Base-modified thymidine and thymine analogs with low cytotoxicity effectively obstruct DNA replication in papovaviridae

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Abstract

Background: Current chemotherapeutic antimetabolites often exhibit severe side effects that limit their use as drugs; therefore, we designed nucleoside compounds with mechanisms of action focusing on inhibiting DNA replication rather than targeting multiple pathways. We previously discovered cytotoxic base-modified thymidine and thymine analogs that show higher selectivity against cancerous versus normal cells compared to the current antimetabolites used in cancer chemotherapy. We anticipated these antimetabolites have the potential to effectively inhibit viral DNA replication while showing low cytotoxicity.

Methods: Base-modified thymidine and thymine analogs were synthesized and their anti-viral activity was evaluated in human cells infected with human papilloma, John Cunningham, and BK viruses using quantitative DNA polymerase chain reaction assay. In addition, their toxicity toward host cells was determined using CellTiter-Glo assay, and compared to cytotoxicity toward human breast cancer cells.

Results: Novel lead compounds with high activity against human papilloma (HPV) and John Cunningham (JCV) viruses have been identified. Their EC50 values lie in low micromolar range (1-2 µM), which is significantly less than that of cidofovir (9-10 µM), a current drug used against DNA viruses. Cytotoxicity of the leads toward the host cells was found to be in 200-300 µM range, which is generally higher than that observed toward MCF-7 human breast cancer cells. None of the tested compounds significantly inhibited BK viral DNA replication.

Conclusion: The lead compounds affect the viruses substantially more selectively than the host cells, which makes them a novel class of bioactive compounds with the potential to become effective anti-viral drugs.

Keywords: Nucleosides, antimetabolites, DNA replication, anti-viral agents, anti-cancer agents, human papillomavirus, John Cunningham virus, breast cancer, chemotherapeutics

Introduction

Papovaviridae is a family of DNA viruses that are associated with serious diseases in patients with compromised immune system. Of those, human papillomavirus (HPV) is the most common sexually transmitted infection worldwide [1]. The high risk types HPV (e.g., 16,18) cause cervical cancer [2-4], while the low risk types HPV (e.g., 6,11) incur respiratory diseases, sometimes fatal, in spite of the current treatment [5]. John Cunningham virus (JCV) is known to cause progressive multifocal leuкоencephalopathy [6], which is usually deadly. BK virus, a close analog of JCV, is implicated in nephropathy [7] in renal transplant patients. Currently, there are no effective drugs that inhibit or cure these viral infections without off-target toxicity. Therefore, developing novel therapeutic agents against these viruses with low cytotoxicity can be lifesaving.

In the past half century, modified nucleobase and nucleoside analogs [8], otherwise termed antimetabolites, have substantially impacted treatment of cancer [9] and infectious diseases [10,11]. Physiologically, they readily undergo cellular uptake by nucleoside transporters [12], followed by metabolism into nucleotides [11], the active species capable of affecting a number of intracellular targets, such as enzymes producing nucleic acids [13-15] and single nucleotides [16,17], and targeting mitochondria leading to apoptosis [18]. Incorporation of antimetabolites into DNA results in damage signaling [19], obstruction of DNA synthesis [20] and repair [21]. Although inhibition of viral DNA
replication without affecting the host DNA is more likely
[22] than selective targeting of neoplastic DNA, occasionally,
antimetabolite anti-viral agents do incur severe side effects,
such as hepatotoxicity [23], hematopoietic toxicity [24], my-
elosuppression [25], hyperlactatemia, and lactic acidosis [26].

Recently, we have discovered base-modified thymidine
anlogs that show higher cytotoxicity toward cancerous cells
compared to benign [27]. Although the modifying moiety at-
tached to the nucleobase obstructs further DNA synthesis upon
incorporation of the 5’-triphosphate of such nucleoside into
a partial double helix DNA primer [28], the preliminary insight
into the mechanism of action strongly suggested inhibition of
a DNA polymerase instead [27]. However, it was still evident
that termination of DNA synthesis occurs only in the presence
of a certain bulky group attached at the 5-methyl group of the
thymine nucleobase. Considering the high selectivity of the
novel species toward cancer cells versus normal cells, we have
decided to investigate the possibility of selective targeting of
viral DNA replication versus killing the host cell by the newly
developed nucleoside analogs. In this paper, we report the
evaluation of 16 thymidine and 15 thymine analogs (Figure 1)
with modifications at the 5-methyl group in their ability to
obstruct DNA replication of HPV and JC viruses, and compare
the antiviral activity to the toxicity toward the host cells. The
lead compounds identified from these structure-activity rela-
tionship studies for both viruses exhibit diminutive cytotoxicity,
which opens the new passage to further drug development.
Our contribution to the field of anti-viral drug discovery is
significant as it gives the promise to access new agents for
these viral diseases that currently have no non-toxic drugs
available for treatment.

![Figure 1. Synthesis of 5-modified thymines and thymidines](image)

Materials and methods

**Synthesis**

All chemicals, reagents, and solvents were purchased from Sigma-Aldrich Inc., TCI, and Fisher Scientific, Inc., and used
as received unless stated otherwise. All reactions were car-
rried out under an atmosphere of dry argon in oven-dried
glassware. Indicated reaction temperatures refer to those of
the reaction bath, while room temperature (rt) is noted as
25°C. Pure reaction products were typically dried under high
vacuum in the presence of phosphorus(V) oxide. Analytical
thin layer chromatography (TLC) was performed using glass
backed silica plates (5x20 cm, 60 Å, 250 μm). Visualization
was accomplished using a 254 nm UV lamp. 1H and 13C
NMR spectra were recorded on either a Bruker Avance 400 MHz or Bruker
DPX 500 MHz spectrometer using solutions of samples in ei-
ther of the deuterated solvents: DMSO, methanol, acetonitrile.
Chemical shifts are reported in ppm with tetramethylsilane as
standard. Data are reported as follows: chemical shift, number
of protons, multiplicity (s=singlet, d=doublet, dd=doublet of
doublet, t=triplet, q=quartet, b=broad, m=multiplet, abq=ab
quartet), and coupling constants. High resolution mass spectral
data were recorded on a Shimadzu Q-TOF 6500 instrument.
All novel compounds were characterized by 1H, 13C, DEPT 13C
NMR spectroscopy and high resolution mass spectrometry.
The identity of previously made nucleoside derivatives was
confirmed by comparison of their 1H NMR to the published
data (reference provided). HPLC analysis of final products
was performed on an Agilent 1200 HPLC with UV detection.
Compounds biologically tested were at least 95% pure as
judged by 1H NMR and HPLC.

**General procedure for preparation of base-modified thymidines**

5-Bromomethyl-3-N-(tert-butyloxy)carbonyl-3’, 5’-bis-O-
(tert-buty) dimethylsilyl-2’-deoxyuridine (1) [28] and appro-
priate alcohol (4-20 eq.) were heated neat at 110-120°C for the
period between 15 minutes to 2 hours under argon atmosphere.
The mixture was cooled down to room temperature, dissolved
in tetrahydrofuran (ca 5 ml), and to this solution chilled at 0°C
tetra-n-butylammonium fluoride trihydrate (TBAF) was added
(ca 2.5 eq.). The reaction mixture was stirred for 2 hours while
gradually warming up to room temperature. The solvent was
removed under reduced pressure and the residue was purified
by silica gel (chloroform/methanol=1:0 to 10:1) and then by C8
reverse-phase column chromatography (water/methanol=19:1
to 1:4) to yield the product as a waxy solid. Compounds 3a-
11a and 14a-18a were obtained as reported previously [27].

5-[1- (phenyl)-1-hexyloxymethyl]-2’-deoxyuridine (12a)

Heating 1 (103mg, 0.158mmol) with α-n-hexylbenzyl alcohol
(1-phenyl-1-hexanol, 376mg, 1.580 mmol) for 2 hours at 116°C
followed by purification of bis- and mono-TBS products with
subsequent treatment with TBAF (161mg, 0.457mmol) afforded
after purification 18mg (27%) of product as 1:1 mixture of
diastereomers. 1H NMR (400 MHz, CD3OD) for diastereomers:
δ 7.96 (s, 1 H), 7.31 (m, 5 H), 6.28 (m, 1 H), 4.42 (m, 1 H), 4.36 (m,
1 H), 4.11 (m, 2 H), 3.95 (m, 1 H), 3.80 (ABq, 1 H), 2.28 (m, 1 H),
2.21 (m, 1 H), 1.82 (m, 1 H), 1.62 (m, 1 H), 1.28 (m, 8 H), 0.89 (m, 3
H). 13C NMR (100 MHz, CD3OD) for diastereomers δ 163.65 (C),
150.69 (C), 142.52 (C), 139.21 (CH), 120.05 (CH), 127.17 (CH), 126.49 and 126.43
(CH), 111.38 (C), 87.56 (CH), 85.16 and 85.09 (CH), 82.11 and 82.00 (CH), 70.91 and 70.85 (CH), 63.11 and 62.91 (CH3), 61.50 (CH3), 39.99 (CH3), 37.89 and 37.86 (CH2), 31.58 (CH2), 28.89 (CH2), 25.44 and 25.39 (CH2), 22.27 (CH3), 13.04 (CH3).

5-[1-(2-nitro)phenyl]-1-heptoxymethyl]-2'-deoxouridine (13a)

Heating 1 (97mg, 0.149mmol) with α-hexyl-2-nitrobenzyl alcohol (1-(2-nitro)phenyl-1-heptanol) (142mg, 0.597mmol) for 15 minutes at 112-114°C followed by purification 18mg (25%) of product as a 1:1 mixture of diastereomers. 1H NMR (400 MHz, CD3OD) δ 8.02 and 7.98 (2 s, 1 H), 7.92 (d, J=8.4 Hz, 1 H), 7.80 (d, J=8.0 Hz, 1 H), 7.69 (m, 1 H), 7.48 (m, 1 H), 7.31 (m, 6 H), 4.05 (s, 1 H), 3.97 (AB d, 2 H, J=12.1 Hz), 0.82 (s, 9 H). HRMS (ESI) for [MNa]+ calculated: 455.21581, observed: 455.21523.

5-chloromethyluracil (2)

Treatment of 1 (57mg, 0.355mmol) with 660mg (4.018mmol) of tert-butylbenzyl alcohol (2,2-dimethyl-tert-butyl-2-nitrobenzyl alcohol) (1-(2-nitro)phenyl-1-heptanol) (142mg, 0.597mmol) for 2.5 hours afforded after purification 25mg of product (31%). 1H NMR (400 MHz, DMSO-d6) δ 11.09 (br. s, 1 H, D2O exchangeable) 11.09 (br. s, 1 H, D2O exchangeable), 7.88 (d, 1 H, J=7.9 Hz), 7.70 (m, 2 H), 7.56 (t, 1 H, J=7.4 Hz), 7.41 (s, 1 H), 4.79 (s, 1 H), 4.08 (AB d, 2 H, J=11.6 Hz), 3.94 (AB d, 2 H, J=11.6 Hz), 0.79 (s, 9 H). 13C NMR (100 MHz, CD3OD) δ 160.04 (C), 151.73 (C), 150.85 (C), 141.28 (C), 133.65 (C), 132.76 (CH), 130.08 (CH), 129.17 (CH), 124.22 (CH), 109.04 (C), 80.96 (CH2), 64.42 (CH3), 36.53 (C), 25.97 (CH3). HRMS (ESI) for [MH]+ calculated: 334.13975, observed: 334.13977; for [MNa]+ calculated: 356.12169, observed: 356.12173.

5-[1-(2-methoxyphenyl)-2,2-(dimethyl)propoxymethyl] uracil (5b)

Treatment of 2 (50mg, 0.311mmol) with 290mg (1.5mmol) of racemic α-tet-butyl-2-methoxybenzyl alcohol (2,2-dimethyl-1-(2-methoxy)phenyl)-1-propanol) (1-propanol) (142mg, 0.597mmol) for 1 hour afforded after purification 30mg of product (31%). 1H NMR (400 MHz, DMSO-d6) δ 11.05 (br. s, 1 H, D2O exchangeable) 10.78 (br. s, 1 H, D2O exchangeable), 7.25 (m, 3 H), 6.96 (m, 2 H), 4.52 (s, 1 H), 3.98 (d, 1 H, J=12.0 Hz), 3.77 (m, 4 H), 0.83 (s, 9 H). HRMS (ESI) for [MNa]+ calculated: 341.14718, observed: 341.14723; for [M-H] calculated: 317.15086, observed: 317.15058.

5-[1-(3-methoxyphenyl)-2,2-(dimethyl)propoxymethyl] uracil (6b)

Treatment of 2 (50mg, 0.311mmol) with 190mg (1.0mol) of racemic α-tet-butyl-3-methoxybenzyl alcohol (2,2-dimethyl-1-(3-methoxy)phenyl)-1-propanol) (1-propanol) (142mg, 0.597mmol) for 1 hour afforded after purification 33mg of product (33%). 1H NMR (400 MHz, DMSO-d6) δ 11.08 (br. s, 1 H, D2O exchangeable) 10.64 (br. s, 1 H, D2O exchangeable), 6.77 (m, 4 H), 5.11 (m, 1 H), 4.44 (s, 1 H), 4.20 (m, 1 H), 3.73 (m, 4 H), 0.88 (s, 9 H). 13C NMR (100 MHz, CD3OD) δ 164.29 (C), 157.02 (C), 151.20 (C), 143.15 (CH), 138.46 (C), 130.25 (CH), 128.27 (CH), 113.84 (CH), 112.15 (CH), 112.05 (C), 74.53 (CH), 65.94 (CH2), 54.79 (CH3), 36.87 (C), 26.16 (CH3).

5-[1-(4-methoxyphenyl)-2,2-(dimethyl)propoxymethyl] uracil (7b)

Treatment of 2 (50mg, 0.311mmol) with 252mg (1.3mmol) of racemic α-tet-butyl-4-methoxybenzyl alcohol (2,2-dimethyl-1-(4-methoxy)phenyl)-1-propanol) (1-propanol) (142mg, 0.597mmol) for 2 hours afforded after purification 30mg of product (31%). 1H NMR (400 MHz, CD3CN) δ 8.86 (br. s, 2 H) 7.20 (m, 2 H), 6.89 (m, 2 H), 4.00 (m, 1 H), 3.80 (m, 4 H), 0.84 (s, 9 H). 13C NMR (100 MHz, CD3CN) δ 167.11 (C), 159.84 (C), 139.76 (CH), 130.48 (CH), 119.27 (C), 113.78 (CH), 111.32 (C), 89.43 (CH), 64.08 (CH2), 55.80 (CH3), 36.21 (C), 26.52 (CH3). HRMS (ESI) for [MNa]+ calculated: 341.14718, observed: 341.14724; for [M-H] calculated: 317.15068, observed: 317.15055.

5-[1-(2-bromophenyl)-2,2-(dimethyl)propoxymethyl] uracil (9b)

Treatment of 2 (50mg, 0.311mmol) with 134mg (0.55mmol) of racemic α-tet-butyl-2-bromobenzyl alcohol (2,2-dime-
thyl-1-(2-bromophenyl)-1-propanol) for 2 hours afforded after purification 22 mg of product (20%). ¹H NMR (400 MHz, DMSO-δ6) δ 11.08 (br. s, 1 H, D2O exchangeable) 10.83 (br. s, D2O exchangeable), 7.28 (m, 6 H), 4.03 (AB d, 1 H, J=6.0 Hz), 3.95 (AB d, 1 H, J=12.0 Hz), 3.86 (AB d, 2 H, J=12.0 Hz), 1.84 (m, 1 H), 0.89 (AB d, 3 H, J=6.5 Hz) 0.68 (AB d, 3 H, J=6.5 Hz). ¹C NMR (100 MHz, CD3OD) δ 164.08 (C), 151.71 (C), 141.50 (C), 140.16 (CH), 128.46 (CH), 127.70 (CH), 127.62 (CH), 109.93 (C), 86.55 (CH), 63.40 (CH3), 34.60 (CH3), 19.27 (CH3), 19.03 (CH3). HRMS (ESI) for [M+Na]+ calculated: 297.12096, observed: 297.12102.

5-[1-phenyl-2-(methyl)propoxymethyl]uracil (16b)

Treatment of 2 (50mg, 0.311mmol) with 467mg (3.114mmol) of commercial racemic α-isopropylbenzyl alcohol (2-methyl-1-phenyl-1-propanol) for 0.5 hours afforded after purification 60mg of product (70%). ¹H NMR (400 MHz, DMSO-δ6) δ 11.10 (br. s, D,O exchangeable) 10.83 (br. s, D,O exchangeable), 7.28 (m, 6 H), 4.03 (AB d, 1 H, J=6.9 Hz), 3.95 (AB d, 2 H, J=12.0 Hz), 3.86 (AB d, 2 H, J=12.0 Hz), 1.84 (m, 1 H), 0.89 (AB d, 3 H, J=6.5 Hz) 0.68 (AB d, 3 H, J=6.5 Hz). ¹C NMR (100 MHz, CD3OD) δ 164.08 (C), 151.71 (C), 141.50 (C), 140.16 (CH), 128.46 (CH), 127.70 (CH), 127.62 (CH), 109.93 (C), 86.55 (CH), 63.40 (CH3), 34.60 (CH3), 19.27 (CH3), 19.03 (CH3). HRMS (ESI) for [M+Na]+ calculated: 297.12096, observed: 297.12102.

Quantitative PCR analysis

Human embryonic kidney cells HEK293 were infected with human papilloma (strain: HPV11), human foreskin fibroblast HFF cells with BK (strain: Gardner), and human embryonic kidney 293TT cells with John Cunningham (strain: MAD -1) viruses. The infected cells were treated with DMSO solutions of test compounds and cidofovir, a known anti-viral drug (®Vistide), as positive control at concentrations 0, 0.1, 1, 10, 50, 100, 200, and 300 µM. The cells were incubated for 72 hours. After incubation, the cells were harvested and their DNA was isolated followed by quantitative real-time PCR analyses using FastStart Universal Probe Master (ROX) (Roche Applied Science). PCR
was performed with an initial denaturation reaction at 95°C for 1 minute and then amplified with 40 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. The amplification was monitored on Step One Plus (Applied Biosystems Inc.). A 5 μL aliquot of the resulting solution was loaded into each well containing a 12% denaturing PAGE gel, which was subsequently run at constant 18 Watts for 35 minutes. The gel was visualized using the Odyssey Infrared Imaging System (LiCor) with 169 μm resolution and the 700-channel laser source which has a solid-state laser diode at 680 nm and ImageQuant 5.0 software was used to determine density measurements. Experiments were performed in triplicate. EC_{50} and EC_{90} values were determined by comparison to the vehicle (negative control). The concentrations of compounds causing 50% and 90% inhibition of HPV and JCC viral DNA replication are summarized in Tables 1 and 2, respectively.

**Cell viability**
The CellTiter-Glo® Luminescent Cell Viability Assay was used. Upon incubation, cells were treated with solution of beetle luciferin in the presence of ATP. Luminescence was recorded 10 minutes after reagent addition using a GloMax®-Multi+Detection System. The luminescent signal from the host cells was compared to the background signal from serum-supplemented medium without cells. The CC_{50} curves were determined by plotting viability versus compound concentration. Kaleidagraph software was used to calculate the R value for each logarithmic curve fitting. The results are outlined in Tables 1 and 2.

### Table 1. Inhibition of human papillomavirus DNA replication (EC_{50}, EC_{90}), toxicity in human host cells (CC_{50}) and breast cancer cells (IC_{50}) [27, 29].

| R1  | R2  | Cpd | R3  | EC_{50} | EC_{90} | CC_{50} | SL_{50} | SI_{90} | IC_{50} | Cpd | R  | EC_{50} | EC_{90} | CC_{50} | SL_{50} | SI_{90} | IC_{50} |
|-----|-----|-----|-----|--------|--------|--------|--------|--------|--------|-----|-----|--------|--------|--------|--------|--------|--------|--------|
| HO  |     | 3a  | HO  | 92.70  | 193.01 | >300   | >3     | 2       | 188    | 3b  | H  | >300   | >300   | >300   | 1      | 1      | 53     |
| HO  |     | 4a  | HO  | 52.34  | >300   | >300   | >6     | 1       | 42     | 4b  | H  | 178.01 | >300   | >300   | >2     | 1      | 141    |
| HO  |     | 5a  | HO  | 2.40   | 234.34 | >300   | >125   | >1     | 88     | 5b  | H  | 262.01 | >300   | >300   | >1     | 1      | 100    |
| HO  |     | 6a  | HO  | 69.05  | >300   | >300   | >4     | 1       | 66     | 6b  | H  | 2.40   | >300   | >300   | >125   | 1      | 150    |
| HO  |     | 7a  | HO  | 5.21   | 107.99 | 289.43 | 55     | 3       | 160    | 7b  | H  | >300   | >300   | >300   | 1      | 1      | >200   |
| Br  |     | 8a  | HO  | >300   | >300   | >300   | 1      | 1       | 80     | 8b  | H  | N.A.   | N.A.   | N.A.   | N.A.   | N.A.   | N.A.   |
| Cl  |     | 9a  | HO  | 22.04  | 48.64  | 256.05 | 12     | 5       | 72     | 9b  | H  | N.D.   | N.D.   | N.D.   | N.D.   | N.D.   | N.D.   |
| HO  |     | 10a | HO  | 2.4    | 2.4    | >300   | >125   | >125   | 88     | 10b | H  | >300   | >300   | >300   | 1      | 1      | 76     |
| HO  |     | 11a | HO  | 2.4    | 241.83 | 293.83 | >122   | >1     | 74     | 11b | H  | 178.01 | >300   | >300   | <2     | 1      | N.D.   |
| HO  |     | 12a | HO  | 123.96 | >60    | 270.90 | 2      | <3     | 48     | 12b | H  | 42.94  | 55.40  | 225.46 | 6      | 5      | >200   |
| Cl  |     | 13a | HO  | 9.11   | >60    | 134.58 | 15     | <2     | 60     | 13b | H  | N.A.   | N.A.   | N.A.   | N.A.   | N.A.   | N.A.   |
| HO  |     | 14a | HO  | 21.73  | 54.07  | 134.58 | >14    | >5     | 123    | 14b | H  | 2.85   | 6.02   | >50    | >21    | >10    | 43     |
| HO  |     | 15a | HO  | >300   | >300   | >300   | 1      | 1      | 160    | 15b | H  | >300   | >300   | >300   | 1      | 1      | >200   |
| HO  |     | 16a | HO  | >300   | >300   | >300   | 1      | 1      | 150    | 16b | H  | 278.13 | >300   | >300   | >1     | 1      | 200    |
| HO  |     | 17a | HO  | 2.4    | 247.24 | >300   | >125   | 1.2    | >200   | 17b | H  | >60    | >60    | 60     | 1      | 1      | >300   |
| HO  |     | 18a | HO  | >300   | >300   | >300   | 1      | 1      | >200   | 18b | H  | 167.11 | >300   | >300   | >2     | 1      | >300   |

**Cidofovir (control)** 9.49  >300  >300  >32  1

*All concentrations are in μM; ^aSI_{50} = CC_{50}/EC_{50}; ^bSI_{90} = CC_{50}/EC_{90}; ^cCpd could not be synthesized; ^dCpd was not tested. ^eRef. 27; ^fRef. 29.
The bioactive compounds were obtained by heating 5-bromo-2’-deoxyuridine (5a) and 5-bromomethyl-2’-deoxyuridine (5b), dimethylsilyl-O-2’-deoxyuridine (3a) or 5-chloromethyluracil (2) with an appropriate alcohol under neat, anhydrous conditions, as reported previously [27]. This transformation yielded nucleobases 3b-18b; removal of the residual TBS groups using tetra-n-butylammonium fluoride yielded nucleoside derivatives 3a-18a (Figure 1). The PCR DNA and CellTiter-Glo assays were performed using standard protocols.

The data reflecting quantitative inhibition of human papilloma virus replication is summarized in Table 1. The trends revealed by structure-activity relationship are quite different from those observed for cytotoxicity toward cancer cells [27]. First, the α-tert-butyl and 2-nitrobenzyl groups were not the best substituents, as evidenced by the higher EC_{50} of 3a compared to 10a, as well as 4a versus 11a, and 4a versus 5a. Furthermore, when the methoxy group in the benzyl substituent in 5a was moved across the ring, the activity of the meta-substituted analog (6a) was decreased, while restored for the para-substituted analog 7a, which is opposite of the anti-cancer activity trend [27]. Second, the presence of an aryl group as either R1 or R2 was not critical, as evidenced by the high activity of 16a. And third, modified nucleobases demonstrated superior activity compared to the corresponding base-modified nucleosides in greater number of instances, particularly, 6b compared to 6a, 12b to 12a, and 14b to 14a. The most impressive potency, however, was demonstrated by the nucleoside derivative 10a a definite hit compound for further drug development, given significant side effects of cidofovir [30,31]. Surprisingly, the chloro-analog of 9a, compound 8a, was only somewhat active, and the activity of 10a, the lead compound for HPV, was rather modest against JCV. Neither of these nucleoside and nucleobase derivatives significantly inhibited DNA replication of the BK virus (see supporting information).

### Results and discussion

The bioactive compounds were obtained by heating 5-bromomethyl-3-N-(tert-butoxy)carbonyl-3’S,5’R-bis-(tert-butyldimethylsilyl)-O-2’-deoxyuridine (1) [28] or 5-chloromethyluracil (2) with an appropriate alcohol under neat, anhydrous conditions, as reported previously [27]. This transformation yielded nucleobases 3b-18b; removal of the residual TBS groups using tetra-n-butylammonium fluoride yielded nucleoside derivatives 3a-18a (Figure 1). The trend discovered for inhibition of JC viral DNA replication was somewhat different (Table 2). The three most active compounds turned out to be 5-(α-tert-butyl-ortho-bromobenzylxoy) methyl-2’-deoxyuridine (9a) and 5-(α-methylbenzylxoy)methyluracil (18b) showing, respectively, moderate and low activity against HPV DNA replication (Table 1). Notably, nucleoside 18a showed 14 fold lower potency compared to the nucleobase, which is unprecedented in the anti-cancer activity of this type of species [27,29]. Surprisingly, the chloro-analog of 9a, compound 8a, was only somewhat active, and the activity of 10a, the lead compound for HPV, was rather modest against JCV. Neither of these nucleoside and nucleobase derivatives significantly inhibited DNA replication of the BK virus (see supporting information).

### Conclusions

We have examined the activity of 5-substituted thymidine and thymine derivatives with respect to inhibition of DNA replication in human papilloma, John Cunningham, and BK viruses. Studies of the structure-activity relationship revealed hits for HPV and JCV, but none against BKV. Importantly, these bioactive species with high anti-viral activity have low cytotoxicity, which makes them novel lead compounds with the possibility of further development.
potential to pursue further drug development.

Supporting information
Spectral characterization of novel molecules, NIAID-NIH in-vitro antiviral screening reports, and the original 248th ACS National Meeting poster [29]. This material is available free of charge at Supplementary data.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions

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References


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