

植物来源的蛋白磷酸酶 Cdc25C 的新抑制剂^{*}

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摘要: 蛋白磷酸酶 Cdc25C 能够使有丝分裂激酶 CDK1/cyclin B 去磷酸化, 从而促进细胞周期的进程。已经在一些肿瘤细胞中检测到 Cdc25C 的过量表达, 这使得 Cdc25C 成为肿瘤治疗中的潜在靶标。通过随机筛选, 发现了八个 Cdc25C 的天然新抑制剂 (1-8), 其 IC₅₀ 值在 1.66~75.07 μmol/L 之间。肿瘤细胞毒试验结果表明, 其中四个化合物 (化合物 3, 4, 5, 7) 对十种肿瘤细胞株显示一定的细胞毒活性, 其 IC₅₀ 值皆小于 10 μg/mL。

关键词: Cdc25C; 磷酸酶; 天然抑制剂; 细胞周期; 肿瘤

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New Inhibitors of Dual-specificity Protein Phosphatase Cdc25C from Plants^{*}

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Abstract: Protein phosphatase Cdc25C dephosphorylates the mitotic kinase CDK1/cyclin B, and then triggers the cell cycle progression. Overexpression of Cdc25C is detected in some cancer cells, which made it to be a potential target for anti cancer drugs. Through Cdc25C assay, compounds 1-8 were found to be new natural inhibitors against Cdc25C with IC₅₀s of 1.66-75.07 μmol/L. Moreover, 3, 4, 5 and 7 exhibited cytotoxicities on a panel of ten cancer cell lines with IC₅₀s below 10 μg/mL.

Key words: Cdc25C; Phosphatase; Natural inhibitor; Cell cycle; Cancer

Common features shared by all cancers are a disordered cell cycle and irregularities in the molecules that control this cycle (Kristjansdottir and Rudolph, 2004). In eukaryotic cells, cell cycle progression is controlled by cyclin dependent kinases (CDKs), which are maintained in an inactive state through phosphorylation by Wee1/Mik1/Myt1 protein kinases and in an

active state through dephosphorylation by protein phosphatases Cdc25s (Ham *et al.*, 1998; Donzelli and Draetta, 2003). Cdc25s are a family of dual-specificity protein phosphatases, which contain three homologues named Cdc25A, Cdc25B and Cdc25C in human cells. Each homologue controls cell cycle progression at distinct checkpoints to promote cell proliferation (Eck-

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stein, 2000; Tamura *et al.*, 2000). Cdc25A is mainly thought to activate CDK2/cyclin E and thereby triggers the G1/S transition of the cell cycle. Cdc25B appears to play a role in both G1 and G2 phases, while Cdc25C specifically dephosphorylates CDK1/cyclin B and triggers the G2/M transition (Loukaci *et al.*, 2001). For their important role in cell cycle regulation, Cdc25s have been attractive targets for drug development (Sohn *et al.*, 2003; Prevost *et al.*, 2003). Inhibitors of these enzymes may become potential chemotherapeutic agents in the field of tumor treatment. Several natural and synthetic compounds were found to be inhibitors of Cdc25s, such as dysidiolide and its analogs (Gunasekera *et al.*, 1996; Shimazawa *et al.*, 2004), vitamin K3 and quinone derivatives (Ham *et al.*, 1997; Cao *et al.*, 2005; Lavergne *et al.*, 2006), sulfircin (Cebula *et al.*, 1997) and vanadate (Huyer *et al.*, 1997) etc.

In our program of searching for inhibitors of Cdc25C, hundreds of natural products in our compound library were screened. Results indicated that compounds **1–8** (Fig. 1) demonstrated inhibitory activities against Cdc25C, and **3**, **4**, **5** and **7** also showed cytotoxicities on several cancer cell lines.

1 Materials and methods

1.1 Materials

Cdc25C was presented by Bayer AG (Germany). Substrate O-methyl fluorescein phosphate and product fluorescent O-methyl fluorescein are from Sigma (M 2629, M 7004). Vanadium oxide (V_2O_5) (Sigma, V-6881) was used as a reference compound for Cdc25C assay. Human cancer cell lines A549 (lung cancer), BGC 823 (gastric cancer cell line), SGC-7901 (gastric cancer cell line), DU-145 (prostate cancer cell line), MDA-MB 231 (breast cancer cell line), HT-29 (colon cancer cell line), BEL-7402 (hepatic cancer cell line), MCF-7 (breast cancer cell line) and U-251 (glioma) were purchased from the Cell Culture Centre of Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Perking, China).

1.2 Chemistry and Pharmacology

Compounds **1–8** (Fig. 1) were isolated from various plants by us with their purities > 95%. Detailed purifications and identifications of these compounds were described previously (Shang *et al.*, 1994; Mortenogro *et al.*, 2003; Peng *et al.*, 1990; Wang *et al.*, 2000; Xu *et al.*, 2005; Iwao *et al.*, 1985; Itokawa *et al.*, 1991). Inhibition data were expressed as IC_{50} values, which were calculated by dose response curves with at least four concentrations (dilution ratio = 1/2), the highest tested concentration for Cdc25C and cytotoxicity assay are 40 $\mu\text{g/mL}$ and 10 $\mu\text{g/mL}$ respectively. Results are expressed as mean IC_{50} values \pm standard deviations.

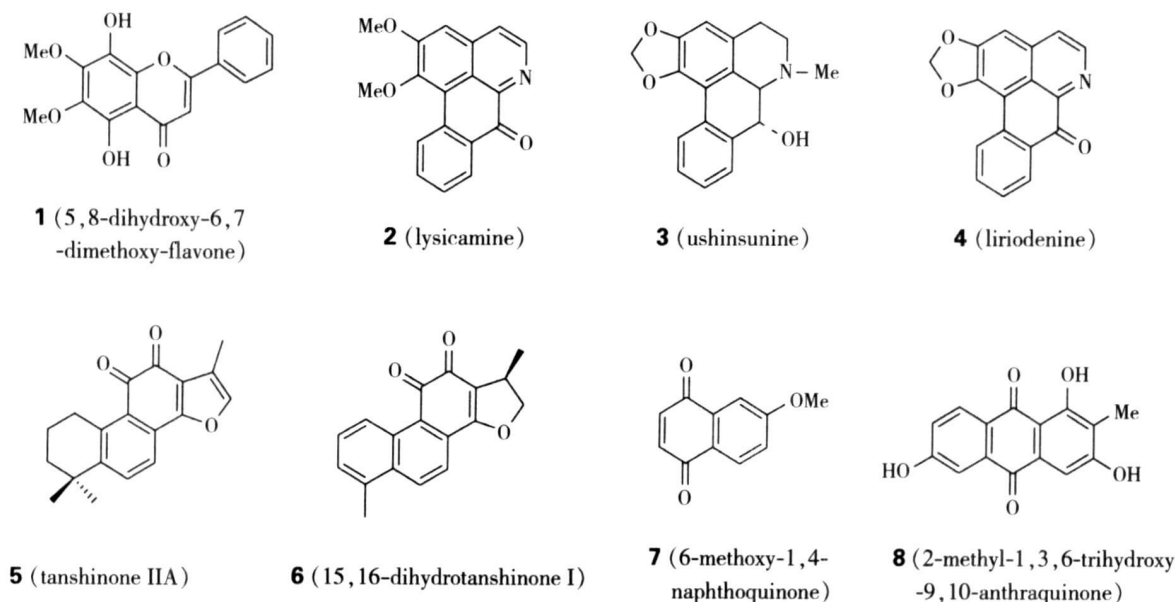


Fig. 1 Chemical structures of compounds **1–8**

Plant resources: 1. from *Fissistigma kwangsiense* (Annonaceae); 2. from *Uvaria tonkinensis* (Annonaceae); 3. from *Michelia yunnanensis* (Magnoliaceae); 4. from *Tsoongiodendron odorum* (Magnoliaceae); 5. from *Salvia castanea* f. *tomentosa* (Labiatae); 6. from *Salvia castanea* f. *tomentosa* (Labiatae); 7. from *Impatiens chungtienensis* (Balsaminaceae); 8. from *Sabina gaussonii* (Cupressaceae).

1.3 Cdc25C Assay

Compound inhibitory activity against Cdc25C was measured as the method described previously (Ducruet *et al.* , 2000) . Test compounds were diluted with the reaction buffer (pH 8.2, 100 mmol/L TrisBase, 40 mmol/L NaCl, 0.01% BSA, 5 mmol/L DTT and 20% Glycerol), then 1 mmol/L OMFP and 24 μg/mL enzyme were added to start the reaction. After incubation for 120 min at 25℃, fluorescence was monitored at 530/40 nm after excitation at 490/40 nm in a Cytofluor II fluorescence plate reader (Perseptive Biosystems) .

1.4 Cell Growth Inhibition Assay

All cancer cells were cultured in RPMI 1640 containing 10% fetal bovine serum and 5 CC Per Strep. Compound cytotoxicity was assessed by the sulforhodamine B (SRB) assay described before (Skehan *et al.* , 1990) . Firstly, 3000– 7000 cells/well were plated in 96 well plates. Twenty four hours later, compounds were added to a final concentration of 10 μg/mL. After incubated for 48 h, cells were fixed by the addition of 50% ice cold trichloroacetic acid and left at 4℃ for 1 h. Then Plates were washed 5 times in water, air dried, and stained for 15 min with 100 μl 0.4% SRB in 1% glacial acetic acid. Excessive dye was removed by washing 5 times in 1% glacial acetic acid. After plates were air dried, SRB was resuspended in 100 μl 10 mmol/L Tris and the OD values were read at 560 nm on a 96-well plate reader (Molecular Devices, SPECTRA MAX 340) .

2 Results and discussion

Through the inhibition assay, eight natural inhibitors containing flavones (1), alkaloids (2– 4) and quinone derivatives (5– 8) were found against Cdc25C (Fig. 1) with IC₅₀ values ranging from 1.66 to 75.07 μmol/L (Table 1) . The structure activity relationship of these compounds was found. In alkaloids, compound 2, which lacked methylenedioxy, was active with IC₅₀ values between compound 3 and 4, which suggested

that methylenedioxy is not necessary for Cdc25C inhibitory activity. But compared with compound 4, saturation of compound 3 was important for its activity. Compound 5 and 6 contain naphthoquinone structures, which may be the reason for their inhibitory activities against Cdc25C. Compound 7 and 8 belong to quinone derivatives, which are proved to be the potent inhibitors of Cdc25 family. Further studies towards structure activity relationship are in progress. Result from cell growth inhibition assay indicated that compounds 3, 4, 5 and 7 showed cytotoxicities with IC₅₀ values below 50 μmol/L (Table 2) . It suggested that these compounds may exert anti-tumor activity by blocking cell cycle progression. Especially, compound 7 inhibited cell proliferation in a broad spectrum of cancer cell lines, in which DU145 was the most sensitive one with IC₅₀ of 8.83 μmol/L, while compound 4 selectively affected A549 with IC₅₀ of 20.44 μmol/L.

Compounds inhibiting Cdc25 dual specificity phosphatase activities might be as potent anticancer agents. With random screening, we found eight new natural inhibitors of Cdc25C, which broaden the structural diversity of Cdc25 inhibitors. Moreover, some inhibitors exhibited efficacious and broad anti-tumor activities in various cancer cell lines. All the results sug-

Table 1 Inhibitory activities of 1–8 against Cdc25C

Comp.	IC ₅₀ (μmol/L)	Comp.	IC ₅₀ (μmol/L)
1	19.46±1.11	5	41.84±2.41
2	11.82±0.93	6	15.61±0.72
3	1.66±2.24	7	12.50±2.29
4	25.27±2.98	8	75.07±3.48
V ₂ O ₅	0.44±0.11		

IC₅₀ values are means of three experiments.

Table 2 Cytotoxicities of compounds 1–8 against cancer cell lines

Cell lines	IC ₅₀ (μmol/L)							
	1	2	3	4	5	6	7	8
A549	NA	NA	17.76±2.20	20.44±2.69	4.63±0.95	NA	17.50±2.39	NA
BGC-823	NA	NA	26.78±3.08	NA	7.11±1.16	NA	18.78±2.45	NA
SGG-7901	NA	NA	NA	NA	NA	NA	NA	NA
DU145	NA	NA	NA	NA	26.90±1.80	NA	8.83±1.54	NA
MDA-MB-231	NA	NA	19.63±2.78	NA	NA	NA	NA	NA
HF-29	NA	NA	NA	NA	NA	NA	33.83±4.15	NA
BEL-7402	NA	NA	NA	NA	NA	NA	21.06±2.50	NA
MCF-7	NA	NA	NA	NA	16.77±1.90	NA	15.74±1.33	NA
U251	NA	NA	NA	NA	22.62±3.27	NA	NA	NA

The IC₅₀ values are means of three experiments; NA, not active

gested that these new natural inhibitors for Cdc25C will facilitate the development of potential chemotherapeutic agents in tumor prevention.

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