Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 615–617

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis of a Novel Class of cdc25A Inhibitors from Vitamin D_3

Kosuke Dodo,[a] Masato Takahashi,[a] Yuji Yamada,[b] Yoshikazu Sugimoto,[b] Yuichi Hashimoto[a] and Ryuichi Shirai[c,*]

[a]*Institute of Molecular and Cellular Biosciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan*
[b]*Cancer Research Lab., Hanno Research Center, Taiho Pharmaceutical Co., Ltd., 1-27 Misugidai, Hanno-shi, Saitama 357-8527, Japan*
[c]*Research and Education Center for Materials Science, Nara Institute of Science and Technology,* 8916-5 *Takayama-cho, Ikoma-shi, Nara 630-0101, Japan*

Received 8 December 1999; accepted 20 January 2000

Abstract—We have developed a novel class of cdc25A inhibitors by drastic modification of the hydrophobic and hydrophilic substructures of dysidiolide. The unsaturated derivative **3b** strongly inhibited cdc25A ($IC_{50} = 7.7$ μM) and caused G1 arrest of HL60 cells.

A novel group of dual-specificity protein phosphatases, consisting of three homologues termed cdc25A, -B and -C, has been shown to play crucial roles in human cell proliferation.[1,2] Cdc25A is expressed in the early G1 phase of the cell cycle and is responsible for G1–S transition.[3] Accumulating evidence suggests that inappropriate amplification or activation of cdc25A is characteristic of a number of human cancers.[4,5] Thus, increased cdc25A activity may contribute to the growth of certain types of cancer. Consequently, inhibitors of cdc25A are candidates for new therapeutic agents to treat human cancers. Dysidiolide is the first natural inhibitor of cdc25A to be discovered ($IC_{50} = 9.4$ μM); it was also shown to inhibit the growth of lung carcinoma and murine leukemia cell lines.[6] Total synthesis of dysidiolide was accomplished by us and other groups,[7–10] but the structure–activity relationship remains unclear. It has been suggested that the γ-hydroxybutenolide moiety (hydrophilic substructure) serves as a surrogate phosphate and the long side chain (hydrophobic substructure) occupies a hydrophobic binding pocket when the molecule is bound to cdc25A (Fig. 1). Modification of these substructures may provide novel cdc25A inhibitors.

Here, we report the design, synthesis and biological activities of novel inhibitors of cdc25A phosphatase. These analogues have a carboxylic acid moiety as the hydrophilic substructure and a perhydroindan framework, derived from Vitamin D_3, as the hydrophobic substructure. The complete ozonolysis of Vitamin D_3 furnished **1**, while partial ozonolysis provided a mixture of **1** and **2**. According to standard procedures as illustrated in Scheme 1, **3a**–**4b**[11] were prepared from **1**. Compounds **5** and **6** were prepared from **2** in the same manner (Fig. 2). Compounds **4a**, **4b** and **6** were obtained as inseparable mixtures of epimers.

Compounds **3a**–**6** were tested for cdc25A-inhibitory activity[12] and in vitro antitumor activity.[13] As shown in Table 1, compounds **3b** and **6** showed strong cdc25A-inhibitory activity, while **4b** and **5** had weak activity. The methyl ester analogues **3a** and **4a** did not inhibit cdc25A at all. Compound **3b** has antitumor activity as well as cdc25A-inhibitory activity. It was suggested that **3b** may exert antitumor activity by blocking cell cycle progression.

Next, to establish the effect of these compounds on cell cycle progression, flow cytometric analysis was performed.[14] HL60 (human leukemia cell line) cells were treated for 20 h with these compounds at 50 μM concentration. The results are presented in Figure 3. Simultaneous increase of G1 phase population and decrease of S phase population indicates G1 arrest, an inhibition of G1–S progression. As expected, compound **3b**, which had strong cdc25A-inhibitory activity, caused

*Corresponding author. Tel.: +81-743-72-6171; fax: +81-743-72-6179; e-mail: rshirai@ms.aist-nara.ac.jp

0960-894X/00/$ - see front matter
PII: S0960-894X(00)00065-2

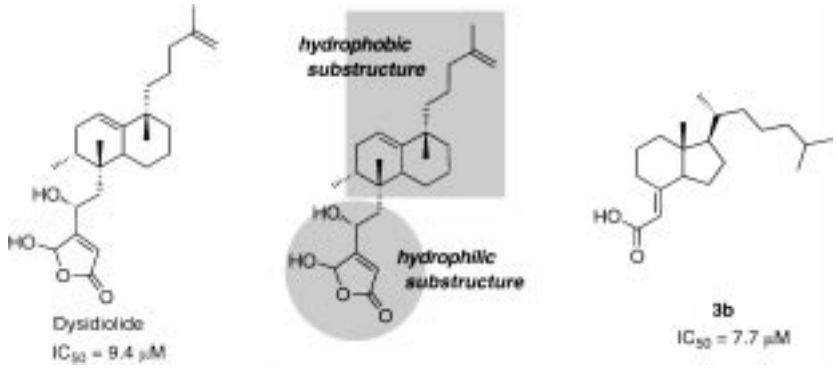
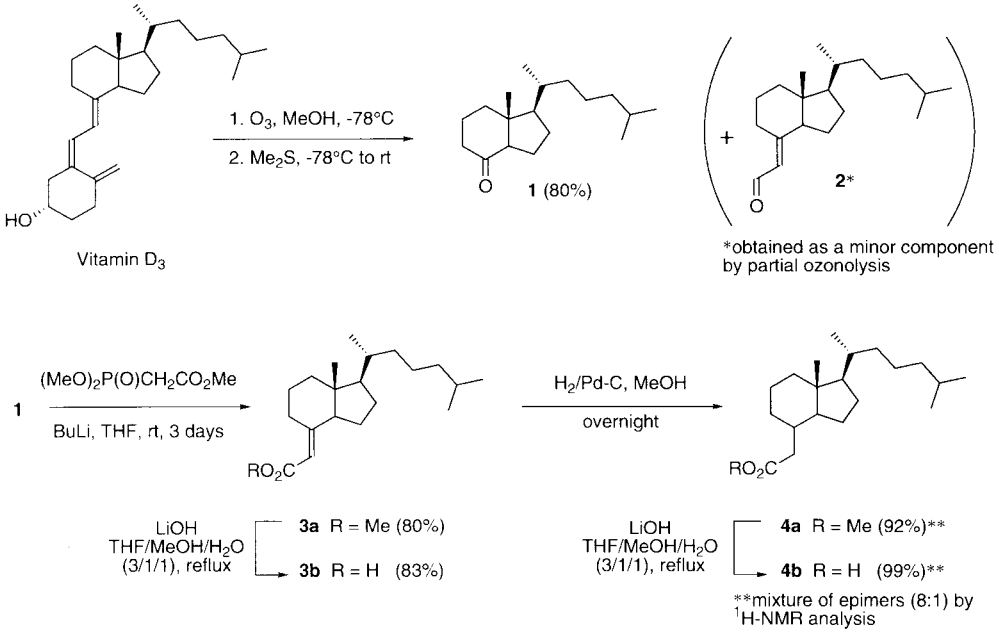


Figure 1.



Scheme 1.

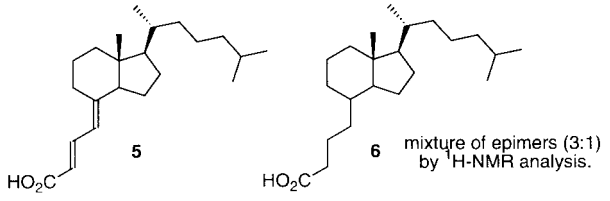


Figure 2.

Table 1. Inhibitory activity of cdc25A and antitumor activity[12,13]

Compound	cdc25A inhibition IC_{50} (μM)	Antitumor activity IC_{50} (μM)/SBC-5
3a	>300	71.0
3b	7.7	47.0
4a	>300	70.0
4b	32.0	>100
5	94.0	42.0
6	9.0	>100

G1 arrest. Interestingly, the methyl ester **3a** also caused G1 arrest, though it showed no cdc25A-inhibitory activity. Membrane-permeable **3a** might be metabolized by endogenous esterases to afford the active carboxylic acid **3b**.

The hydrophobic and hydrophilic substructure model turned out to be useful to design cdc25A inhibitors. It is also demonstrated that perhydroindan framework, an easily available intermediate from Vitamin D_3, is quite effective to construct such molecules. While this project was undergoing, Zalkow and co-workers reported the synthesis of **7**, a potent inhibitor of cdc25A having characteristic nitrile side chain (Fig. 4).[15] We believe our strategy should open the easy access to the future cdc25A inhibitors with minimal structure. Exploration of the structure–activity relationship is continuing.

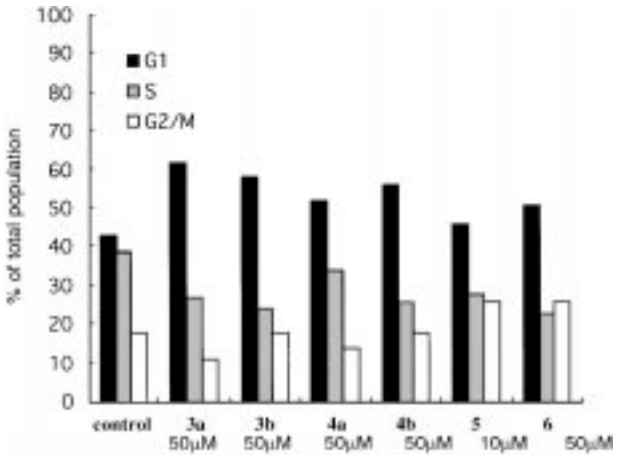
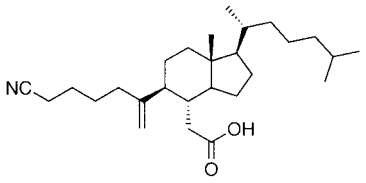


Figure 3. Flow cytometric analysis of HL60 cells. G1 Arrest on cell cycle effected by the drugs.



7
IC_{50}=2.2 μM

Figure 4.

Acknowledgements

We are grateful to Professor T. Tsuruo and Dr. A. Tomida (Institute of Molecular and Cellular Biosciences, The University of Tokyo) for helpful discussions about the flow cytometric analysis. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan.

References and Notes

1. Galaktionov, K.; Beach, D. *Cell* **1991**, *67*, 1181.
2. Jinno, S.; Suto, K.; Nagata, A.; Igarashi, M.; Kanaoka, Y.; Nojima, H.; Okayama, H. *EMBO J.* **1994**, *13*, 1549.
3. Hoffmann, I.; Draetta, G.; Karsenti, E. *EMBO J.* **1994**, *13*, 4302.
4. Galaktionov, K.; Lee, A. K.; Eckstein, J.; Draetta, G.; Meckler, J.; Loda, M.; Beach, D. *Science* **1995**, *269*, 1575.
5. Gasparotto, D.; Maestro, R.; Piccin, S.; Vukosavljievic, T.; Barzan, L.; Sulfaro, S.; Boiocchi, M. *Cancer Res.* **1997**, *57*, 2366.
6. Gunasekera, S. P.; Kelly-Broger, M.; Lobkobsky, E.; Clardy, J. *J. Am. Chem. Soc.* **1996**, *118*, 8759.
7. Takahashi, M.; Dodo, K.; Hashimoto, Y.; Shirai, R.; *Tetrahedron Lett.*, in press.
8. Corey, E. J.; Roberts, B. E. *J. Am. Chem. Soc.* **1997**, *119*, 12425.
9. Boukouvalas, J.; Cheng, Y.; Robichaud, J. *J. Org. Chem.* **1998**, *63*, 228.
10. Magnuson, S. T.; Sepp-Lorenzino, L.; Rosen, N.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1998**, *120*, 1615.
11. ^{1}H NMR (500 MHz, $CDCl_3$) data are as follows: **3a** (clear oil): δ 5.47 (1H, s), 3.82–3.87 (1H, m), 3.69 (3H, s), 2.10 (1H, t, J=8 Hz), 2.02 (1H, br d, J=6 Hz), 0.93 (3H, d, J=6 Hz), 0.87 (3H, d, J=7 Hz), 0.86 (3H, d, J=7 Hz), 0.57 (3H, s). **3b** (colorless crystals, mp. 117–118 °C): δ 5.50 (1H, s), 3.81–3.87 (1H, m), 2.13 (1H, t, J=7 Hz), 2.03 (1H, br d, J=6 Hz), 0.93 (3H, d, J=6 Hz), 0.87 (3H, d, J=7 Hz), 0.86 (3H, d, J=7 Hz), 0.59 (3H, s).
12. cdc25A phosphatase assay: catalytic domain protein of human cdc25A (283–523 aa) was produced from *Escherichia coli* strain DH5α using pGEX-2T glutathione-*S*-transferase (GST)-fusion protein expression vector (Pharmacia) according to the instructions provided by the manufacturer. Phosphatase activity of cdc25A was assayed in 100 μL of buffer containing 10 mM HEPES (pH 8.0), 50 mM NaCl, and 1 mM dithiothreitol (DTT), with 10 mM *p*-nitrophenol phosphate (Sigma) as a substrate, using 96-well microtiter plates.
13. In vitro antitumor activity evaluation: SBC-5 (human non-small lung carcinoma cell line) cells were plated at the density of 1×10^3/well in flat-bottomed 96-well microplates and cultured overnight. The cells were incubated with the test compound at various concentrations for 72 h. The IC_{50} value was defined as the drug concentration needed to cause 50% inhibition of cell growth with respect to the control.
14. Flow cytometric analysis: HL60 cells (5×10^5 cells/mL) were treated with compounds for 20 h at 10 μM (**5**) or 50 μM (others) concentration. Cell cycle distributions were determined by using a Becton Dickinson fluorescence-activated cell analyzer. Data were interpreted using the ModFit LT software provided by the manufacturer. Compound **5** induced apotosis at 50 μM concentration.
15. Peng, H.; Zalkow, L. H.; Abraham, R. T.; Powis, G. *J. Med. Chem.* **1998**, *41*, 4677.