HETEROCYCLES, Vol. 89, No. 7, 2014, pp. 1606 - 1619. © 2014 The Japan Institute of Heterocyclic Chemistry
Received, 7th April, 2014, Accepted, 2nd May, 2014, Published online, 12th May, 2014
DOI: 10.3987/COM-14-12999

THE SYNTHESIS AND EVALUATION OF NEW CARBOCYCLIC PYRROLO[2,3-*d*]PYRIMIDINE NUCLEOSIDE ANALOGS

Jongbok Lee,[a] Hyewon Seo,[b] Sangeun Yoon,[a] Kowoon Choi,[b] Chul-Hoon Lee,[b]* and Hakjune Rhee[a,c]*

[a]Department of Bionanotechnology, Hanyang University, Sa 3-Dong 1271, Ansan, Kyunggi-Do, 426-791, Korea. E-mail: hrhee@hanyang.ac.kr
[b]Department of Pharmacy, Hanyang University, Sa 3-Dong 1271, Ansan, Kyunggi-Do, 426-791, Korea. E-mail: chhlee@hanyang.ac.kr
[c]Department of Chemistry and Applied Chemistry, Hanyang University, Sa 3-Dong 1271, Ansan, Kyunggi-Do, 426-791, Korea.

Abstract - New carbocyclic pyrrolo[2,3-*d*]pyrimidine nucleoside analogs were synthesized with the key intermediate, 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (**2**), by S_N2 reaction. One of the products, 4-amino-6-bromo-1-cyclopentyl-1*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (**9**), showed significant anti-proliferative activity to the human ovarian cancer PA-1 cells (IC_{50}: 3.9 μM). Based on the biological effects and the functional group characteristics of the compound **9**, other carbocyclic nucleoside analogs related to the compound **9** were synthesized with key intermediate **2** by a Pd(0)-catalyzed coupling reaction. As expected, *syn*-4-amino-6-bromo-7-[4-(methoxymethyl)-2-cyclopenten-1-yl]-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (**15**) showed very similar anti-proliferative activity (IC_{50}: 2.6 μM) when compared to compound **9**.

INTRODUCTION

Carbocyclic nucleosides are analogs of naturally occurring nucleosides in which a methylene group has replaced the oxygen atom of the furanose ring. These analogs have good metabolic stability because they are unaffected by hydrolases and phosphorylases that cleave the glycosidic bond of natural nucleosides.[1] Interestingly, the analogs are recognized by the same enzymes that recognize the natural nucleosides.[2] Carbocyclic nucleosides have received considerable attention as potential antiviral and antitumor agents, especially those with purine or pyrimidine bases.[2-4] For example, carbovir, the carbocyclic analog of 2',3'-didehydro-2',3'-dideoxyguanosine, has been the subject of intensive research

due to its selective anti-HIV-1 activity and hydrolytic stability.[5-8] Moreover, its congener abacavir, (±)-*cis*-4-[2-amino-6-(cyclopropylamino)-9*H*-purin-9-yl]-2-cyclopentene-1-methanol] demonstrates the importance of structure-activity relationship (SAR) studies because it has a higher oral bioavailability than carbovir and has been used in cocktail therapy for the treatment of AIDS.[9-12]

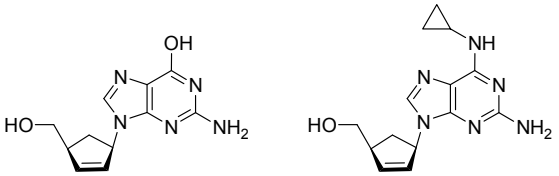


Figure 1. The structures of carbovir and abacavir

Pyrrolo[2,3-*d*]pyrimidine analogs have also attracted attention since toyocamycin and sangivamycin were isolated.[13-17] Sangivamycin, (4-amino-5-carboxamido-7-(D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine), isolated from *Streptomyces rimosus*, has antiviral and antitumor activity.[18,19] A number of its analogs have also been synthesized and evaluated as potential anticancer agents against a variety of human cancers such as mammary carcinoma and leukemia.[20-22] Although such analogs can inhibit the growth of various human cancer cells, none of them are used in a clinical setting because of their toxicity to normal human cells.[23] In order to overcome the weakness of these analogs, the selective inhibition of cancer cell growth must be achieved.

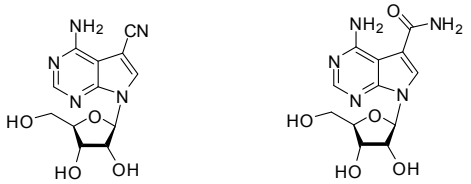


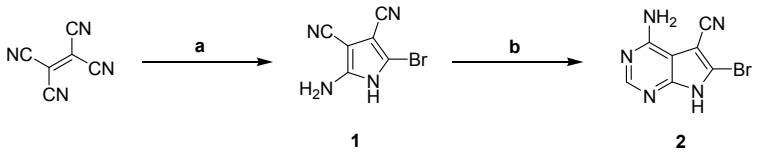
Figure 2. The structures of toyocamycin and sangivamycin

In the current study, we describe the synthesis of carbocyclic pyrrolo[2,3-*d*]pyrimidine nucleoside analogs and its biological activity.

RESULTS AND DISCUSSION

To synthesize carbocyclic pyrrolo[2,3-*d*]pyrimidine nucleoside analogs, the key intermediate, 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (**2**), was synthesized by previously reported methods.[24,25] 2-Amino-5-bromo-3,4-dicyanopyrrole (**1**) was prepared by the reaction of tetracyanoethylene in the presence of an excess amount of hydrogen bromide (**Scheme 1**). Compound **1** was reacted with foramidine acetate in 2-ethoxyethanol to give key intermediate **2** as a pale yellow solid which was used

without further purification for the next steps.[13,26] Although an improved method for the synthesis of compound **2** has been reported, the reaction was carried out under harsh conditions.[27]



1 2

Scheme 1. Synthesis of key intermediate **2**. Reagents and conditions:
(a) HBr in AcOH (33 wt%), acetone, EtOAc, 0 °C, 1.5 h, 53%;
(b) formamidine acetate, 2-ethoxyethanol, reflux, 36 h, 63%.

The reaction of key intermediate **2** with cyclopentyl bromide in the presence of sodium hydride as a base produced the two regioisomers **3** and **4** (**Scheme 2**). To analyze the two regioisomers (**3** and **4**), the nuclear Overhauser effect (NOE) between hydrogen on the 2-position of the pyrrolo[2,3-*d*]pyrimidine and hydrogens on the cyclopentyl ring was examined by 2-D NMR spectroscopy. The aromatic proton and protons on the cyclopentyl ring in the 1-substituted pyrrolo[2,3-*d*]pyrimidine (**3**) appeared as cross peaks in the NOESY spectrum, whereas the cross peaks in the 7-substituted pyrrolo[2,3-*d*]pyrimidine (**4**) were not detected because the two protons are spatially too far away. The structure of 1-substituted pyrrolo[2,3-*d*]pyrimidine (**3**) was also confirmed by X-ray crystallography (**Figure 3**).

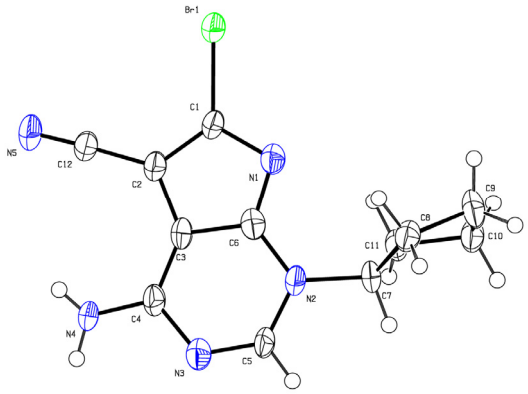
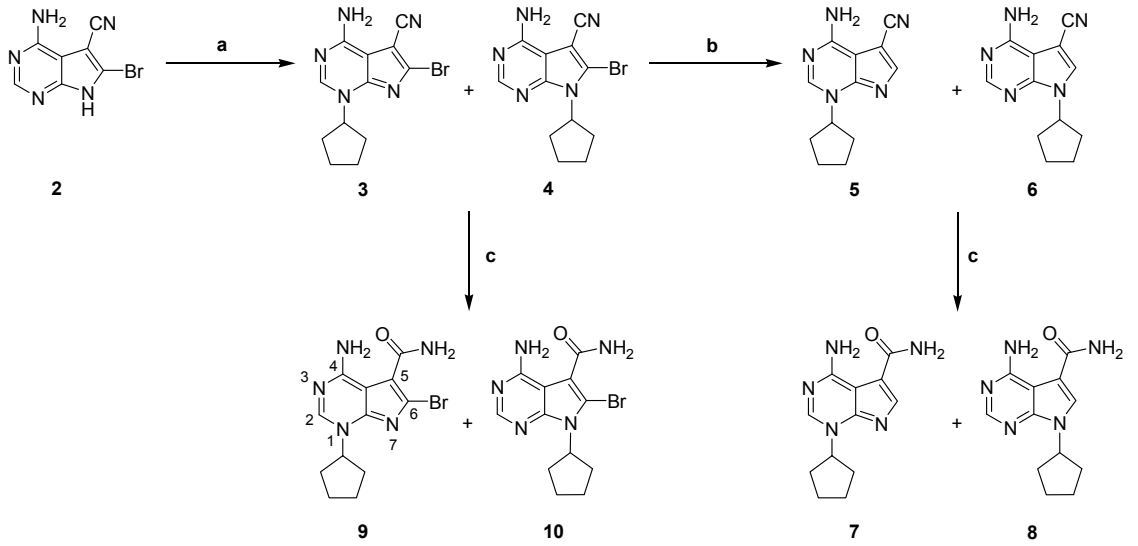


Figure 3. ORTEP representation of the X-ray structure of 4-amino-6-bromo-1-cyclopentyl-1*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (**3**).[28]

Compounds **7** and **8** were synthesized by debromination of isolated compounds **3** and **4** and the subsequent oxidative hydrolysis of compounds **5** and **6**.[29,30] Compound **9** and **10** were obtained in excellent yields by oxidative hydrolysis of compounds **3** and **4**, respectively (**Scheme 2**).

To determine whether the synthesized carbocyclic pyrrolo[2.3-*d*]pyrimidine nucleoside analogs (compounds **3-10**) can selectively inhibit the cell proliferation, we performed the MTT assay using a human ovarian cancer PA-1 cells. As a result, compound **9** significantly demonstrated anti-proliferative



Scheme 2. Synthesis of carbocyclic pyrrolo[2,3-*d*]pyrimidine nucleoside compounds (**3-10**). Reagents and conditions: (a) i) NaH, dimethylacetamide, 60 ^{o}C, 30 min. ii) cyclopentyl bromide, 60 ^{o}C, 24 h, 37% for **3**, 20% for **4**; (b) HCO_2NH_4, Pd/C, EtOH, reflux, 1h, 94% for **5**, 94% for **6**; (c) H_2O_2 (30 wt%), K_2CO_3, DMSO, rt, 1 h, 83% for **7**, 76% for **8**, 97% for **9**, 95% for **10**.

effect in a dose-dependent manner (IC_{50}: 3.9 μM for 24 h). However, the other compounds **3-8**, and **10** did not successfully inhibit the cancer cell proliferation or were shown to have no effect (**Figure 4**). Thus, we recognized that the amide group in compound **9** is much more effective in terms of cell growth inhibition, while the nitrile group in compound **3** has no effect. In contrast to other reported analogs of sangivamycin, which are usually debrominated compounds and 7-substituted compounds, compound **9**, brominated and 1-substituted carbocyclic pyrrolo[2,3-*d*]pyrimidine, demonstrated excellent anti-proliferative activity in PA-1 cells.

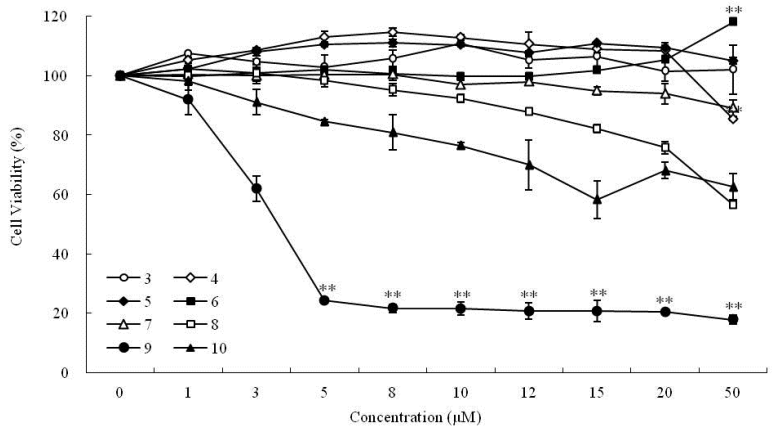
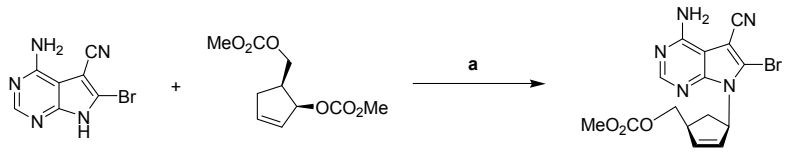


Figure 4. Anti-proliferative effect of compounds **3-10** in human ovarian cancer PA-1 cells. PA-1 cells were incubated with 1 - 50 μM of each compound for 24 h. The cell viability was determined by MTT assay. The results represent the mean ± SD (standard deviation) of three independent experiments. (*p<0.05, **p<0.01)

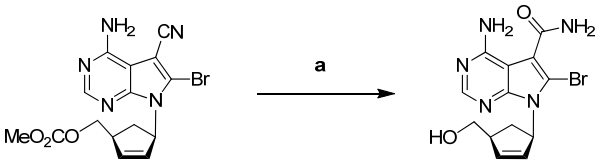
With this interesting result, we focused on the position of the cyclopentyl ring, which has hydrophobic character. In order to determine the effect of the hydrophobic character on the anti-cancer activity, we decided to select and examine 7-substituted compounds which are well known carbocyclic and normal nucleoside analogs as carbovir, abacavir, toyocamycin, and sangivamycin. Our group has previously reported on the synthesis of carbocyclic nucleosides by a Pd(0)-catalyzed coupling reaction. The *syn*-dicarbonate (**11**) was prepared by the known procedure.[31] The Pd(0)-catalyst, which produced π-allylpalladium complex with the dicarbonate (**11**), was prepared *in situ* from $Pd(OAc)_2$ with triisopropylphosphite in the presence of *n*-BuLi in anhydrous THF under argon. As several research groups reported,[32-34] the π-allylpalladium complex was coupled with intermediate **2** by nucleophilic attack to give the *syn*-coupling product (**12**) (**Scheme 3**).[31-36] The 1,4-substitutied product was favorably obtained over the 1,2-substituted product due to the steric hindrance of the nonbonding interaction with the carbonate moiety in the cyclopentene ring.[32-34,37]



2 **11** **12**

Scheme 3. Synthesis of compound **12**. Reagents and conditions: (a) i) $Pd(OAc)_2$, $(i\text{-PrO})_3P$, THF, rt, 10 min. ii) *n*-BuLi, rt, 5 min. iii) NaH, **2**, DMSO, rt. iv) **11**, DMSO, rt, 2 h, 50%.

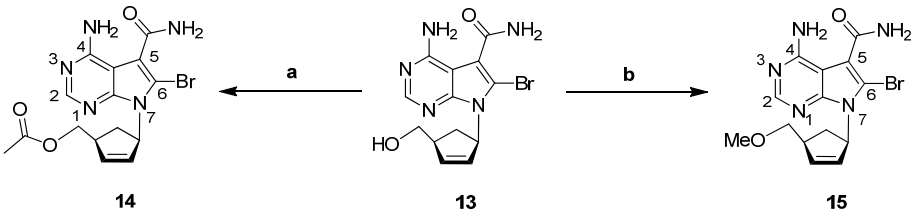
Oxidative hydrolysis of compound **12**, followed by the hydrolysis of the carbonate moiety, produced the carbocyclic nucleoside (**13**) (**Scheme 4**).



12 **13**

Scheme 4. Synthesis of compound **13**. Reagents and conditions: (a) i) H_2O_2 (30 wt%), K_2CO_3, DMSO, rt, 2 h. ii) aq. MeOH, rt, 2 h. 75%.

In order to provide hydrophobic characters, the hydroxyl group of compound **13** was treated with acetic anhydride to give acetylated compound **14**. Compound **13** was also reacted with dimethyl sulfate to produce the etherificated compound **15** (**Scheme 5**).



Scheme 5. Synthesis of compound **14** and **15**. Reagents and conditions:
(a) Ac_2O, pyridine, DMAP, DMSO, rt, 6 h, 90%; (b) Me_2SO_4, NaH, DMSO, rt, 1 h, 75%.

We expected that the C-N bond at 7-position can freely rotates to provide the hydrophobic environment around the 1-position as shown in compound **14** and **15**. To examine the cytotoxicity of the synthesized carbocyclic pyrrolo[2,3-*d*]pyrimidine nucleoside analogs (compounds **12-15**) in PA-1 cells, we also performed the MTT assay. In contrast to compound **12,** which did not inhibit the proliferation of PA-1 cells, compound **15** showed significant cell growth inhibition with 1 - 50 μM in a dose-dependent manner and very similar biological activity when compared to compound **9**. The IC_{50} value of compound **15** for growth inhibition for 24 h was 2.6 μM. Compounds **13** and **14** showed very similar biological activity. The IC_{50} values for compounds **13** and **14** were 10.5 μM and 8.8 μM, respectively (**Figure 5**). Such similarities were observed because compound **14** would undergo *in vitro* hydrolysis to compound **13**. With this result, we realized that the hydrophobic character adjacent to the 1-position of carbocyclic pyrrolo[2,3-*d*]pyrimidine nucleoside contributes considerably to the biological activity.

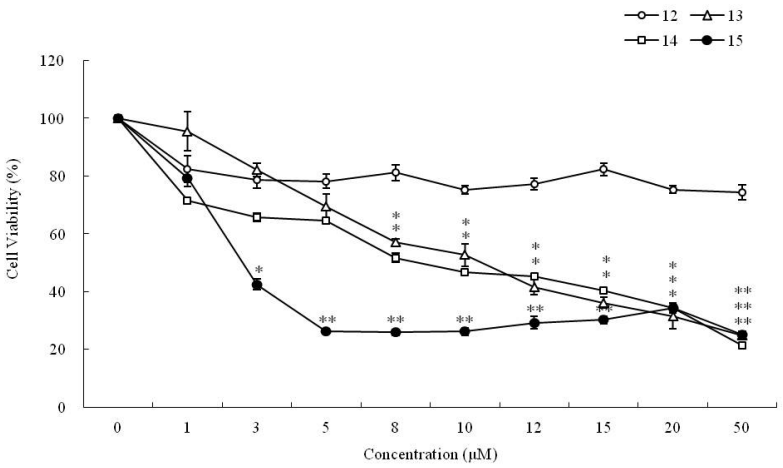


Figure 5. Anti-proliferative effect of compounds **12-15** in human ovarian cancer PA-1 cells. PA-1 cells were incubated with 1 - 50 μM of each compound for 24 h. The cell viability was determined by MTT assay. The results represent the mean ± SD (standard deviation) of three independent experiments. (*p<0.05, **p<0.01)

CONCLUSIONS

We synthesized new carbocyclic pyrrolo[2,3-*d*]pyrimidine nucleoside analogs and evaluated the *in vitro* cytotoxicity of the compounds. With key intermediate **2**, 1-substituted carbocyclic nucleosides were synthesized by S_N2 reaction. Compound **9** significantly inhibited the growth of human ovarian cancer cells (IC_{50}: 3.9 μM). We also synthesized and examined other analogs, namely 7-substituted carbocyclic nucleosides that are related to compound **9,** by a Pd(0)-catalyzed coupling reaction. Similar to compound **9,** compound **15** showed excellent anti-proliferative activity (IC_{50}: 2.6 μM). According to the biological activity data, the amide functional group, the existence of the bromine atom, and the position of the hydrophobic character would have an effect on the anti-cancer activity. We believed that our synthesized compounds would be potent anti-cancer agents and provide basic data of SAR study for human ovarian cancer cell, PA-1.

EXPERIMENTAL

General. ^{1}H and ^{13}C NMR spectra were obtained in DMSO-d_6 with Bruker and Varian spectrometer operating at 400 MHz and 500 MHz for ^{1}H and 100 MHz and 125 MHz for ^{13}C with TMS as an internal standard. Infrared spectra were recorded on a Bruker Alpha FT-IR spectrometer. Melting points were determined with a Sanyo Gallenkamp melting point apparatus. High-resolution mass spectra (HRMS) were obtained on a JMS 700 mass spectrometer with double-focusing mass analyzers. Analytical thin layer chromatography (TLC) was conducted on E. Merck 60 F254 aluminum-backed silica gel plates (0.2 mm) with a fluorescent indicator. Flash column chromatography was performed using Merck silica gel 60 (230-400 mesh) under positive pressure. All starting materials, reagents, and the solvents were of reagent grade, and solvents were purified by a known procedure before use.[38]

Synthesis of 2-amino-5-bromo-3,4-dicyanopyrrole (1).[24,25] To a stirred solution of tetracyanoethylene (10.24 g, 80.0 mmol) in the mixture of acetone (60 mL) and EtOAc (120 mL) at 0 ^{o}C was added 33 wt% HBr in AcOH (59 mL, 240.0 mmol) over a period of 1 h. The reaction mixture was stirred for an additional 30 min. The resulting pale yellow solid was collected by vacuum filtration and then washed with EtOAc (100 mL). The solid was air dried on filter, and the suspended in ice water (100 mL). The pH of the suspension was adjusted to 11 with 50% aq. NaOH at which point, all of the solid was dissolved. AcOH was then added to cooled solution until the pH 5, and precipitate was filtered by vacuum filtration. The tan powder was dried under vacuum to afford crude product **1** (8.95 g, 53%) which was used for next step without further purification. ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.31 (bs, 1H), 6.49 (bs, 2H).

Synthesis of 4-amino-6-bromo-5-cyanopyrrolo[2,3-d]pyrimidine (2).[13,26-27] To a stirred solution of compound **1** (2.1 g, 10.0 mmol) in 2-ethoxyethanol (50 mL) was added formamidine acetate (4.16 g, 40.0 mmol). The reaction mixture was then refluxed for 36 h. The solvent was evaporated, and the solid was dissolved in NH_4OH/EtOH then treated with charcoal. The solution was filtered by vacuum filtration and added AcOH until pH was near 7. The precipitate was filtered by vacuum filtration and dried under vacuum to afford crude product **2** (1.50 g, 63%) as a yellow powder, which was used for next step without further purification. ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.81 (bs, 1H), 8.21 (s, 1H), 7.17 (bs, 2H).

Synthesis of 4-amino-6-bromo-1-cyclopentyl-1*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (3) and 4-amino-6-bromo-7-cyclopentyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (4). Compound **2** (0.26 g, 1.1 mmol) and NaH (0.05 g, 1.2 mmol) were dissolved in dimethylacetamide (5 mL). The reaction mixture was stirred for 30 min at 60 ^{o}C. Cyclopentyl bromide (0.24 mL, 1.3 mmol) was then added to the reaction mixture and stirred at 60 ^{o}C for 24 h. The solvent was evaporated and crude mixture was purified by flash column chromatography eluting with 30 to 50% EtOAc/Hex to afford product **3** (0.12 g, 37%) as a white powder, R_f = 0.22 (50% EtOAc/Hex) and product 4 (0.07 g, 20%) as a white powder, R_f = 0.45 (50% EtOAc/Hex). For product **3**: mp 275-277 ^{o}C (dec.); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.48 (s, 1H), 8.36 (bs, 1H), 7.10 (bs, 1H), 5.12 (quintet, J = 8.0 Hz, 1H), 2.19-2.12 (m, 2H), 2.10-2.02 (m, 2H), 1.93-1.85 (m, 2H), 1.72-1.65 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 155.9, 146.6, 144.0, 132.6, 116.4, 105.3, 83.6, 61.1, 31.6, 24.2; IR (KBr): 3541, 3302, 3119, 2958, 2924, 2206, 1662, 1611, 1413, 1245 cm^{-1}; HRMS(EI+): *m/z* $[M]^+$ calcd for $C_{12}H_{12}BrN_5$: 305.0276; found: 305.0276. For product **4**: mp 262-264 ^{o}C (dec.); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.20 (s, 1H), 6.88 (bs, 2H), 5.07 (quintet, J = 8.8 Hz, 1H), 2.35-2.29 (m, 2H), 2.03-1.99 (m, 4H), 1.70-1.68 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 166.1, 156.9, 152.1, 149.8, 112.0, 110.8, 102.0, 57.3, 29.9, 24.5; IR (KBr): 3482, 3301, 3105, 2938, 2920, 2218, 1684, 1594, 1482, 1285 cm^{-1}; HRMS(EI+): *m/z* $[M]^+$ calcd for $C_{12}H_{12}BrN_5$: 305.0276; found: 305.0276.

Synthesis of 4-amino-1-cyclopentyl-1*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (5) and 4-amino-7-cyclopentyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (6). To a stirred solution of compound **3** (0,06 g, 0.2 mmol) and ammonium formate (0.13 g, 2.0 mmol) in 20 mL of EtOH was added 10% Pd/C (10 mg). The mixture was then refluxed for 1 h. The hot reaction mixture was filtered through Celite and washed with hot EtOH (20 mL). The solution was evaporated and the residue was diluted with EtOAc (20 mL), washed with water (2 X 10 mL), dried over anhydrous $MgSO_4$, and evaporated to

provide crude mixture. The crude mixture was purified by flash column chromatography eluting with 25% Hex/EtOAc to afford product **5** (0.04 g, 94%) as a white solid, R_f = 0.20 (25% Hex/EtOAc). Product **6** (0.04 g, 94%) was obtained from compound **4** as a white solid in the same manner, R_f = 0.30 (25% Hex/EtOAc). For compound **5**: mp 252 °C (dec.); ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 8.49 (s, 1H), 7.84 (s, 1H), 7.40-6.60 (bs, 2H), 5.18 (quintet, *J* = 8.0 Hz, 1H), 2.17-2.13 (m, 4H), 2.00-1.87 (m, 2H), 1.74-1.63 (m, 2H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ 156.7, 147.1, 146.2, 143.4, 117.4, 103.7, 81.2, 60.9, 30.9, 23.8; IR (KBr): 3444, 3314, 3112, 2954, 2204, 1662, 1616, 1431, 1161 cm^{-1}; HRMS(FAB+): *m/z* $[M+H]^+$ calcd for $C_{12}H_{14}N_5$: 228.1249; found: 228.1248. For compound **6**: mp 198 °C; ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 8.34 (s, 1H), 8.21 (s, 1H), 6.79 (bs, 2H), 5.03 (quintet, *J* = 8Hz, 1H), 2.15-2.09(m, 2H), 1.90-1.85 (m, 4H), 1.72-1.64 (m, 2H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ 156.9, 153.2, 149.9, 132.4, 115.7, 101.3, 81.5, 55.9, 32.1, 23.5; IR (KBr): 3466, 3306, 3084, 2964, 2220, 1654, 1592, 1309, 1222 cm^{-1}; HRMS(FAB+): *m/z* $[M+H]^+$ calcd for $C_{12}H_{14}N_5$: 228.1249; found: 228.1250.

Synthesis of 4-amino-1-cyclopentyl-1*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (7) and 4-amino-1-cyclopentyl-1*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (8): To a stirred solution of compound **5** (0.14 g, 0.6 mmol) in 5 mL of DMSO, cooled in an ice bath, were added 30 wt% H_2O_2 (0.5 mL) and K_2CO_3 (0.2 g). The mixture was then stirred at room temperature for 1 h. 50 mL of water was added to precipitate the product. The precipitate was filtered off and dried over under vacuum to afford product **7** (0.12 g, 83%) as a white solid. Product **8** (0.11 g, 76%) was obtained from compound **6** as a white solid in the same manner. For product **7**: mp 243 °C (dec.); ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 9.90 (bs, 1H), 8.33 (s, 1H), 7.90 (s, 1H), 7.82 (bs, 1H), 7.64 (bs, 1H), 6.91 (bs, 1H), 5.17 (quintet, *J* = 8.0 Hz, 1H), 2.15-2.09 (m, 4H), 1.96-1.86 (m, 2H), 1.74-1.63 (m, 2H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ 167.8, 157.7, 147.7, 142.5, 140.9, 110.7, 102.9, 60.0, 31.0, 23.8; IR (KBr): 3468, 3320, 3148, 2962, 1634, 1584, 1442, 1181 cm^{-1}; HRMS(FAB+): *m/z* $[M+H]^+$ calcd for $C_{12}H_{16}N_5O$: 246.1355; found: 246.1356. For product **8**: mp 229 °C (dec.); ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 9.20-8.60 (bs, 2H), 8.09 (s, 1H), 8.07 (s, 1H), 7.84 (bs, 1H), 7.27 (bs, 1H), 5.04 (quintet, *J* = 7.0 Hz, 1H), 2.16-2.09 (m, 2H), 1.92-1.85 (m, 2H), 1.83-1.74 (m, 2H), 1.72-1.68 (m, 2H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ 166.5, 158.0, 152.5, 150.5, 125.3, 109.7, 100.9, 54.7, 32.4, 23.7; IR (KBr): 3548, 3127, 2966, 1655, 1605, 1445, 1221 cm^{-1}; HRMS(FAB+): *m/z* $[M+H]^+$ calcd for $C_{12}H_{16}N_5O$: 246.1355; found: 246.1356.

Synthesis of 4-amino-6-bromo-1-cyclopentyl-1*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (9) and 4-amino-6-bromo-7-cyclopentyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (10). To a stirred

solution of compound **3** (0.18 g, 0.6 mmol) in 5 mL of DMSO, cooled in an ice bath, were added 30 wt% H_2O_2 (0.5 mL) and K_2CO_3 (0.2 g). The mixture was then stirred at room temperature for 1 h. 50 mL of water was added to precipitate the product. The precipitate was filtered off and dried over under vacuum to afford product **9** (0.19 g, 97%) as a white solid. Product **10** (0.18 g, 95%) was obtained from compound **4** as a white solid in the same manner. For product **9**: mp 233-235 ^oC (dec.); ^1H NMR (400 MHz, DMSO-d_6): δ 9.89 (bs, 1H), 8.36 (s, 1H), 8.07 (bs, 1H); 7.57 (bs, 1H), 7.08 (bs, 1H), 5.12 (quintet, J = 8.0 Hz, 1H), 2.19-2.11 (m, 2H), 2.09-2.00 (m, 2H), 1.94-1.84 (m, 2H), 1.74-1.65 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.3, 156.7, 145.7, 142.7, 125.5, 107.9, 104.1, 59.6, 31.0, 23.6; IR (KBr): 3541, 3304, 3148, 2960, 1668, 1648, 1450, 1238 cm^{-1}; HRMS(EI+): *m/z* $[M]^+$ calcd for $C_{12}H_{14}BrN_5O$: 323.0382; found: 323.0380. For product **10**: mp 242 ^oC (dec.); ^1H NMR (500 MHz, DMSO-d_6): δ 8.09 (s, 1H), 7.90 (bs, 1H), 7.62 (bs, 1H), 7.74-7.34 (bs, 2H), 5.09 (quintet, J = 8.7 Hz, 1H), 2.39-2.36 (m, 2H), 2.01-1.95 (m, 4H), 1.68-1.67 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 167.8, 157.7, 147.7, 142.5, 140.9, 110.7, 102.9, 60.0, 31.0, 23.8; IR (KBr): 3476, 3264, 3118, 2954, 1643, 1603, 1474, 1275 cm^{-1}; HRMS(EI+): *m/z* $[M]^+$ calcd for $C_{12}H_{14}BrN_5O$: 323.0382; found: 323.0384.

Synthesis of *syn*-[4-(4-amino-6-bromo-5-cyano-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-2-cyclopenten-1-yl]methyl methyl carbonate (12). To a stirred solution of $Pd(OAc)_2$ (0.046 g, 0.2 mmol) and (i-PrO)$_3$P (0.15 mL, 0.61 mmol) in 5 mL of THF under argon (air and moisture free-condition) was slowly added n-BuLi (1.6 N in hexane, 0.38 mL, 0.61 mmol) at room temperature. After 10 min, the solution of compound **2** (0.48 g, 2.0 mmol) and NaH (0.088 g, 2.2 mmol) in DMSO (5 mL) was added into the reaction flask by using double-tipped needle. The solution of the dicarbonate (**11**) (0.41 g, 1.8 mmol) in DMSO (3 mL) was added into the reaction flask by using double-tipped needle. After 2 h, the reaction mixture was diluted with CH_2Cl_2 (30 mL), washed with water (2 X 10 mL), dried over anhydrous $MgSO_4$, and evaporated to provide crude mixture. The crude mixture was purified by flash column chromatography eluting with 50% EtOAc/Hex to afford product **12** (0.35 g, 50%) as a white solid, R_f = 0.15 (35% Hex/EtOAc). mp 222-224 ^oC (dec.); ^1H NMR (400 MHz, DMSO-d_6): δ 8.48 (bs, 1H), 8.19 (s, 1H), 7.19 (bs, 1H), 6.22-6.21(m, 1H), 6.04-6.03 (m, 1H), 5.92-5.91 (m, 1H), 4.18-4.16 (m, 2H), 3.67 (s, 3H), 3.16-3.14 (m, 1H), 2.88-2.80 (m, 1H), 1.70-1.63 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 155.6, 155.4, 146.3, 142.9, 138.9, 132.6, 129.2, 116.3, 104.8, 83.6, 69.6, 64.4, 54.9, 44.4, 34.2; IR (KBr): 3315, 3197, 2955, 2207, 1746, 1639, 1440, 1255 cm^{-1}; HRMS(EI+): *m/z* $[M]^+$ calcd for $C_{15}H_{14}BrN_5O_3$: 391.0280; found: 391.0276.

Synthesis of syn-4-amino-6-bromo-7-[4-(hydroxyl methyl)-2-cyclopenten-1-yl]-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (13). To a stirred solution of compound **12** (0.45 g, 1.1 mmol) in 5 mL of DMSO, cooled in an ice bath, were added 30 wt% H_2O_2 (0.5 mL) and K_2CO_3 (0.1 g). The mixture was then stirred at room temperature until starting material was completely consumed by TLC monitoring. 3 mL of 20% MeOH in water was added and stirred for 2 h. The solvent was evaporated, and the resulting solid was washed with 10 mL of water and 10 mL of Et_2O ether on filter and dried over under vacuum to afford product **13** (0.29 g, 75%) as a white solid, R_f = 0.35 (10% MeOH/CH_2Cl_2). mp 245-250 ^oC (dec.); ^1H NMR (400 MHz, DMSO-d_6): δ 9.89 (bs, 1H), 8.16 (s, 1H), 8.09 (bs, 1H), 7.58 (bs, 1H), 7.08 (bs, 1H), 6.24-6.23 (m, 1H), 5.93-5.91 (m, 2H), 4.71 (t, J = 5.2 Hz, 1H), 3.52-3.47 (m, 1H), 3.43-3.38 (m, 1H), 2.92-2.90 (m, 1H), 2.78-2.70 (m, 1H), 1.64-1.58 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.3, 156.8, 145.7, 142.2, 140.5, 128.1, 125.5, 108.0, 103.9, 63.3, 63.1, 47.6, 34.0; IR (KBr): 3543, 3410, 3296, 3177, 2881, 1633, 1456, 1238, 1094, 764 cm^{-1}; HRMS(EI+): *m/z* $[M]^+$ calcd for $C_{13}H_{14}BrN_5O_2$: 351.0331; found: 351.0327.

Synthesis of *syn*-[4-(4-amino-6-bromo-5-carbamoyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-2-cyclopenten-1-yl]methyl acetate (14). To a stirred solution of compound **13** (0.035 g, 0.1 mmol) in DMSO (1 mL) were added Ac_2O (0.011 mL, 0.11 mmol), pyridine (0.010 mL, 0.12 mmol), and dimethylaminopyridine (0.001 g, 0.01 mmol). The reaction mixture was stirred for 6 h at room temperature, diluted with CH_2Cl_2 (20 mL), washed with water (2 X 10 mL), dried over anhydrous $MgSO_4$, and evaporated to give the crude material. The crude material was purified by flash column chromatography eluting with 15% Hex/EtOAc to afford product **14** (0.035 g, 90%) as a white solid, R_f = 0.15 (15% Hex/EtOAc). mp 172-174 ^oC (dec.); ^1H NMR (400 MHz, DMSO-d_6): δ 9.92 (bs, 1H), 8.16 (bs, 1H), 8.11 (s, 1H), 7.62 (bs, 1H), 7.09 (bs, 1H), 6.23-6.21 (m, 1H), 6.03-6.02 (m, 1H), 5.91-5.90 (m, 1H), 4.12-4.03 (m, 2H), 3.14-3.12 (m, 1H), 2.88-2.80 (m, 1H), 1.98 (s, 3H), 1.64-1.58 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.3, 166.3, 156.9, 145.6, 142.0, 138.7, 129.1, 125.6, 108.1, 104.0, 65.9, 63.1, 44.0, 34.3, 20.6; IR (KBr): 3448, 3285, 3157, 2925, 1726, 1616, 1441, 1236, 1038, 769 cm^{-1}; HRMS(EI+): *m/z* $[M]^+$ calcd for $C_{15}H_{16}BrN_5O_3$: 393.0437; found: 393.0435.

Synthesis of *syn*-4-amino-6-bromo-7-[4-(methoxymethyl)-2-cyclopenten-1-yl]-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (15): To a stirred solution of compound **13** (0.030 g, 0.09 mmol) in DMSO (1 mL) was added NaH (0.004 g, 0.11 mmol). After 5 min, dimethyl sulfate (0.01 mL, 0.1 mmol) was added into the reaction mixture and stirred for 1 h at room temperature. The solvent was evaporated to afford the crude mixture. The crude mixture was purified by HPLC on Xterra^{TM} RP_{18}7μm

column (19 X 300 mm) eluting with 30% MeOH/H_2O (flow rate: 5.0 mL/min; λ = 220 nm; t_{major} = 58 min; t_{minor} = 31 min) to afford product **15** (0.025 g, 75%) as a white solid. mp 212-214 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.65 (bs, 1H), 8.27 (s, 1H), 7.65 (bs, 1H), 7.12 (bs, 1H), 6.24-6.23 (m, 1H), 5.93-5.92 (m, 2H), 4.73 (bs, 1H), 3.51-3.48 (m, 1H), 3.42-3.38 (m, 1H), 3.05 (s, 3H), 2.92-2.90 (m, 1H), 2.79-2.71 (m, 1H), 1.64-1.58 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.8, 155.8, 145.2, 142.7, 141.0, 128.3, 125.2, 108.1, 104.8, 63.6, 63.5, 47.8, 34.4, 27.6; IR (KBr): 3461, 3314, 3219, 2934, 1649, 1456, 1245, 1088 cm^{-1}; HRMS(EI+): *m/z* $[M]^+$ calcd for $C_{14}H_{16}BrN_5O_2$: 365.0487; found: 365.0484.

SUPPLEMENTARY DATA

^{1}H NMR and ^{13}C NMR spectra for products, ^{1}H-^{1}H NOESY spectra for **3** and **4**, X-ray crystallographic data for **3**, and anti-proliferative effect of compounds **3-10**, **12-15** in human ovarian cancer PA-1 cells are provided as supplementary data.

ACKNOWLEDGEMENTS

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0070920 and 2010-0012838). J. Lee and S. Yoon acknowledge financial support from the Korean Ministry of Education through the second stage of the BK21 and the BK21-Plus project for the Hanyang University graduate program. We thank Prof. H. Chun for X-ray crystallography.

REFERENCES AND NOTES

1. C. Desgranges, G. Razaka, M. Rabaud, H. Bricaud, J. Balzarini, and E. De Clercq, *Biochem. Pharmacol*., 1983, **32**, 3583.
2. V. E. Marquez and M.-I. Lim, *Med. Res. Rev*., 1986, **6**, 1.
3. S. D. Patil, M. Hosoya, R. Snoeck, G. Andrei, J. Balzarini, and E. De Clercq, *J. Med. Chem.*, 1992, **35**, 3372.
4. M. D. Migliore, N. Zonta, C. McGuigan, G. Henson, G. Andrei, R. Snoeck, and J. Balzarini, *J. Med. Chem.*, 2007, **50**, 6485.
5. R. Vince and M. Hua, *J. Med. Chem.*, 1990, **33**, 17.
6. N. Katagiri, M. Takebayashi, H. Kokufuda, C. Kaneko, K. Kanehira, and M. Torihara, *J. Org. Chem.*, 1997, **62**, 1580.
7. M. E. Jung and H. Rhee, *Tetrahedron Lett*., 1993, **34**, 4449.

8. B. M. Trost, L. Li, and S. D. Guile, *J. Am. Chem. Soc*., 1992, **114**, 8745.
9. M. S. Daluge, S. S. Goog, B. M. Faletto, H. W. Miller, H. M. Clair, R. L. Boone, M. Tisdale, R. N. Parry, E. J. Reardon, E. R. Dornsife, R. D. Averett, and A. T. Krenitsky, *Antimicrob. Agents Chemother.*, 1997, **41**, 1082.
10. D. W. Kimberlin, D. M. Coen, K. K. Biron, J. I. Cohen, R. A. Lamb, M. Mckinlay, E. A. Emini, and R. J. Whitley, *Antiviral Res*., 1995, **26**, 369.
11. S. S. Good, S. M. Daluge, S. V. Ching, K. M. Ayers, W. B. Mahony, M. B. Faletto, B. A. Domin, B. S. Owens, R. E. Dornsife, J. A. McDowell, S. W. LaFon, and W. T. Symond, *Antiviral Res.*, 1995, **26**, A229.
12. S. M. Daluge, M. T. Martin, B. R. Sickles, and D. A. Livingston, *Nucleosides Nucleotides Nucleic Acids*, 2000, **19**, 297.
13. R. L. Tolman, R. K. Robins, and L. B. Townsend, *J. Am. Chem. Soc.*, 1968, **90**, 524.
14. E. C. Taylor and R. W. Hendess, *J. Am. Chem. Soc*., 1965, **87**, 1995.
15. P. Leonard, S. A. Ingale, P. Ding, X. Ming, and F. Seela, *Nucleosides Nucleotides Nucleic Acids*, 2009, **28**, 678.
16. P. K. Gupta, S. Daunert, M. R. Nassiri, L. L. Wotring, J. C. Drach, and L. B. Townsend, *J. Med. Chem.*, 1989, **32**, 402.
17. P. K. Gupta, M. R. Nassiri, L. A. Coleman, L. L. Wotring, J. C. Drach, and L. B. Townsend, *J. Med. Chem.*, 1989, **32**, 1420.
18. D. E. Bergstrom, A. J. Brattesani, M. K. Ogawa, P. A. Reddy, M. J. Schweickert, J. Balzarini, and E. De Clercq, *J. Med. Chem*., 1984, **27**, 285.
19. S. R. Turk, C. Shipman, M. R. Nassiri, G. Genzlinger, S. H. Krawczyk, L. B. Townsend, and J. C. Drach, *Antimicrob. Agents Chemother.*, 1987, **31**, 544.
20. R. K. Robins and G. R. Revankar, *Med. Res. Rev.*, 1985, **5**, 273.
21. M. T. Migawa, J. C. Drach, and L. B. Townsend, *J. Med. Chem.*, 2005, **48**, 3840.
22. Y. Ding, H. An, Z. Hong, and J.-L. Girardet, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 725.
23. E. Gunic, J.-L. Girardet, Z. Pietrzkowski, C. Esler, and G. Wang, *Bioorg. Med. Chem.*, 2001, **9**, 163.
24. E. E. Swayze, J. M. Hinkley, and L. B. Townsend, 'Nucleic Acid Chemistry,' Vol. 4, ed. by L. B. Townsend and R. S. Tipson, Wiely, New York, 1991, pp. 16-18.
25. W. J. Middleton, V. A. Engelhardt, and B. S. Fisher, *J. Am. Chem. Soc.*, 1958, **80**, 2822.
26. R. L. Tolman, R. K. Robins, and L. B. Townsend, *J. Am. Chem. Soc.*, 1969, **91**, 2102.
27. A. R. Porcari and L. B. Townsend, *Synth. Commun.*, 1998, **28**, 3835.
28. CCDC-853547 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk

/data_request/cif.

29. A. R. Porcari and L. B. Townsend, *Nucleosides Nucleotides Nucleic Acids*, 2004, **23**, 31.
30. A. R. Katritzky, B. Pilarski, and L. Urogdi, *Synthesis*, 1989, 949.
31. G.-i. An and H. Rhee, *Nucleosides Nucleotides Nucleic Acids*, 2003, **22**, 437.
32. B. M. Trost, L. Li, and S. D. Guile*, J. Am. Chem. Soc.*, 1992, **114**, 8745.
33. M. E. Jung and H. Rhee, *J. Org. Chem.*, 1994, **59**, 4719.
34. N. Katagiri, M. Takebayashi, H. Kokufuda, C. Kaneko, K. Kanehira, and M. Torihara, *J. Org. Chem.*, 1997, **62**, 1580.
35. B. M. Trost, *Angew. Chem., Int. Ed. Engl*., 1986, **25**, 1.
36. J. Tsuji, *Tetrahedron*, 1986, **42**, 4361.
37. H. Rhee, D.-O. Yoon, and M. E. Jung, *Nucleosides Nucleotides Nucleic Acids*, 2000, **19**, 619.
38. W. L. F. Armarego and C. L. L. Chai, ed. 'Purification of Laboratory Chemicals,' 6th ed., Elsevier, Oxford, 2009.