalyze the removal of phosphate groups from phosphoserine/phosphothreonine and phosphotyrosine residues, respectively. Recently, a novel group of dual specificity protein phosphatases, CDC25 phosphatases, which dephosphorylate contiguous phosphothreonine and phosphotyrosine residues on the cyclin-dependent kinases (cdk), has been shown to play crucial roles in cell proliferation.[1,2]

In human cells, CDC25 consists of three phase-specific isoforms termed CDC25A, CDC25B, and CDC25C. CDC25A is expressed early in the G1 phase of the cell cycle and is responsible for the activation of at least two cyclin-dependent kinases required for G1-to-S phase progression.[1,2] CDC25A dephosphorylates the catalytic cdk subunit at Tyr-15 and Thr-14 near the ATP-binding site, allowing binding of ATP to the cyclin−cdk complex and rendering the complex catalytically active. The dephosphorylation of both cyclin E−cdk2 and cyclin A−cdk2 complexes by CDC25A is tightly regulated in normal cells. However, accumulating evidence suggests that inappropriate amplification or activation of CDC25A is characteristic of a number of human cancers, including breast cancers.[3,4] Thus, increased CDC25A activity may contribute to the dysregulated growth of certain types of cancer cells. Small molecule inhibitors of CDC25A may possess novel antitumor activities.[5]

Except for the widely used broad-spectrum protein phosphatase inhibitor vanadate,[6] few dual specificity protein phosphatase inhibitors have been reported. Dysidiolide (Figure 1) was the first natural CDC25A phosphatase inhibitor (IC_{50} = 9.4 μM) with antitumor activity discovered.[7] The promising biological activities of this compound soon resulted in total syntheses by three groups.[8] A combinatorial library of small molecule phosphatase inhibitors based on the pharmacophore of natural PSTPase inhibitors has been reported. SC-ααδ9, the best CDC25A inhibitor in the library, showed an IC_{50} of 15 μM.[9]

Thus, a need remains for the discovery and development of more potent CDC25A inhibitors. We have sought novel structures, active as signal transduction and cell cycle inhibitors, with antitumor activities. To shorten the time from initial discovery to clinic, we have investigated unusual chemical transformations of readily available complex natural product scaffolds. It has been suggested[7] that in dysidiolide, the γ-hydroxybutenolide moiety likely serves as a surrogate phosphate, while the long side chain occupies a hydrophobic binding pocket associated with the normal substrate. We visualized that a cholesteryl moiety might provide the scaffold for constructing a CDC25A inhibitor, with the C and D rings and attached C8 side chain mimicking the hydrophobic rings, with attendant side chains, of dysidiolide, and the surrogate phosphate could be constructed from the A and B rings via some type of fragmentation reaction of these rings. The C-3 acetoxy group and C-5 double bond of cholesteryl acetate would obviously serve as entries in this approach.

Herein, we report the discovery of a group of novel CDC25A inhibitors, synthesized by pyrolysis of a readily available natural product derivative, 3-α-azido-B-homo-

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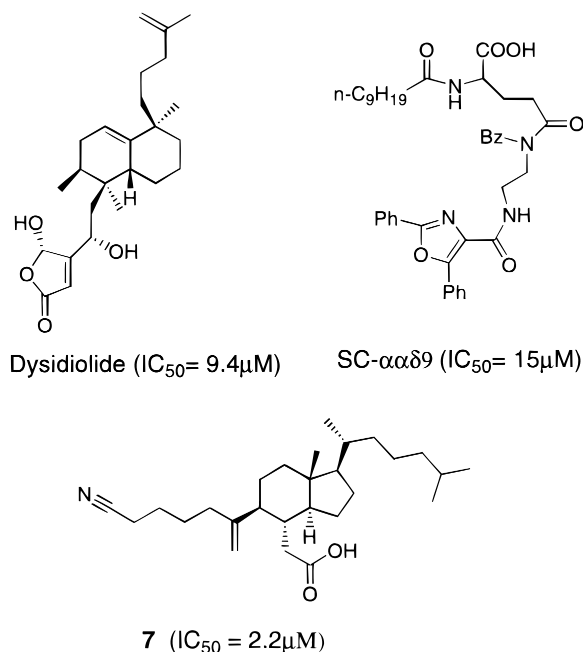
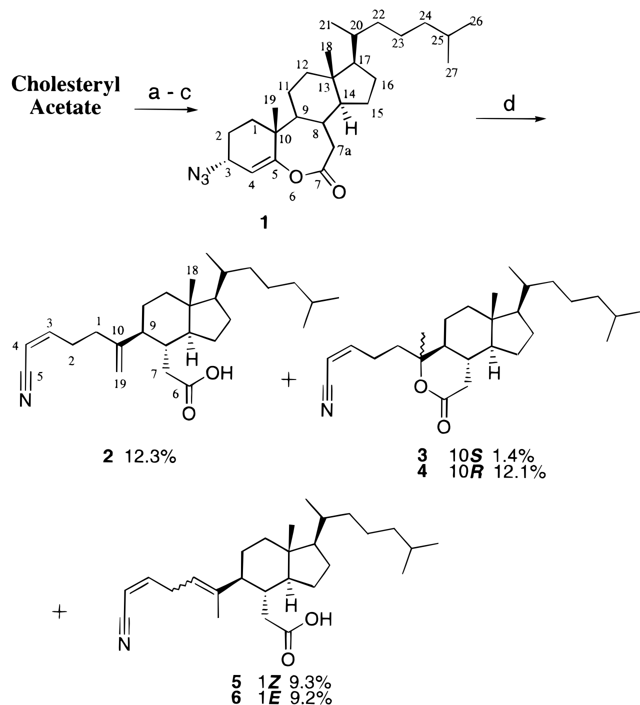


Figure 1. Chemical structures of dysidiolide, SC-ααδ9, and compound **7**.

Scheme 1[a]



[a] (a) O_3, −60 °C, petroleum ether, 2 M HCl, 72%; (b) $SOCl_2$, CH_2Cl_2, 2 h, 84%; (c) 2 equiv of NaN_3 (10%), acetone, 1 h, 98%; (d) compound **1** was coated on silica gel, heated at 180 °C, 1 h.

6-oxa-4-cholesten-7-one (**1**). Compound **7,** the best inhibitor in this series, inhibited the dephosphorylation of fluorescein diphosphate by CDC25A with an IC_{50} of 2.2 μM and is thus far the most potent CDC25A inhibitor to be reported.

Results and Discussion. As illustrated in Scheme 1, pyrolysis precursor **1** was prepared by ozonolysis of cholesteryl acetate,[10] followed by conversion of the resulting keto acid to 3β-actoxy-B-homo-6-oxa-4-cholesten-7-one and exposure of the latter to sodium azide (10% aqueous) at room temperature. The structure of compound **1** was identified by its IR spectrum (azide, 2094 cm^{-1}; enol lactone, 1763 cm^{-1}) and confirmed by ^{1}H NMR, ^{13}C NMR, DEPT spectra, and high-resolution CIMS spectroscopy. Silica gel (0.040−0.063 mm; EM Science) was evenly coated with **1** (ethyl acetate solution) to give 20 wt % solid, after evaporation of the solvent. Heating of this solid at 180 °C for 1 h, followed by extraction (ethyl acetate), silica gel column chromatography, and reverse-phase HPLC, gave products **2**−**6**.

Scheme 2. Proposed Mechanism for Formation of Compounds **2**−**6**

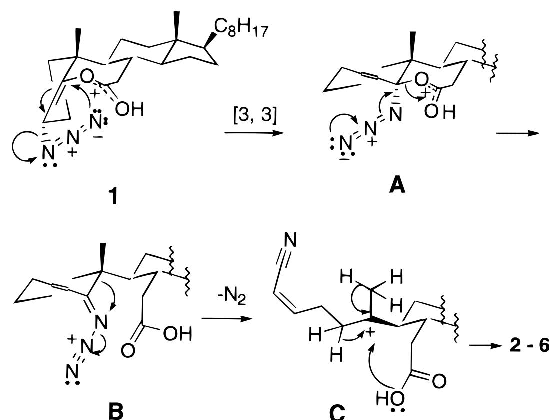


Table 1. Chemical Shifts for H-19 of Compounds **2**−**6**

	2	**3**	**4**	**5**	**6**
δ_{H-19}	4.93, s 4.84, s	1.34, s	1.43, s	1.67, brs[a]	1.60, brs[a]

[a] brs stands for broad singlet.

These products can be visualized as arising from the common carbocation intermediate **C** as illustrated in Scheme 2. Thus, heating of compound **1** on silica gel would lead to **A** by 3,3-sigmatropic rearrangement and protonation. Fragmentation of **A** would be initiated by opening of ring **A** to give **B**, which after loss of molecular nitrogen would give **C**, the common intermediate in the formation of products **2**−**6**. These compounds share the structural features of a cholesteryl side chain and intact C and D rings, while rings A and B have been opened to give one chain containing a conjugated cyano group exhibiting absorptions around 2220−2230 cm^{-1} in the IR spectra. Elemental analysis and high-resolution EIMS confirmed the same molecular formula, $C_{27}H_{43}NO_2$, for compounds **2**−**6**. Compound **2** was hydrogenated to remove the conjugated double bond using Pd on carbon to give compound **7**.

The structures of compounds **2**−**7** were determined by IR, MS, and a series of NMR experiments, including ^{1}H NMR, ^{13}C NMR, DEPT, COSY, HMBC, and HMQC. As shown in Table 1, the chemical shifts of H-19 are indicative of the structures. The configurations of the conjugated double bonds in these compounds were assigned as cis based on the coupling constants. The configurations of the double bonds between C-1 and C-10 in acids **5** and **6** were assigned from the NOSEY spectrum of **5**. The relative stereochemistry of the lactone epimers **3** and **4** was determined by the single-crystal X-ray diffraction[11] of **3** and the NOESY spectrum of **4**.

Compounds **2**−**7** were tested for their CDC25A inhibitory activity by measuring the inhibition of dephos-

Table 2. CDC25A Inhibition by Compounds **2**–**7**

	2	**3**	**4**	**5**	**6**	**7**	dysidiolide	SC-ααδ9
IC_{50} (μM)	9.7[a]	13.3[a]	>50[a]	36.3[a]	24.2[a]	2.2[a]	9.4[b]	15[c], 4[d]

[a] IC_{50} value obtained by assays using fluorescein diphosphate as substrate. [b] IC_{50} value reported in ref 7 by assays using *p*-nitrophenylphosphate as substrate. [c] IC_{50} value reported in ref 9 by assays using fluorescein diphosphate as substrate. [d] IC_{50} value reported in ref 9 by assays using *p*-nitrophenylphosphate as substrate.

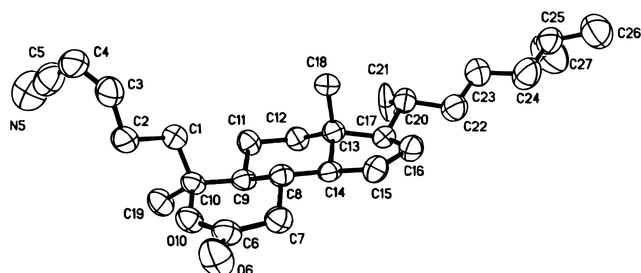


Figure 2. ORTEP drawing of the X-ray structure for compound **3**.

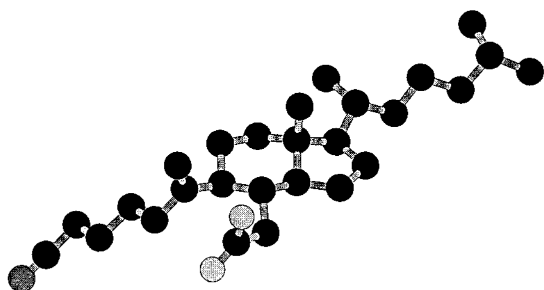


Figure 3. Computed low-energy conformation of compound **7**.

phorylation of fluorescein diphosphate by this enzyme as described previously.[9] Results of the in vitro CDC25A inhibition assay of compounds **2**–**7** presented in Table 2 revealed the importance of the orientations of the cyano-containing side chains in these compounds. With the 10*S* configuration (Figure 2), lactone **3** showed very little inhibitory activity (IC_{50} > 50 μM), while its 10*R* isomer, compound **4**, is approximately 4-fold more potent (IC_{50} = 13.3 μM). A similar tendency can be observed within the acid series of compounds **2** and **5**–**7**, wherein the different configurations of double bonds vary the positioning of the cyano group and result in differentiated inhibitory activities (IC_{50} = 9.7, 36.3, and 24.2 μM for **2**, **5**, and **6,** respectively). When the conjugated double bond was hydrogenated to give compound **7,** a significant enhancement of CDC25A inhibitory activity was obtained (IC_{50} = 2.2 μM).

Some common structure features can be observed among compounds **2**–**7**, dysidiolide, and SC-ααδ9. They all contain long alkyl side chains that may fit into a hydrophobic pocket in the substrate binding site, and they either contain a carboxyl group (compounds **2**, **5**–**7**, and SC-ααδ9) or a lactone moiety (compounds **3**, **4**, and dysidiolide), which may interact with the active site arginine in place of a phosphate. Figure 3 illustrates one of the lowest-energy conformations of compound **7** found by a conformational search using the MM+ force field.[12] This conformation shows a flattened structure which is very different from the 3D conformation observed in the X-ray structure[7] of dysidiolide, wherein the diaxial orientations of the two side chains lead to their almost vertical projections from the plain formed by the two rings. This suggests that a flattened structure may fit better into the binding site of CDC25A. The recent publication of the crystal structure of CDC25A[13] and the deposition of the atomic coordinates in the Brookhaven Protein Data Bank have permitted us to initiate the modeling studies on the compounds described in this communication, and these studies will be reported in a future publication.

At this time, only compounds **2** and **4** have been tested for their growth inhibition activities against tumor cell cultures, as described previously.[14] Acid **2** and lactone **4** were found to suppress growth of HT-29 colon cancer cells with IC_{50} values of 10.9 and 12.0 μM, respectively. Lactone **4** also inhibited the growth of A-549 lung adenocarcinoma cells with an IC_{50} of 7.0 μM.

In conclusion, we have employed silica gel-supported pyrolysis of an azido-homo-oxa steroid to synthesize a group of novel, small molecule inhibitors of human CDC25A phosphatase, using the readily available natural product cholesteryl acetate as starting material. Acid **7**, the best inhibitor in this group, inhibited the activity of CDC25A protein phosphatase reversibly and more potently (IC_{50} = 2.2 μM) than the well-known marine natural product dysidiolide. Compounds **2** and **4** inhibited proliferation of some human tumor cell lines in a dose-dependent manner. These compounds provide unique leads for the design of more potent CDC25A inhibitors as potential anticancer agents, and the synthesis and screening of structural variants based on these motifs are currently underway.

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Supporting Information Available: Full experimental details and spectroscopic data for compounds listed in Table 2 and atomic coordinate information for compound **3** (19 pages). Ordering information is given on any current masthead page.

References

(1) Galaktionov, K.; Beach, D. Specific activation of cdc25 tyrosine phosphatases by B-type cyclins: evidence for multiple roles of mitotic cyclins. *Cell* **1991**, *67*, 1181–1194.
(2) (a) Jinno, S.; Suto, K.; Nagata, A.; Igrashi, M.; Kanaoka, Y.; Nojima, H.; Okayama, H. Cdc25A is a novel phosphatase functioning early in the cell cycle. *EMBO J.* **1994**, *13*, 1549–1556. (b) Hoffmann, I.; Draetta, G.; Karsenti, E.; Activation of the phosphatase activity of human cdc25A by a cdk2-cyclin E dependent phosphorylation at the G1/S transition *EMBO J* **1994**, *13*, 4302–4310.
(3) (a) Galaktionov, K.; Lee, A. K.; Eckstein, J.; Draetta, G.; Meckler, J.; Loda, M.; Beach, D. CDC25 phosphatases as potential human oncogenes. *Science* **1995**, *269*, 1575–1577. (b) Gasparotto, D.; Maestro, R.; Piccinin, S.; Vukosavljievic, T.; Barzan, L.; Sulfaro, S.; Boiocchi M. Overexpression of CDC25A and CDC25B in head and neck cancers. *Cancer Res.* **1997**, *57*, 2366–2368.
(4) Galaktionov, K.; Chen, X.; Beach, D. Cdc25 cell-cycle phosphatase as a target of c-myc. *Nature* **1996**, *382*, 511–517.
(5) Draetta, G.; Eckstein, J. Cdc25 protein phosphatases in cell proliferation. *Biochim. Biophys. Acta* **1997**, *1332*, M53–62.
(6) Baratte, B.; Meijer, L.; Galaktionov, K.; Beach, D. Screening for antimitotic compounds using the cdc25 tyrosine phosphatase, an activator of the mitosis-inducing p34cdc2/cyclin Bcdc13 protein kinase. *Anticancer Res.* **1992**, *12*, 873–880.

(7) Gunasekera, S. P.; McCarthy, P. J.; Kelly-Broger, M.; Lobkovsky, E.; Clardy, J. Dysdiolide: A novel protein phosphatase inhibitor from Caribbean Sponge *Diysidea ehterria* de Laubenfels. *J. Am. Chem. Soc.* **1996**, *118*, 8759−8760.

(8) (a) Corey, E. J.; Roberts, B. E. Total Synthesis of Dysidiolide. *J. Am. Chem. Soc.* **1997**, *119*, 12425−12431. (b) Magnuson, S. T.; Sepp-Lorenzino, L.; Rosen, N.; Danishefsky, S. J. A Concise Total Synthesis of Dysidiolide through Application of a Dioxolenium-Mediated Diels−Alder Reaction. *J. Am. Chem. Soc.* **1998**, *120*, 1615−1616. (c) Boukouvalas, J.; Cheng, Y.; Robichaud, J. Total synthesis of (+)-Dysidiolide. *J. Org. Chem.* **1998**, *63*, 228−229.

(9) Rice, R. L.; Rusnak, J. M.; Yokokawa, F.; Yokokawa, S.; Messner, D.; Boynton, A. L.; Wipf, P.; Lazo, J. S. A targeted library of small-molecule, tyrosine, and dual-specificity phosphatase inhibitors from a rational core design and random side chain variation. *Biochemistry* **1997**, *36*, 15965.

(10) Lettre, H.; Mathes, K.; Wagner, M. *Liebigs Ann. Chem.* **1967**, *703*, 147.

(11) Compound **3** crystallized in space group *P*212121 with $a = 6.467(2)$ Å, $b = 34.103(1)$ Å, $c = 35.479(1)$ Å and refined to a conventional factor $R = 0.0069$ for 867 parameters and 10 471 reflections with $F_o > 4\sigma(F_o)$. Data collection was at 248 K, with $Z = 12$. There was some disorder in the side chains of all three unique molecules.

(12) Compound **7** was computer-built using ISIS/Draw (version 2.1; MDL Information System, Inc., San Leandro, CA) and input into Hyperchem Molecular Modeling Package (version 5.1; Hypercube Inc., Gainesville, FL). Conformation search was performed by varying torsion angles defined by atoms 16−17−20−21, 6−7−8−9, and 1−10−9−11 as well as those in the C and D rings to reach a rms gradient of <0.01 with the MM+ force field. Using usage-directed searching method, a total of 1175 iterations and 1000 optimizations yielded 132 low-energy conformations, wherein five lowest-energy conformations were picked and further geometry-optimized using MM+ force field with Polak−Ribiere optimizer to a gradient of <0.01. The conformation of the lowest-energy (below that of the fifth lowest conformation by 0.253 77 kcal/mol) obtained is shown in Figure 3.

(13) Fauman, E. B.; Cogswell, J. P.; Lovejoy, B.; Rocque, W. J.; Holmes, W.; Momtana, V. G.; Piwnica-Worms, H.; Rink, M. J.; Saper, M. A. Crystal structure of the catalytic domain of the human cell cycle control phosphatase, cdc25A. *Cell* **1998**, *93*, 617−625.

(14) Powis, P. G.; Abraham, R. T.; Ashendel, C. L.; Zalkow, L. H.; Dvorakova, K.; Salmon, S.; Harrison, S.; Worzalla, J. Inhibition of intracellular Ca^{2+} signaling, cytotoxicity and antitumor activity of the herbicide oryzalin and its analogues. *Cancer Chemother. Pharmacol.* **1997**, *41*, 22−28.

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