



Study on the Synthesis and PKA-I Binding Activities of 5-Alkynyl Tubercidin Analogues

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Abstract—5-Alkynyl tubercidin analogues were synthesized and their biological activities were evaluated. It was found that protein kinase A could be activated by 5-alkynyl tubercidin (**9a**) and cAMP-binding ability to PKA-I was selectively inhibited by it. Molecular modeling showed that the interaction of **9a** and PKA-I was associated with the existence of hydrophobic alkynyl group. During the synthesis of tubercidin analogues, a pair of 2'-carbonyl participating abnormal coupling products (**11a**, **11b**) was obtained, the structure was identified by X-ray crystalline diffraction. © 2002 Elsevier Science Ltd. All rights reserved.

Introduction

It was discovered that malfunctions of cellular signaling have been associated with many diseases including cancer and diabetes.^{1–3} Many therapeutic strategies have been developed through the design and synthesis of compounds which activate or inactivate protein kinases. Cyclic adenosine monophosphate (cAMP) dependent pathway is one of the major signal transduction pathways in mammalian cells. cAMP is called second messenger, it acts by binding to the regulatory subunits of protein kinase A (PKA). PKA is a tetramer composed of a regulatory subunits (R) dimer and two catalytic (C) subunits. The regulatory subunits of isozyme I and II (RI and RII, respectively) are dissimilar. RI subunit overexpresses in cancer cell lines and tumor specimens, decreasing RI/RII ratios and/or down-regulation of RI correlate with growth inhibition and cellular differentiation.^{4–6}

Regulations of signal transduction by synthetic molecules have been subjects of intense research in the industrial setting as well as in academics. Several studies have demonstrated that different antisense oligonucleotides targeting the RI α subunit of PKA-I expression causes cell growth arrest and differentiation in a variety of cancer cell lines.^{7,8} It has also been shown that the selective regulation of PKA-I or PKA-II by site selective

cAMP analogues leads to inhibition of cancer cell growth in a wide variety of cancer cell types in vitro and in vivo. From amongst those cAMP analogues, 8-Cl-cAMP, in particular, has proven strong antitumor activity and currently is in clinical Phase II trials as an anticancer agent, and it is suggested as a potential partner for co-treatment with classical chemotherapeutics.⁴ Upon on the intensive investigation, it has found that 8-Cl-cAMP is relatively easy to be metabolized in vivo, and the metabolite 8-Cl-adenosine also demonstrates very active antitumor activity. Though the action mode of 8-Cl-adenosine is not well understood, it is reported to decrease the RI-subunit of PKA in lung epithelial cells and also induce HL-60 promyelocytic leukemia cells apoptosis.^{9,10} The biological activity of 8-Cl-adenosine reveals that nucleoside might also modulate protein kinase behavior as its cyclic nucleotide does, and encourage us to find other more active nucleoside which target the signal pathway.

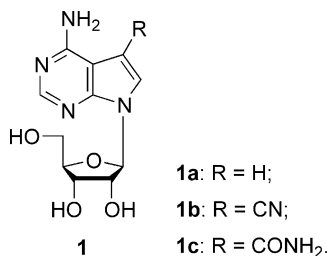
Tubercidin (7-deaza-adenosine) (**1a**) as well as 5-substituted derivatives—toyocamycin (**1b**) and sangivamycin (**1c**)—are naturally occurring antibiotics (Scheme 1),¹¹ their analogues have been synthesized in the past years, some of them exhibited antitumor activities and showed mild effect on PKA activity.^{12–15} In this study, a hydrophobic alkynyl group was introduced to the 5-position of tubercidin and the designed compound **9** was synthesized and the biological activities were evaluated primarily.

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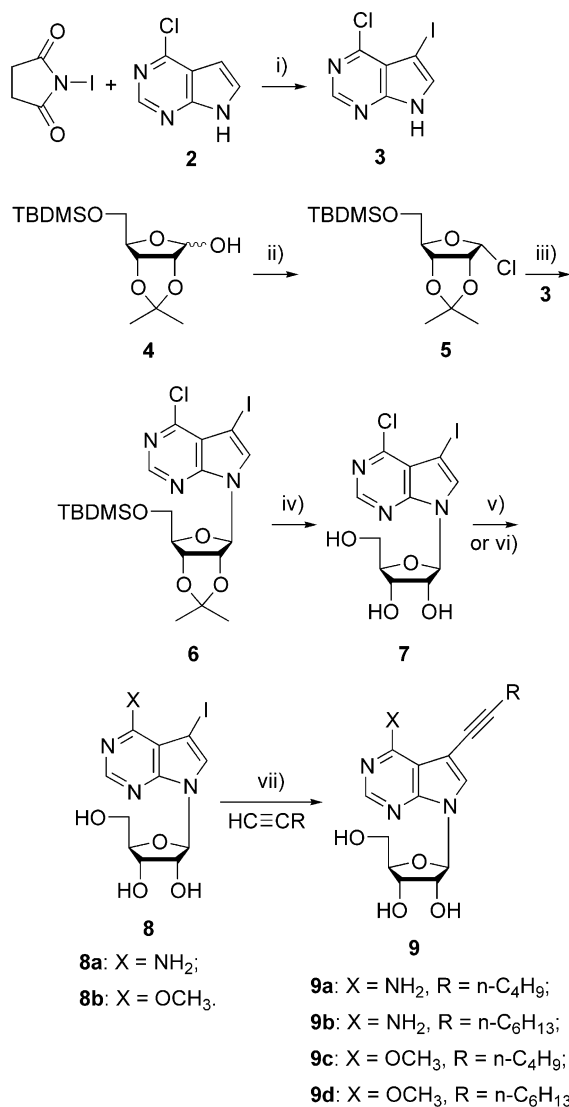
Results and Discussion

Synthesis

5-alkynyl tubercidin analogues (**9**) were synthesized via the routine as demonstrated in Scheme 2. 4-Chloro-5-iodo-7H-pyrrolo[2,3-*d*]pyrimidine (**3**) reacted with freshly prepared 1-chloro-5-*O*-*tert*-butyldimethylsilyl-2,3-*O*-isopropylidene- α -D-ribofuranose (**5**) to give 4-chloro-5-iodo-(5'-*O*-*tert*-butyldimethylsilyl-2',3'-*O*-isopropylidene- β -D-ribofuranosyl)-7H-pyrrolo[2,3-*d*]pyri-



Scheme 1.

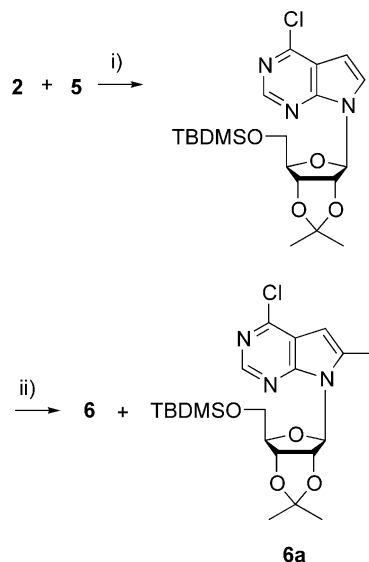


Scheme 2. (i) NaH, rt, 20 h; (ii) CCl₄, P(NMe₂)₃, -78 °C, 1 h; (iii) NaH, rt, 24 h; (iv) CF₃COOH, rt, 1 h; (v) NH₃/CH₃OH, 110 °C, 22 h; (vi) K₂CO₃/MeOH, rt, 12 h; (vii) Pd(Ph₃)₄, CuI, DMF, rt, 16 h.

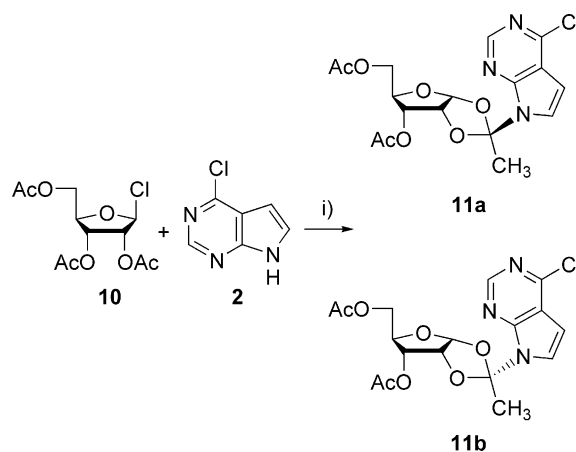
midine (**6**) and α -anomer was also generated (α/β 1:12). **6** was treated with CF₃COOH and the deprotected product **7** was obtained almost quantitatively. Amino-lyzing **7** with saturated NH₃/CH₃OH, then alkynylating with 1-hexyne or 1-octyne under the existence of Pd(PPh₃)₄ and CuI, the alkynylated product **9a** and **9b** were obtained in high yields. In another case, if **7** was hydrolyzed in methanol in the presence of K₂CO₃, compound **8b** was formed, the alkynylated product **9c** and **9d** could be obtained (Scheme 2).

For the synthesis of compound **7**, protected α -D-ribofuranosyl chloride **5** was coupled with 4-chloro-7H-pyrrolo[2,3-*d*]pyrimidine (**2**) followed by iodolization with ICl. Unfortunately, a mixture of **6** and **6a** was generated (Scheme 3). The result indicated that the ribosyl group affected the nucleophilicity of C-5 and C-6 in tubercidin analogue.

When using acetyl as the protecting group in β -D-ribofuranosyl chloride (**10**) for the coupling reaction with 4-chloro-7H-pyrrolo[2,3-*d*]pyrimidine (**2**), an abnormal



Scheme 3. (i) NaH, MeCN, rt, 24 h; (ii) ICl, rt, 72 h.



Scheme 4. (i) NaH, CH₂Cl₂, rt, 48 h.

coupling reaction was observed (Scheme 4). **10** was reacted with **2** in dichloromethane at room temperature under the existence of NaH, two compounds were obtained with the yield of 41% (**11a**) and 21% (**11b**), respectively. NMR results demonstrated that neither **11a** nor **11b** were consistent with the desired product. ^{13}C NMR showed that only two carbonyl groups existed in both compounds and a new peak appeared at δ 114.9 (**11a**) or 115.8 (**11b**). HMBC and HMQC identified that this carbon was connected to a methyl group. After **11a** or **11b** was treated with saturated ammonia methanol solution, two signals of carbonyl groups disappeared in ^{13}C NMR but signal of 114.9 or 115.8 still existed in the deprotected product. All spectrometric data of **11a** or **11b** were consistent with the structure in which the heterocyclic base was connected to the carbon of 1,2-*O*-ethylidene moiety of 3,5-diacetyl-ribose. A crystalline sample of **11a** was obtained from petroleum–dichloromethane solution, and its X-ray diffraction results confirmed the structure and showed that new chiral center, C-7 of **11a**, was in *S* configuration (Fig. 1). Since the spectrometric data of **11b** was nearly identical to **11a**, it should be a stereo isomer of **11a** with *R* configuration of its C-7.

The formation of **11a** and **11b** could be explained by the neighboring participation effect of acetyl group. The heterocyclic base attacked the carbon of the cation intermediate (path **a**) instead of the ribosyl C-1 carbon (path **b**) (Fig. 2). This abnormal coupling reactions were occasionally observed when weak nucleophilic hetero-

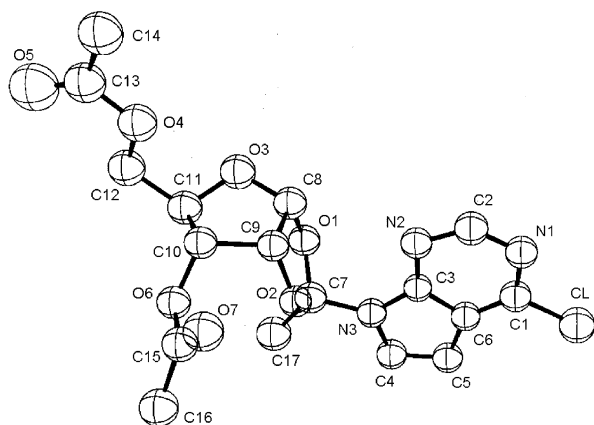


Figure 1. ORTEP drawing of X-ray diffraction structure of compound **11a**.

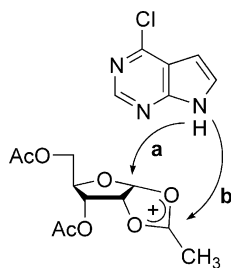


Figure 2. Schematic representation of neighboring acetic group participating effect on formation of normal (path **a**) and abnormal (path **b**) products.

cyclic bases were used as reactants. Similar coupling products were reported by Cristalli and Revankar.^{16,17} De Clercq also proposed an abnormal coupling structure in 1995, in which an O-5 carbonyl other than O-2 carbonyl participating product was suggested.¹⁸ Crystal structure presented in this work clarified the O-2 carbonyl neighboring participation mechanism.

Biological evaluation

Compounds **9a–d** and **11a–b** were screened by culture of tumor cells, **9a** and **9b** exhibited relatively high inhibition effects on the proliferation of KB, Hela and Bel-7402 cells (Table 1), otherwise, **9c**, **9d**, **11a** and **11b** did not show obvious effects on cell growth. It indicated that the tubercidin substituted skeleton and the 4-amino group was essential to the inhibition activities of tumor cell growth.

The effects on protein kinase A activity by compound **9a** in the concentration range of 0.1–10 μM was investigated (Fig. 3). The results showed that PKA was activated by **9a**, which was in accordance with most cyclic-AMP derivatives and 8-Cl-adenosine, it seemed that **9a** acted in the similar way as they did. Whereas it was reported that toyocamycin and tubercidin inhibited PKA activity with IC_{50} of 20 and >100 μM , respectively,¹⁴ it indicated that **9a** and tubercidin affected PKA activity in a different way. The V-shaped variation of PKA activities might be resulted from the binding differences of **9a** to PKA-I and PKA-II. The selective binding ability of compound **9a** to PKA was examined by competitive cAMP-[^3H] binding assay (Fig. 4). The result showed that **9a** could inhibit cAMP binding to PKA-I very effectively and exhibited concentration dependence, meanwhile no obvious difference to control was observed in PKA-II case. It might be concluded that the alkynyl tubercidin could target to PKA-I selectively.

Table 1. Growth inhibition of tubercidin and its derivatives to various tumor cells

Compound	IC_{50} (μM)		
	KB	Hela	Bel-7402
9a	0.3	0.4	0.55
9b	0.1	0.2	0.25
Tubercidin	1.8	0.45	0.25

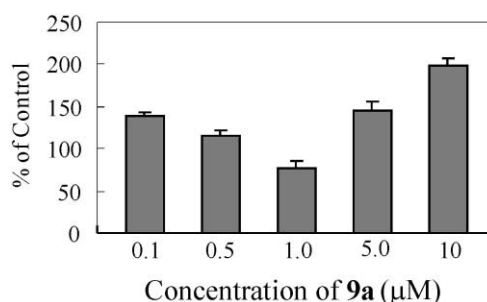


Figure 3. Effect of compound **9a** on PKA activity ($p < 0.05$).

For understanding the mechanism more intensively, molecular modeling was used to simulate the interaction of 5-alkynyl tubercidin with PKA. Three-dimensional structure of the regulatory subunit R_{α} of PKA clearly showed that in the cAMP binding site there was a relatively large hydrophobic area next to N-7 position of cAMP. If the cAMP was replaced by 5-alkynyl tubercidin, the alkynyl group could fit this pocket tightly by the hydrophobic interactions with the adjacent amino acid residues, such as Leu 112 (Fig. 5). It implied that the hydrophobic substitution at 5-position of tubercidin could be contributed to the selective binding activity to PKA-I. It would be interesting to provide a strategy for the development of new antitumor candidates from the structural modification of tubercidin.

Experimental

Materials and methods

D-Ribose, 1-hexyne, 1-octyne, $\text{Pd}(\text{PPh}_3)_4$ and other main materials were purchased from ACROS. CH_3CN , pyridine, CH_2Cl_2 , DMF were dried by distillation from CaH_2 . Chromatographic purifications were carried out using silica gel (200–400 mesh, Qingdao Chemicals).

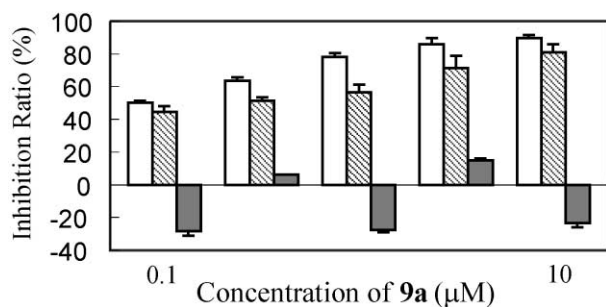


Figure 4. Effect of compound **9a** on cAMP binding with PKA (blank), PKA-I (cross) and PKA-II (gray) ($p < 0.05$).

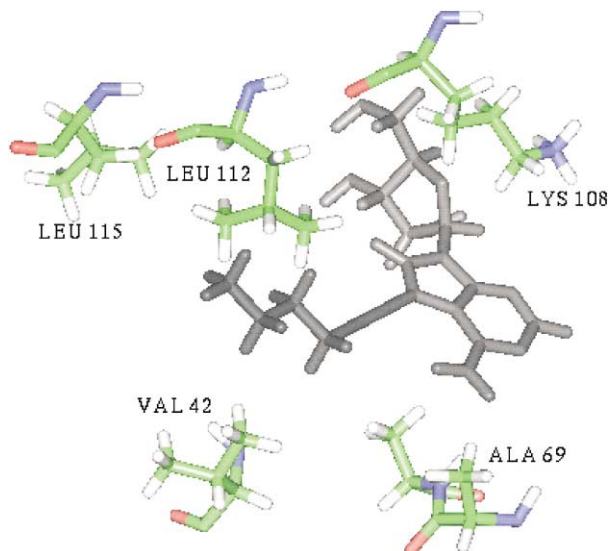


Figure 5. Molecular modeling showing the interaction of compound **9a** with amino acid residues of PKA-I $_{\alpha}$.

4-Chloro-5-iodo-7H-pyrrolo[2,3-*d*]pyrimidine (**3**) was synthesized according to refs 19 and 20. 4-Chloro-5-iodo-(β -D-ribofuranosyl)-7H-pyrrolo[2,3-*d*]pyrimidine (**7**) was synthesized according to references starting from the coupling of 5-*O*-*tert*-butyldimethylsilyl-2,3-*O*-isopropylidene-D-ribofuranose (**4**) and compound **3**.^{12,21} PKA assay kit (Spinzyme formate) was purchased from Pierce. cAMP-[^3H] assay kit was purchased from the Institute of Atomic Power of China.

NMR spectra were recorded on Varian 300 MHz spectrometer. Mass spectra were obtained on ZAB-HS and HRMS were recorded on APEX II FTICR (Bruker) mass spectrometer. Optical rotation was measured on Perkin-Elmer 243B polarimeter. Elemental analyses were performed on PE-240C analyzer.

Molecular modeling

Molecular modeling was performed on SGI Indy workstation and MSI Insight II suite was used. Three-dimensional structure of PKA-I R_{α} subunit was from Weber's theoretical model (Protein Data Bank entry 1APK).²² Tubercidin derivative was placed to PKA-I R_{α} cAMP binding site by modifying cAMP structure, and was subjected to molecular mechanics energy minimization (1000 steps steepest descent and 1000 steps conjugate gradient) consequently. In the computation, the amino acid residues which were 5 Å away from tubercidin derivative were fixed and others were unrestrained, and the cvff forcefield was used.

4-Amino-5-iodo-7-(β -D-ribofuranosyl)-7H-pyrrolo[2,3-*d*]pyrimidine (8a**).** Compound **7** (475 mg, 1.15 mmol) was dissolved in 50 mL of saturated $\text{NH}_3/\text{CH}_3\text{OH}$ solution. After stirred at 116 °C in a sealed vessel for 22 h, the mixture was evaporated and separated with silica gel column. 400 mg (yield 88.7%) of oil-like product was collected. MS/EI: 391 ($M-1$). ^1H NMR ($\text{DMSO}-d_6$, ppm). 8.09 (s, 1H, H-2); 7.66 (s, 1H, H-6); 6.68 (s, 2H, NH_2); 6.01 (d, 1H, $J_{1',2'} = 6.0$ Hz, H-1'); 4.34 (m, 1H, H-2'); 4.03 (m, 1H, H-3'); (m, 1H, H-4'); 3.57 (m, 2H, H-5').

4-Methoxy-5-iodo-7-(β -D-ribofuranosyl)-7H-pyrrolo[2,3-*d*]pyrimidine (8b**).** Compound **7** (440 mg, 1.0 mmol) was dissolved in 12 mL of CH_3OH . 300 mg (2.0 mmol) of K_2CO_3 was added and stirred at room temperature for 12 h. The mixture was evaporated and separated with silica gel column. 250 mg (yield 88.7%) of solid product was collected. Mp 212–213 °C (dec). MS/EI: 407 (M). ^1H NMR ($\text{DMSO}-d_6$, ppm). 8.44 (s, 1H, H-2); 7.88 (s, 1H, H-6); 6.13 (d, 1H, $J_{1',2'} = 6.0$ Hz, H-1'); 4.37 (m, 1H, H-2'); 4.05 (m, 4H, H-3', OCH_3); 3.95 (m, 1H, H-4'); 3.57 (m, 2H, H-5').

General procedure for the synthesis of 4-amino-5-alkynyl-7-(β -D-ribofuranosyl)-7H-pyrrolo [2,3-*d*] pyrimidine (9**).** 0.57 mmol of compound **7** was dissolved in 8 mL of anhydrous DMF, 5.7 mmol of 1-hexyne or 1-octyne, 0.16 mL (1.15 mmol) of triethyl amine and 75 mg (0.065 mmol) of $\text{Pd}(\text{PPh}_3)_4$ were added under Ar atmosphere. After the solution was stirred at room temperature for

16 h, the mixture was evaporated under reduced pressure. MeOH was added to dissolve residue and insoluble material was removed by filtration. Filtrate was evaporated and separated with silica gel column, the desired product was collected.

Compound 9a. Yield 56.8%. Mp 214–215 °C (dec). $[\alpha]_D^{19}$ (c 0.165, MeOH) –49.6. UV (MeOH): $\lambda_{\max}$ 277 nm (ϵ 11,000). ^1H NMR (CD_3OD , ppm). 8.07 (s, 1H, H-2); 7.48 (s, 1H, H-6); 5.97 (d, 1H, $J_{1',2'} = 6.3$ Hz, H-1'); 4.57 (m, 1H, H-2'); 4.26 (m, 1H, H-3'); 4.09 (m, 1H, H-4'); 3.70 (m, 2H, H-5'); 2.48 (t, 2H, $J = 6.6$ Hz, CCCH_2); 1.63–1.46 (m, 4H, CH_2); 0.97 (t, 3H, $J = 7.2$ Hz, CH_3). HRMS calcd for $\text{C}_{17}\text{H}_{22}\text{N}_4\text{O}_4$ (M+1) 347.1713, found 347.1708.

Compound 9b. Yield 50.8%. Syrup. $[\alpha]_D^{19}$ (c 0.190, MeOH) –62.1. UV (MeOH): $\lambda_{\max}$ 276 nm (ϵ 7700). ^1H NMR (CD_3OD , ppm). 8.05 (s, 1H, H-2); 7.47 (s, 1H, H-6); 5.97 (d, 1H, $J_{1',2'} = 6.3$ Hz, H-1'); 4.56 (m, 1H, H-2'); 4.25 (m, 1H, H-3'); 4.08 (m, 1H, H-4'); 3.81 (m, 2H, H-5'); 2.45 (t, 2H, $J = 6.9$ Hz, CCCH_2); 1.62–1.30 (m, 4H, CH_2); 0.91 (t, 3H, $J = 6.9$ Hz, CH_3). HRMS calcd for $\text{C}_{19}\text{H}_{26}\text{N}_4\text{O}_4$ (M+1) 375.2026, found 375.2022.

Compound 9c. Yield 54.3%. Mp 123–125 °C (dec). $[\alpha]_D^{19}$ (c 0.245, MeOH) –61.1. UV (MeOH): $\lambda_{\max}$ 272 nm (ϵ 7500). MS/EI: 361 (M). ^1H NMR (CD_3OD , ppm). 8.44 (s, 1H, H-2); 7.87 (s, 1H, H-6); 6.12 (d, 1H, $J_{1',2'} = 6.0$ Hz, H-1'); 4.36 (m, 1H, H-2'); 4.07 (m, 4H, H-3', OCH_3); 3.90 (m, 1H, H-4'); 3.63 (m, 2H, H-5'); 2.44 (t, 2H, $J = 6.6$ Hz, CCCH_2); 1.50 (m, 4H, CH_2); 0.94 (t, 3H, $J = 6.6$ Hz, CH_3). Anal. calcd for $\text{C}_{18}\text{H}_{23}\text{N}_3\text{O}_5$: C 59.82, H 6.41, N 11.63; found C 59.68, H 6.49, N 11.47.

Compound 9d. Yield 46.0%. Mp 119–123 °C (dec). $[\alpha]_D^{19}$ (c 0.190, MeOH) –48.4. UV (MeOH): $\lambda_{\max}$ 268 nm (ϵ 7500). MS/EI: 389 (M). ^1H NMR (CD_3OD , ppm). 8.44 (s, 1H, H-2); 7.86 (s, 1H, H-6); 6.12 (d, 1H, $J_{1',2'} = 6.0$ Hz, H-1'); 4.22 (m, 1H, H-2'); 4.09 (m, 4H, H-3', OCH_3); 3.91 (m, 1H, H-4'); 3.56 (m, 2H, H-5'); 2.43 (t, 2H, $J = 6.6$ Hz, CCCH_2); 1.55–1.31 (m, 8H, CH_2); 0.89 (t, 3H, $J = 6.6$ Hz, CH_3). Anal. calcd for $\text{C}_{20}\text{H}_{27}\text{N}_3\text{O}_5$: C 61.68, H 6.99, N 10.79; found C 61.79, H 7.14, N 10.58.

3,5-Di-O-acetyl-1,2-O-(methyl-1-(4-chloro-pyrrolo[2,3-d]-pyrimidin-7-yl) methylene)- α -D-funarose (11). 31 mg (0.2 mmol) of 4-chloro-7H-pyrrolo[2,3-d]pyrimidine was suspended in 5 mL of CH_2Cl_2 , then 46 mg (1.0 mmol) of 52% NaH was added. After stirred at room temperature for 30 min, 0.32 g (1 mmol) of 1-chloro-2,3,4-tri-O- β -D-ribose (**9**) (dissolved in 5 mL of CH_2Cl_2) was added and stirred at room temperature for 48 h. After evaporation, ethyl acetate was used to extract the residue for three times (5 mL each). Ethyl acetate solution was combined and separated with silica gel chromatography, 34 mg of **11a** (yield 41.0%) and 17 mg of **11b** (yield 20.5%) were collected, respectively.

Compound 11a. Mp 115–117 °C (dec). $[\alpha]_D^{19}$ (c 0.175, MeOH) +70.8. UV (MeOH): $\lambda_{\max}$ 268 nm (ϵ 6200). MS/FAB: 412 (M+H). ^1H NMR (300 MHz, $\text{DMSO}-d_6$, ppm): 8.69 (s, 1H, H-2); 7.79 (d, 1H, $J_{6,5} = 2.8$ Hz, H-6);

6.66 (d, 1H, $J_{5,6} = 2.8$ Hz, H-5); 6.21 (d, 1H, $J_{1',2'} = 4.2$ Hz, H-1'); 4.93 (m, 1H, H-2'); 4.83 (m, 1H, H-3'); 4.33 (m, 2H, H-4', H-5'); 4.13 (m, 1H, H-5'); 2.22 (s, 3H, CH_3); 2.11 (s, 3H, CH_3); 0.98 (s, 3H, CH_3). ^{13}C NMR ($\text{DMSO}-d_6$, ppm): 170.1, 169.6, 150.9, 150.4, 149.4, 128.1, 118.4, 114.9, 105.2, 98.6, 78.0, 76.0, 62.0, 24.6, 20.5, 20.4. Anal. calcd for $\text{C}_{17}\text{H}_{18}\text{ClN}_3\text{O}_7$: C 49.58, H 4.41, N 10.20; Found: C 50.00, H 4.44, N 10.26.

Compound 11b. Syrup. $[\alpha]_D^{19}$ (c 0.210, MeOH) +109.5. UV (MeOH): $\lambda_{\max}$ 269 nm (ϵ 4200). ^1H NMR (300 MHz, $\text{DMSO}-d_6$, ppm): 8.73 (s, 1H, H-2); 7.78 (d, 1H, $J_{6,5} = 3.9$ Hz, H-6); 6.71 (d, 1H, $J_{5,6} = 3.6$ Hz, H-5); 6.24 (d, 1H, $J_{1',2'} = 4.2$ Hz, H-1'); 5.18 (m, 1H, H-2'); 4.68 (m, 1H, H-3'); 3.95 (m, 3H, H-4', H-5'); 2.00 (s, 3H, CH_3); 1.98 (s, 3H, CH_3); 1.73 (s, 3H, CH_3). ^{13}C NMR ($\text{DMSO}-d_6$, ppm): 169.9, 169.3, 150.9, 150.6, 149.8, 127.8, 118.3, 115.8, 106.3, 98.5, 79.2, 77.9, 71.7, 62.8, 26.4, 20.5, 19.9. HRMS calcd for $\text{C}_{17}\text{H}_{18}\text{ClN}_3\text{O}_7$ (M+H) 412.0901, found 412.0898.

Measurement of biological behaviors

The inhibition of tumor cell growth was measured by SRB assay. PKA activity was measured using the colorimetric PKA assay kit.²³ The treated cell were washed with ice-cold PBS three times, resuspended in lysis buffer (50 mM Tris-HCl, 5 mM EDTA, 10 mM NaF, 10% glycerol, pH 7.2), sonicated, and centrifuged. Supernatants then were taken as lysate. PKA activity assays were performed following the manufacturer's instructions included with the colorimetric PKA assay kit. The cAMP contents were measured using the cAMP-[^3H] assay kit according to the manufacturer's instruction. PKA-I and PKA-II were separated with reference's method.^{24,25}

Acknowledgement

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References and Notes

- Sridhar, R.; Hanson-Painton, O.; Cooper, D. R. *Pharmaceutical Res.* **2000**, *17*, 1345.
- Hunter, T.; Plowman, G. D. *Trends Biochem. Sci.* **1997**, *22*, 18.
- Pawson, T. *Nature* **1995**, *373*, 573.
- Schwede, F.; Maronde, E.; Genieser, H.-G.; Jastorff, B. *Pharmacol. Ther.* **2000**, *87*, 199.
- Propper, D. J.; Saunders, M. P.; Salisbury, A. J.; Long, L.; O'Byrne, K. J.; Braybrooke, J. P.; Dowsett, M.; Taylor, M.; Talbot, D. C.; Ganesan, T. S.; Harris, A. H. *Clin. Cancer Res.* **1999**, *5*, 1682.
- Beebe, S. J. *Cancer Biol.* **1994**, *5*, 285.
- Cho-Chung, Y. S. *Pharmacol. Ther.* **1999**, *82*, 437.
- Tortora, G.; Caputo, R.; Damiano, V.; Bianco, R.; Pepe, S.; Bianco, A. R.; Jiang, Z.; Agrawal, S.; Ciardiello, F. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 12586.
- Lange-Carter, C. A.; Vuillequez, J. J.; Malkinson, A. M. *Cancer Res.* **1993**, *53*, 393.

10. Langeveld, C. H.; Jongenelen, C. A.; Heimans, J. J.; Stoof, J. C. *Cancer Res.* **1992**, 52, 3994.
11. Cristalli, G.; Franchetti, P.; Grifantini, M.; Nocentini, G.; Vittori, S. *J. Med. Chem.* **1989**, 32, 1463.
12. Rosemeyer, H.; Seela, F. *Helv. Chim. Acta* **1988**, 71, 1573.
13. Anderson, J. D.; Bontems, R. J.; Geary, S.; Cottam, H. B.; Larson, S. B.; Matsumoto, S. S.; Smee, D. F.; Robins, R. K. *Nucleosides Nucleotides* **1989**, 8, 1201.
14. Bobek, M.; Boloch, A. *Nucleosides Nucleotides* **1994**, 13, 429.
15. Seela, F.; Zulauf, M.; Chen, S.-F. *Nucleosides Nucleotides Nucleic Acids* **2000**, 19, 237.
16. Ramasamy, K.; Robins, R. K.; Revankar, G. R. *J. Heterocycl. Chem.* **1987**, 24, 863.
17. Cristalli, G.; Vittori, S.; Eleuteri, A.; Volpini, R.; Cola, D.; Camaioni, E.; Gariboldi, P. V.; Lupidi, G. *Nucleosides Nucleotides* **1993**, 12, 39.
18. Serafinowski, P.; Dorland, E.; Balzarini, J.; De Clercq, E. *Nucleosides Nucleotides* **1995**, 14, 545.
19. Davoll, J. J. *Chem. Soc.* **1960**, 131.
20. Bergstron, D.; Brattesani, D. *Nucleic Acids Res.* **1980**, 8, 6213.
21. Seela, F.; Zulauf, M. *Synthesis* **1996**, 726.
22. Weber, I. T.; Steitz, T. A.; Bubis, J.; Taylor, S. S. *Biochemistry* **1987**, 26, 343.
23. Huang, Y.-T.; Lin, J.-K.; Lee, M.-T. *Biochem. Pharmacol.* **1999**, 58, 1639.
24. Robinson-Steiner, A. M.; Corbin, J. D. *J. Biol. Chem.* **1983**, 258, 1032.
25. Tagliaferri, P.; Katsaros, D.; Clar, T.; Ally, S.; Tortora, G.; Neckers, L.; Rubalcava, B.; Parandoosh, Z.; Chang, Y. A.; Revankar, G. R.; Crabtree, G. W.; Robins, R. K.; Cho-Chung, Y. S. *Cancer Res.* **1988**, 48, 1642.