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Synthesis and anti-HBV activity of carbocyclic nucleoside hybrids with salient features of entecavir and aristeromycin†

Ramakrishnamraju Samunuri,[ab] Masaaki Toyama,[c] Renuka Sivasankar Pallaka,[b] Seshubabu Neeladri,[b] Ashok Kumar Jha,[b] Masanori Baba[c] and Chandralata Bal *[a]

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Modified carbocyclic nucleosides (**4a–g**) constituting 7-deazapurine, 4′-methyl, exocyclic double bond and 2′,3′-hydroxy were synthesized. NOE and X-ray studies of **4c** confirmed the α-configuration of 4′-methyl. The anti-HBV assay demonstrated **4e** (IC_{50} = 3.4 μM) without notable cytotoxicity (CC_{50} = 87.5 μM) as a promising lead for future exploration.

Hepatitis B is one of the common viral diseases. As per estimation, 350 million people are chronically infected with hepatitis B virus (HBV) worldwide and are at the risk of developing liver cancer.[1,2] Chronic HBV patients require long-term treatment, which only suppresses the infection and is not efficient in eliminating the virus. Drug-resistant viruses emerging due to the long-term regimen mandates synthesis and efforts to be directed towards finding more potent and less toxic novel anti-HBV agents. Entecavir (**I**, Fig. 1) has become one of the most prescribed anti-HBV drugs for the treatment of chronically infected patients.[3,4] It comprises a carbocyclic framework with a 6′-*exo* double bond, which seems to be an essential pharmacophore.[5] Aristeromycin (**II**, Fig. 1) is a naturally occurring carbocyclic purine nucleoside and its modified derivatives are reported to exhibit a wide range of pharmacological activities against viral infection, cancer *etc.*[6,7] Recently, 4′-substituted nucleosides have attracted consideration as balapiravir (for HCV) and festinavir (for HIV) reached the advanced phase of drug development.[8] Moreover, there are a few reports on 4′-substituted carbocyclic nucleosides analogs (CNAs) in literature.[9,10]

From the last decade, our group has been involved in the synthesis of biologically significant novel CNAs.[11–14] Recently, we described the synthesis of aristeromycin analogs (**III**, Fig. 1) with novel features: the 6′-exocyclic double bond and 4′-α-methyl group.[5] Although none of them demonstrated significant anti-HBV activity, none were strongly cytotoxic (CC_{50} > 100 μM). These results motivated us for further chemical exploration of this new class of CNAs towards improving their medicinal properties. Recently, base-modified nucleosides containing 7-deazapurine have attracted considerable attention.[15,16] Therefore, in this study, we designed a novel class of modified carbocyclic nucleosides (**IV**, Fig. 1) by replacing the adenine moiety of **III** with 7-deazapurine. Herein, we report our efforts toward the synthesis, anti-HBV activity, and cytotoxicity profiles of newly designed molecules.

The carbocyclic sugar intermediate (**1**) as a single diastereomer was achieved in eight synthetic steps from D-ribose with an overall yield of 17–20%.[5] The Cl/Br/I group was successfully substituted at the C-7 position of 6-chloro-7-deazapurine (**2a**) by treating with NCS/NBS/NIS in DMF.[12]

The fluoro derivative (**2b**) was obtained by heating **2a** with selectfluor in a acetonitrile : acetic acid (80 vol, 5 : 1) mixture at 70 °C.[17] The coupling of **1** with 6-chloro-7-deazapurine (**2a**) or its 7-halo derivatives (**2b–e**) under Mitsunobu reaction conditions afforded the corresponding protected coupled products. The deprotection was performed under acidic conditions without further purification to yield **3a–e** (Scheme 1).

The treatment of **3a–e** with methanolic ammonia at 100 °C under a sealed condition yielded the desired carbocyclic nucleosides (**4a–e**).[18]

The 7-vinyl/ethynyl analogs of **4a** were synthesized from the iodo derivative **4e** by palladium-catalyzed cross-coupling reaction to give the desired compounds (**4f–g**) in good yield (Scheme 2).[18] In brief, the 7-ethynyl derivative (**4f**) was synthesized in two steps from **4e** by treating with trimethylsilyl acetylene (TMS-acetylene) and $Pd(PPh_3)_4$ in DMF at 50 °C under the sealed condition, followed by treatment with K_2CO_3 in methanol at ambient temperature. The vinyl derivative (**4g**) was synthesized from **4e** by treating

[a] *Department of Chemistry, Birla Institute of Technology, Mesra, Ranchi, Jharkhand, 835215, India. E-mail: cbal@bitmesra.ac.in*
[b] *Chemistry Services, GVK Biosciences Pvt. Ltd, IDA Nacharam, Hyderabad, India*
[c] *Division of Antiviral Chemotherapy, Joint Research Center for Human Retrovirus Infection, Kagoshima University, 8-35-1, Sakuragaoka, Kagoshima, 890-8544, Japan*
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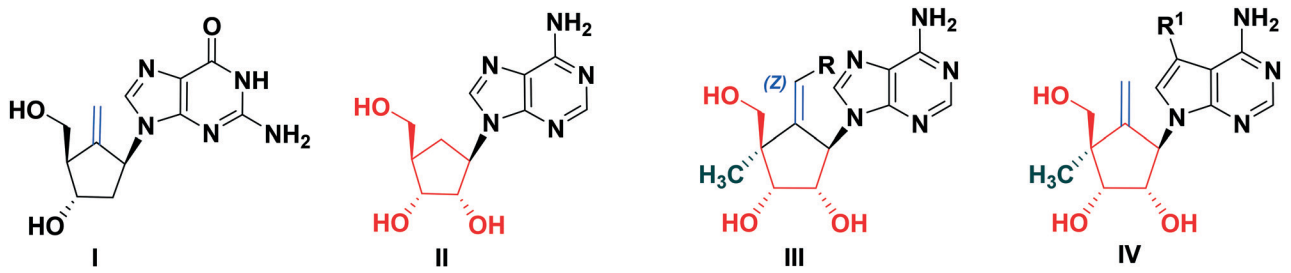


Fig. 1 Chemical structures entecavir (I), aristeromycin (II), aristeromycin analogs (III), and modified carbocyclic nucleosides (IV) for investigation in the present study.

with tributyl vinyl tin and $Pd(PPh_3)_4$ in DMF at 110 °C. All compounds and key intermediates were characterized *via* spectral analyses.

The NOE study of **4c** (Fig. 2) indicated the α-configuration of the methyl group at 4′-position in the carbocyclic sugar ring,[19] which was further confirmed *via* single-crystal X-ray diffraction studies (Fig. 3).

HepG2.2.15.7 cells (1×10^4 cells per well) were inoculated into a microtiter plate. After incubation for 24 h, the cells were cultured in the presence of various concentrations of test compounds (**4a–g**). Then, every three days, the culture medium was replaced by a fresh one containing an appropriate concentration of **4a–g**.

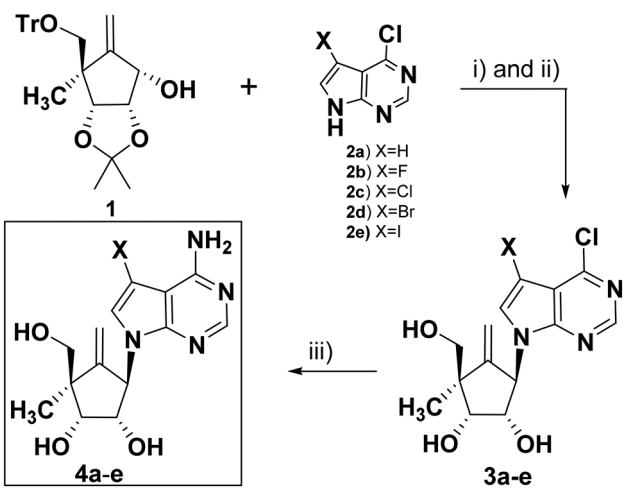
After nine days of incubation, the culture supernatants were collected and examined for HBV DNA levels using real-time PCR. The cells were tested for their viability by the tetrazolium dye method. IC_{50}: 50% effective concentration based on the inhibition of the HBV DNA levels in culture supernatants and CC_{50}: 50% cytotoxic concentration based on the reduction of viable cells was calculated. The results are summarized in Table 1 and Fig. 4. Interestingly, structural modification resulted in a noteworthy antiviral activity (**4c–f**) with IC_{50} ranging from 3.4 to 22.9 μM without considerable cytotoxicity. From the above-mentioned results, **4a–b** and **4g** were inactive towards HBV. It is interesting to note that the introduction of the bulkier halo groups at C-7 of the base (**4e**) remarkably increased the activity.

In summary, we synthesized a new series of modified carbocyclic nucleosides (**4a–g**) from commercially available starting materials in 10–12 synthetic steps. These compounds were evaluated for their antiviral activity against the hepatitis B virus and cytotoxicity properties in the HepG2.2.15.7 cells. Among the screened compounds, **4e** exhibited a noteworthy antiviral activity (IC_{50} = 3.4 μM) without notable cytotoxicity. From these studies, it is evident that these novel carbocyclic nucleosides might be valuable in designing new drugs for the HBV treatment.

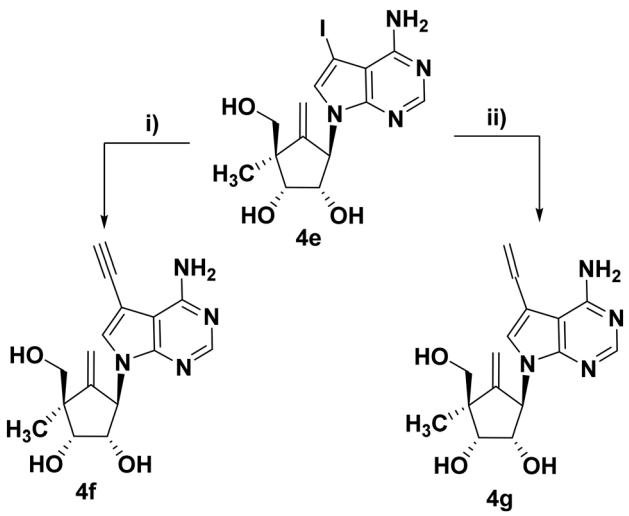
Experimental and spectral data of final compounds

General method for synthesis of 4a–e

A screw-cap vial equipped with a magnetic stirrer bar was charged with NH_3 in methanol (7 M, 7 ml) and appropriate **3a–e** (0.80 mmol), and then sealed with a screw cap. The vial was heated up to 100 °C and stirred for 24 h. The reaction mixture was concentrated under a reduced pressure, and



Scheme 1 Synthesis of **4a–e**. Reagents and conditions: (i) PPh_3, DIAD, THF, 10 °C–rt, 1 h; (ii) TFA : H_2O (8 : 2 ratio), rt, 30 min; (iii) NH_3 in MeOH, 100 °C, sealed tube, 24 h.



Scheme 2 Synthesis of 7-ethynyl/vinyl derivatives (**4f–g**). Reagents and conditions: i) a) trimethylsilyl acetylene, CuI, Et_3N, $Pd(PPh_3)_4$, DMF, 50 °C, 3 h; b) K_2CO_3, MeOH, rt, 30 min; ii) tri-*n*-butyl vinyl tin, $Pd(PPh_3)_4$, DMF, 110 °C, 3 h.

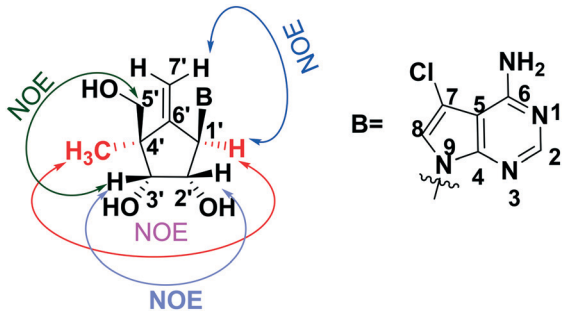


Fig. 2 Compound **4c** showed NOE between 4′-methyl with 1′-*H*, as shown in red arrow to demonstrate α-configuration.

crude was purified by flash chromatography on a silica gel (230–400 mesh, elution gradient 0–9% MeOH in CH_2Cl_2).

(1*S*,2*R*,3*R*,5*R*)-5-(4-Amino-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-3-(hydroxymethyl)-3-methyl-4-methylenecyclopentane-1,2-diol (**4a**): purified yield: 78%, off-white solid, (TLC: Rf 0.2, 10% MeOH in CH_2Cl_2); $[\alpha]_D^{20}$: +2.73 (*c* = 0.25, DMSO); mp: 210–220 °C; UV (MeOH) λ_{max}: 274.25 nm; ^{1}H NMR (400 MHz, CD_3OD) *δ*: 1.19 (s, 3H), 3.51 (d, *J* = 10.8 Hz, 1H), 3.66 (d, *J* = 10.8 Hz, 1H), 4.01 (d, *J* = 5.2 Hz, 1H), 4.55 (d, *J* = 3.2 Hz, 1H), 4.73 (dd, *J* = 4.4 and 9.6 Hz, 1H), 5.07 (d, *J* = 3.2 Hz, 1H), 5.50–5.53 (m, 1H), 6.70 (d, *J* = 3.2 Hz, 1H), 7.27 (d, *J* = 3.2 Hz, 1H), 8.08 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) *δ*: 18.9, 49.2, 62.7, 69.2, 73.5, 74.3, 99.8, 102.0, 107.8, 123.8, 148.3, 149.5, 155.0, 155.3; HRMS (ESI-Orbitrap) *m*/*z*: exact mass calculated for $C_{14}H_{19}N_4O_3$ $[M + H]^+$: 291.1457, found: 291.1422.

(1*S*,2*R*,3*R*,5*R*)-5-(4-Amino-5-fluoro-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-3-(hydroxymethyl)-3-methyl-4-methylenecyclopentane-1,2-diol (**4b**): purified yield: 55%, off-white solid, (TLC: Rf 0.2, 10% MeOH in CH_2Cl_2); $[\alpha]_D^{20}$: −6.43 (*c* = 0.25, DMSO); mp: 243–247 °C; UV (MeOH) λ_{max}: 280.25 nm; ^{1}H NMR (300 MHz, CD_3OD) *δ*: 1.18 (s, 3H), 3.50 (d, *J* = 10.8 Hz, 1H), 3.63 (d, *J* = 10.8 Hz, 1H), 3.98 (d, *J* = 4.8 Hz, 1H), 4.59 (d, *J* = 3.2 Hz, 1H), 4.63 (dd, *J* = 4.5 and 9.6 Hz, 1H), 5.07 (d, *J* = 3.3 Hz, 1H), 5.47–5.51 (m, 1H), 7.00 (d, *J* = 2.1 Hz, 1H), 8.02 (s, 1H); ^{19}F NMR (376 MHz, DMSO-d_6) *δ*: −168.25; ^{13}C NMR (100 MHz, DMSO-d_6) *δ*: 18.9, 49.1, 62.0, 69.1, 73.4, 74.2, 91.9, 105.2, 107.8, 140.3, 146.4, 152.3, 154.2, 155.7; HRMS (ESI-Orbitrap) *m*/*z*: exact mass calculated for $C_{14}H_{18}FN_4O_3$ $[M + H]^+$: 309.1285, found: 309.1325.

(1*S*,2*R*,3*R*,5*R*)-5-(4-Amino-5-chloro-7*H*-pyrrolo[2,3-*d*]-pyrimidin-7-yl)-3-(hydroxymethyl)-3-methyl-4-methylenecyclopentane-1,2-diol (**4c**): purified yield: 80%, off-white solid, (TLC: Rf 0.2, 10% MeOH in CH_2Cl_2); $[\alpha]_D^{20}$: −30.41 (*c* = 0.25, DMSO); mp: 226–229 °C; UV (MeOH) λ_{max}: 281.25 nm; ^{1}H NMR (300 MHz, CD_3OD) *δ*: 1.18 (s, 3H), 3.51 (d, *J* = 11.1 Hz, 1H), 3.65 (d, *J* = 10.8 Hz, 1H), 3.99 (d, *J* = 4.8 Hz, 1H), 4.59 (d, *J* = 2.7 Hz, 1H), 4.68 (dd, *J* = 4.8 and 9.9 Hz, 1H), 5.08 (d, *J* = 3.0 Hz, 1H), 5.46–5.51 (m, 1H), 7.24 (s, 1H), 8.05 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) *δ*: 18.9, 49.2, 62.4, 69.1, 73.5, 74.2, 99.4, 101.4, 107.9, 120.0, 149.6, 152.2, 154.7, 156.7; HRMS (ESI-Orbitrap) *m*/*z*: exact mass calculated for $C_{14}H_{18}ClN_4O_3$ $[M + H]^+$: 325.0989, found: 325.1031.

(1*S*,2*R*,3*R*,5*R*)-5-(4-Amino-5-bromo-7*H*-pyrrolo[2,3-*d*]-pyrimidin-7-yl)-3-(hydroxymethyl)-3-methyl-4-methylenecyclopentane-1,2-diol (**4d**): purified yield: 75%, off-white solid, (TLC: Rf 0.2, 10% MeOH in CH_2Cl_2); $[\alpha]_D^{20}$: +6.14 (*c* = 0.25, DMSO); mp: 228–232 °C; UV (MeOH) λ_{max}: 283.25 nm; ^{1}H NMR (300 MHz, CD_3OD) *δ*: 1.18 (s, 3H), 3.51 (d, *J* = 11.1 Hz, 1H), 3.65 (d, *J* = 11.1 Hz, 1H), 3.98 (d, *J* = 4.5 Hz, 1H), 4.58 (d, *J* = 2.7 Hz, 1H), 4.69 (dd, *J* = 4.8 and 9.9 Hz, 1H), 5.08 (d, *J* = 2.7 Hz, 1H), 5.48–5.51 (m, 1H), 7.30 (s, 1H), 8.04 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) *δ*: 18.9, 49.2, 62.5, 69.1, 73.5, 74.2, 85.4, 100.6, 107.9, 122.5, 150.0, 152.0, 154.7, 156.8; HRMS (ESI-Orbitrap) *m*/*z*: exact mass calculated for $C_{14}H_{18}BrN_4O_3$ $[M + H]^+$: 369.0484, found: 369.0522.

(1*S*,2*R*,3*R*,5*R*)-5-(4-Amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-3-(hydroxymethyl)-3-methyl-4-methylenecyclopentane-1,2-diol (**4e**): purified yield: 80%, off-white solid, (TLC: Rf 0.2, 10% MeOH in CH_2Cl_2); $[\alpha]_D^{20}$: +1.92 (*c* = 0.25, DMSO); mp: 227–228 °C; UV (MeOH) λ_{max}: 290.25 nm; ^{1}H NMR (300 MHz, CD_3OD) *δ*: 1.17 (s, 3H); 3.51 (d, *J* = 11.1 Hz, 1H), 3.65 (d, *J* = 10.8 Hz, 1H), 3.99 (d, *J* = 4.8 Hz, 1H), 4.57 (d, *J* = 2.4 Hz, 1H), 4.70 (dd, *J* = 4.8 and 9.9 Hz, 1H), 5.07 (d, *J* = 3.0 Hz, 1H), 5.40–5.50 (m, 1H), 7.37 (s, 1H), 8.04 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) *δ*: 18.9, 49.2, 50.4, 62.6, 69.2, 73.6, 74.3, 102.8, 107.9, 127.9, 150.7, 151.6, 154.8, 157.1; HRMS (ESI-Orbitrap) *m*/*z*: exact mass calculated for $C_{14}H_{18}IN_4O_3$ $[M + H]^+$: 417.0345, found: 417.0377.

Table 1 Anti-HBV and cytotoxicity of **4a–g** in HepG2.2.15.7 cells

Compound	IC_{50} (μM)	CC_{50} (μM)
4a	>100	>100
4b	>100	>100
4c	22.9	>100
4d	8.3	>100
4e	3.4	87.5
4f	6.3	>100
4g	>100	>100
Aristeromycin	>3	>3
Entecavir	0.18 (nM)	>100 (nM)

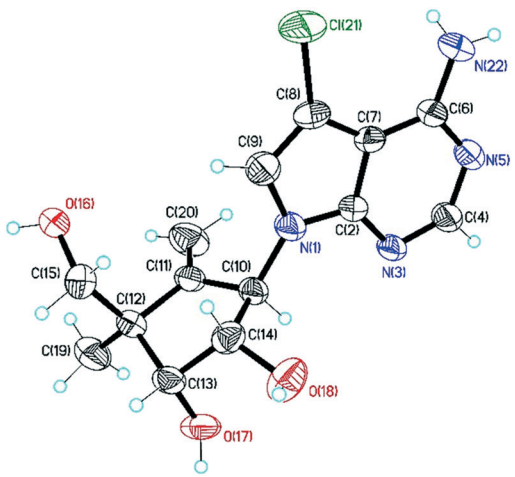
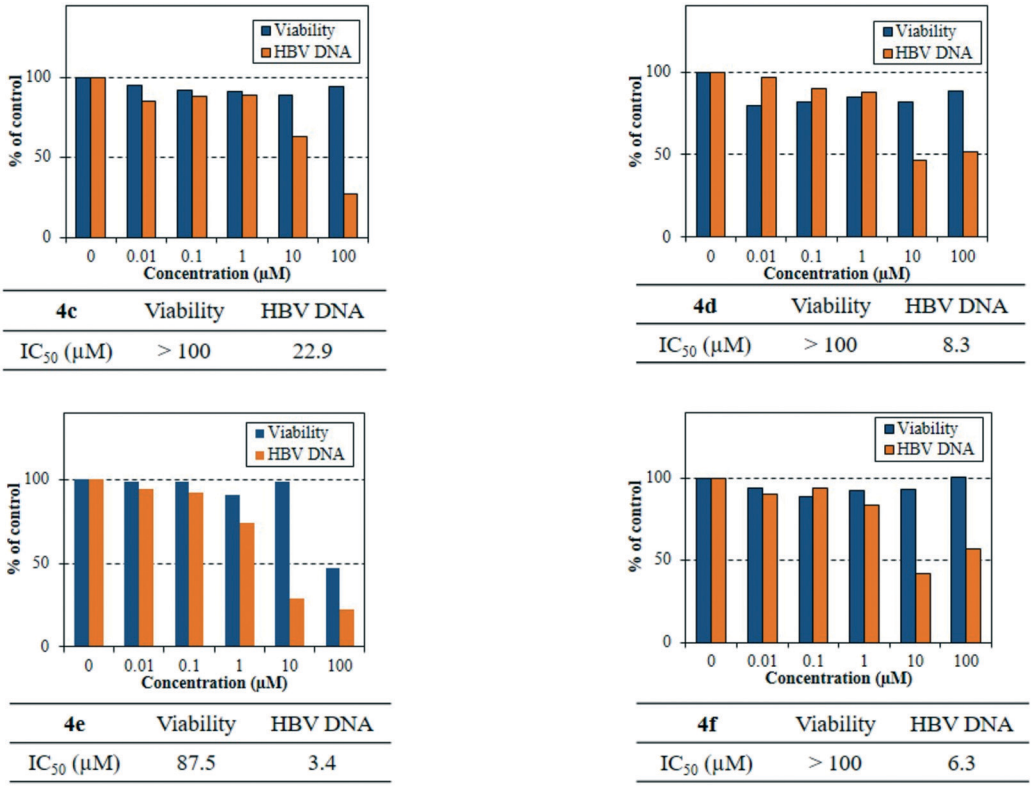


Fig. 3 Single-crystal X-ray structure of **4c**, showing a thermal displacement ellipsoid (50% probability) plot [CCDC no. 1969219].



4c	Viability	HBV DNA
IC_{50} (μM)	> 100	22.9

4d	Viability	HBV DNA
IC_{50} (μM)	> 100	8.3

4e	Viability	HBV DNA
IC_{50} (μM)	87.5	3.4

4f	Viability	HBV DNA
IC_{50} (μM)	> 100	6.3

Fig. 4 The anti-HBV activity of **4c–f**. The cell viability and activity are shown in the bar diagram. IC_{50}: 50% effective concentration based on the inhibition of the HBV DNA levels in culture supernatants. CC_{50}: 50% cytotoxic concentration based on the reduction of viable cells.

Synthetic procedure for 4f

A suspension of **4e** (0.60 mmol), trimethylsilyl acetylene (3.0 mmol), CuI (0.06 mmol), Et_3N (3.0 mmol) and $(PPh_3)_4$-Pd (0.06 mmol) in DMF was stirred at 50 °C under sealed condition for 3 h. The reaction mixture was concentrated under reduced pressure and crude was purified by silica gel (100–200 mesh) column chromatography, elution gradient 0–6% MeOH in CH_2Cl_2 to afford trimethylsilyl protected compound. The deprotection was carried out by stirring in methanol and K_2CO_3 (3.0 mmol) at rt for 30 min. The reaction mixture was concentrated under reduced pressure and purified by flash chromatography on silica gel (230–400 mesh), eluting gradient 0–7% MeOH in CH_2Cl_2.

(1*S*,2*R*,3*R*,5*R*)-5-(4-Amino-5-vinyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-3-(hydroxymethyl)-3-methyl-4-methylenecyclopentane-1,2-diol (**4f**): purified yield: 62%, off white solid. (TLC: Rf 0.1, 10% MeOH in CH_2Cl_2); $[\alpha]_D^{20}$: −2.17 (*c* = 0.25, DMSO); mp: 183–187 °C; UV (MeOH) λ_{max}: 283.25 nm; ^{1}H NMR (400 MHz, CD_3OD) δ: 1.18 (s, 3H), 3.51 (d, *J* = 10.8 Hz, 1H), 3.66 (d, *J* = 10.8 Hz, 1H), 3.70 (s, 1H), 3.99 (d, *J* = 4.4 Hz, 1H), 4.58 (d, *J* = 2.8 Hz, 1H), 4.72 (dd, *J* = 4.4 and 10.0 Hz, 1H), 5.08 (d, *J* = 3.2 Hz, 1H), 5.48–5.44 (m, 1H), 7.48 (s, 1H), 8.06 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 18.9, 49.3, 62.8, 69.2, 73.5, 74.2, 77.7, 82.7, 93.0, 102.1, 108.0, 128.5, 150.0, 152.4, 154.8, 157.4; HRMS (ESI-Orbitrap) *m*/*z*: exact mass calculated for $C_{16}H_{19}N_4O_3$ $[M + H]^+$: 315.1457, found: 315.1418.

Synthetic procedure for 4g

To a suspension of **4e** (0.6 mmol), $(PPh_3)_4Pd$ (0.06 mmol) in anhydrous DMF under argon atmosphere, tri-*n*-butyl(vinyl)tin (1.8 mmol) was added. The resulting mixture was heated at 110 °C for 3 h under sealed condition. Upon completion of reaction, concentrated the volatile under reduced pressure and crude was purified by flash chromatography on silica gel (230–400 mesh), elution gradient 0–7% MeOH in CH_2Cl_2.

(1*S*,2*R*,3*R*,5*R*)-5-(4-Amino-5-ethynyl-7*H*-pyrrolo[2,3-*d*]-pyrimidin-7-yl)-3-(hydroxymethyl)-3-methyl-4-methylenecyclopentane-1,2-diol (**4g**): purified yield: 60%, off white solid, (TLC: Rf 0.1, 10% MeOH in CH_2Cl_2); $[\alpha]_D^{20}$: −14.28 (*c* = 0.25, DMSO); mp: 194–198 °C; UV (MeOH) λ_{max}: 294.25 nm; ^{1}H NMR (300 MHz, CD_3OD) δ: 1.19 (s, 3H), 3.52 (d, *J* = 11.1 Hz, 1H), 3.67 (d, *J* = 11.1 Hz, 1H), 4.00 (d, *J* = 4.8 Hz, 1H), 4.57 (d, *J* = 2.7 Hz, 1H), 4.78 (dd, *J* = 4.8 and 9.9 Hz, 1H), 5.07 (d, *J* = 3.3 Hz, 1H), 5.24 (dd, *J* = 1.5 and 10.8 Hz, 1H), 5.44–5.48 (m, 1H), 5.58 (dd, *J* = 1.8 and 17.4 Hz, 1H), 7.05 (dd, *J* = 10.8 and 11.1 Hz, 1H), 7.35 (s, 1H), 8.02 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 18.9, 49.2, 62.3, 69.2, 73.5, 74.1, 100.2, 107.7, 112.1, 113.3, 120.0, 129.2, 151.1, 155.0, 157.5; HRMS (ESI-Orbitrap) *m*/*z*: exact mass calculated for $C_{16}H_{21}N_4O_3$ $[M + H]^+$: 317.1614, found: 317.1575.

Conflicts of interest

There are no conflicts to declare.

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19 $C_{14}H_{17}C_lN_4O_3$, M = 324.77, monoclinic, space group: $P2(1)$, a = 8.6168(3), b = 6.7974(3), c = 13.2395(4) Å, α = 90.000(0), β = 99.3435(8), γ = 90.000(0), V = 765.17(6) Å3, T = 293(2) K, Z = 2, μ = 0.268 mm^{-1}, $F(000)$ = 340.0, D_c = 1.410 Mg m^{-1}, crystal size 0.35 × 0.25 × 0.20 mm, 15 852 reflections measured, 3621 unique, R_1 = 0.0323 for 2861 $F_o > 4\sigma(F_o)$, and 0.0370 for all 3143 data and 226 parameters. Unit cell determination and intensity data collection was performed at 293(2) K. Structure solutions by direct methods and refinements by full-matrix least-squares methods on F^2 [CCDC NO: 1969219].