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

Kayla M. Borland, Patrick R. Wolfkiel, Matthew P. Burke, Sean M. Lawson, Courtney A. Stockman, Aron P. Bercz, Julia N. Tolstolutskaya and Vladislav A. Litosh*

*Correspondence: Vladislav.Litosh@uc.edu

Department of Chemistry, University of Cincinnati, 301 W. Clifton Ct. Cincinnati, OH 45221, USA.

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 leukoencephalopathy [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], myelosuppression [25], hyperlactatemia, and lactic acidosis [26]. Recently, we have discovered base-modified thymidine analogs that show higher cytotoxicity toward cancerous cells compared to benign [27]. Although the modifying moiety attached 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 relationship 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.

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 carried 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 either of the deuterated solvents: DMSO, methanol, acetoneitrile. 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-butyloxymethyl)carbonyl-3', 5'-bis-O-(tert-butyl) dimethylsilyl-2'-deoxyuridine (1) [28] and appropriate 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-butyllammonium 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, 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
5-[1-(2-nitrophenyl)-1-heptoxymethyl]-2'-deoxyuridine (13a)

Heating 1 (97mg, 0.149mmol) with α-hexyl-2-nitrobenzyl alcohol (1:2-(nitrophenyl)-1-heptanol) (142mg, 0.597mmol) for 15 minutes at 112-114°C followed by purification 18mg (25%) of product as 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=13a) 0.82 (s, 9 H). HRMS (ESI) for [MNa]+ calculated: 311.13661, observed: 311.13660 and 129.60 and 129.56 (CH), 129.34 (CH), 125.23 (C), 125.17 (CH), 111.38 (C), 110.90 (CH), 109.90 (C), 88.61 (CH), 63.73 (CH3), 35.60 (C), 26.52 (CH3). HRMS (ESI) for [MNa]+ calculated: 311.13661, observed: 311.13661.

General procedure for the synthesis of modified thymines

5-Chloromethyluracil (5b) and appropriate alcohol (4-11 eq.) were added. The solvent was removed under reduced pressure and the solid was applied onto a silica gel column. Chromatography (SiO2, CH2Cl2/MeOH=20:1) afforded the product as a powder.

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

Treatment of 2 (50mg, 0.311mmol) with 290mg (1.5mmol) of racemic α-tert-butyl-2-methoxybenzyl alcohol (2,2-dimethyl-1-(2-methoxyphenyl)-1-propanol) for 1 hour afforded after purification 25mg of product (31%). 1H NMR (400 MHz, DMSO-d6) δ 11.09 (br. s, 1 H, D,O exchangeable) 10.9 (br. s, 1 H, D,O exchangeable) 7.88 (d, 1 H, J=7.9 Hz), 7.70 (m, 2 H), 7.65 (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 (CH), 133.65 (C), 132.76 (CH), 130.08 (CH), 129.17 (CH), 124.22 (CH), 109.04 (C), 80.96 (CH), 64.42 (CH2), 36.53 (C), 25.97 (CH3). HRMS (ESI) for [M]+ calculated: 334.13975, observed: 334.13977; for [MNa]+ calculated: 334.12619, observed: 335.12173.

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

Treatment of 2 (50mg, 0.311mmol) with 190mg (1.0mmol) of racemic α-tert-butyl-3-methoxybenzyl alcohol (2,2-dimethyl-1-(3-methoxyphenyl)-1-propanol) for 1 hour afforded after purification 33mg of product (33%). 1H NMR (400 MHz, DMSO-d6) δ 11.08 (br. s, 1 H, D,O exchangeable) 10.64 (br. s, 1 H, D,O 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-(2-nitrophenyl)-2,2-(dimethyl)propoxymethyl] uracil (7b)

Treatment of 2 (50mg, 0.311mmol) with 252mg (1.3mmol) of raceminc α-tert-butyl-4-methoxybenzyl alcohol (2,2-dimethyl-1-(4-methoxyphenyl)-1-propanol) for 2 hours afforded after purification 30mg of product (31%). 1H NMR (400 MHz, DMSO-d6) δ 11.09 (br. s, 1 H, D,O exchangeable) 10.68 (br. s, 1 H, D,O 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), 2.63 (s, 9 H). 13C NMR (100 MHz, CD3OD) δ 160.04 (C), 151.73 (C), 150.85 (C), 141.28 (CH), 133.65 (C), 132.76 (CH), 130.08 (CH), 129.17 (CH), 124.22 (CH), 109.04 (C), 80.96 (CH), 64.42 (CH2), 36.53 (C), 25.97 (CH3). HRMS (ESI) for [M]+ calculated: 341.14718, observed: 341.14723; for [M-H] calculated: 317.15068, observed: 317.15058.

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

Treatment of 2 (50mg, 0.311mmol) with 134mg (0.55mmol) of racemic α-tert-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-d6) δ 11.08 (br. s, 1 H, D2O exchangeable), 10.83 (br. s, 1 H, D2O exchangeable), 7.92 (d, 1 H, J=9.1 Hz), 7.72 (m, 1 H), 7.72 (m, 1 H), 7.65 (m, 1 H), 7.54 (m, 1 H), 7.37 (s, 1 H), 4.62 (d, 1 H, J=6.0 Hz), 3.97 (AB d, 1 H, J=11.7 Hz), 3.87 (AB d, 1 H, J=11.7 Hz), 1.65 (m, 5 H), 1.23 (m, 1 H), 1.07 (m, 1 H). 13C NMR (100 MHz, DMSO-d6) δ 163.60 (C), 151.18 (C), 149.10 (C), 140.71 (CH), 135.72 (C), 132.89 (CH), 129.02 (CH), 128.47 (CH), 123.82 (CH), 108.79 (CH), 79.57 (CH), 63.56 (CH), 43.68 (CH), 28.99 (CH2), 27.65 (CH2), 25.86 (CH3), 25.65 (CH3), 25.43 (CH3).

5-[1-(phenyl)cyclohexyloxymethyl]uracil (11b) Treatment of 2 (50mg, 0.311mmol) with 290mg (1.5mmol) of racemic α-cyclohexyl-2-nitrobenzyl alcohol (1-(2-nitrophenyl)cyclohexanol) for 1 hour afforded after purification 20 mg of product (20%). 1H NMR (400 MHz, DMSO-d6) δ 11.08 (br. s, 1 H, D2O exchangeable), 10.83 (br. s, 1 H, D2O exchangeable), 7.92 (d, 1 H, J=9.1 Hz), 7.72 (m, 1 H), 7.72 (m, 1 H), 7.65 (m, 1 H), 7.54 (m, 1 H), 7.37 (s, 1 H), 4.62 (d, 1 H, J=6.0 Hz), 3.97 (AB d, 1 H, J=11.7 Hz), 3.87 (AB d, 1 H, J=11.7 Hz), 1.65 (m, 5 H), 1.23 (m, 1 H), 1.07 (m, 1 H). 13C NMR (100 MHz, DMSO-d6) δ 163.60 (C), 151.18 (C), 149.10 (C), 140.71 (CH), 135.72 (C), 132.89 (CH), 129.02 (CH), 128.47 (CH), 123.82 (CH), 108.79 (CH), 79.57 (CH), 63.56 (CH), 43.68 (CH), 28.99 (CH2), 27.65 (CH2), 25.86 (CH3), 25.65 (CH3), 25.43 (CH3).
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.
The bioactive compounds were obtained by heating 5-bromo-methyl-3-N-(tert-butyloxy)carbonyl-3’S’-bis-(tert-butyloxy)dimethylsilyl-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 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, 5-(α-cyclohexylbenzyloxy)methyl-2’-deoxyuridine, with the 90% efficiency (EC_{90}) value of 2.4 μM and the 50% cytotoxicity (CC_{50}) value exceeding 300 μM. The outstanding SI_{90} value of over 125 makes 10a a definite hit compound for further drug development, given significant side effects of cidofovir [30,31] whose potency is lower. Expectedly, cytotoxicity of almost all the examined antimetabolites toward host cells is significantly lower than that toward breast cancer cells [29].

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-butyloxy)-orthoo-bromobenzoxyl) methyl-2’-deoxyuridine 9a and 5-(α-methylbenzoxyl)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 potential for 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

| Authors' contributions | KMB | PRW | MPB | SML | CAS | APB | JNT | VAL |
|------------------------|-----|-----|-----|-----|-----|-----|-----|-----|
| Research concept and design | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Collection and/or assembly of data | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Data analysis and interpretation | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Writing the article | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Critical revision of the article | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Final approval of article | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Statistical analysis | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |

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References
1. Ramakrishnan S, Partricia S and Mathan G. Overview of high-risk HPV's 16 and 18 infected cervical cancer: pathogenesis to prevention. Biomed Pharmacother. 2015; 70:103-10. | Article | PubMed
2. Veldman T, Horikawa I, Barrett JC and Schlegel R. Transcriptional activation of the telomerase hTERT gene by human papillomavirus type 16 E6 oncoprotein. J Virol. 2003; 75:4467-72. | Article | PubMed Abstract | PubMed FullText
3. Bulut G, Fallen S, Beauchamp EM, Drebing LE, Sun J, Berry DL, Kallakury B, Crum CP, Toretsky JA, Schlegel R and Uren A. Use of reprogrammed cells to identify therapy for respiratory papillomatosis. N Engl J Med. 2012; 367:1220-7. | Article | PubMed Abstract | PubMed FullText
4. Park WB, Shaddix SC, Chang CH, White EL, Rose LM, Brockman RW, Shortney AJ, Montgomery JA, Secrist JA, 3rd and Bennett LL, Jr. Effects of 2-chloro-9-(2-deoxy-2-fluro-beta-D-arabinofuranosyl)adenine on K562 cellular metabolism and the inhibition of human ribonucleotide reductase and DNA polymerases by its 5’-triphosphate. Cancer Res. 1991; 51:2386-94. | Article | PubMed
5. Peters GL, van der Wilt CL, van Groeningen CJ, Smid K, Meijer S and Pinedo HM. Thymidylate synthase inhibition after administration of fluorouracil with or without leucovorin in colon cancer patients: implications for treatment with fluorouracil. J Clin Oncol. 1994; 12:2035-42. | Article | PubMed
6. Padgett BL, Walker DL, Zuurheim GM, Eckroade RJ and Dessel BH. Cultivation of papova-like virus from human brain with progressive multifocal leuconoecephalopathy. Lancet. 1971; 1:1257-60. | Article | PubMed
7. Fishman JA. BK virus nephropathy—polyomavirus adding insult to injury. N Engl J Med. 2002; 347:527-30. | Article | PubMed
8. Burke MP, Borland KM and Litosh VA. Base-Modified Nucleosides as Chemotherapeutic Agents: Past and Future. Curr Top Med Chem. 2016; 16:1231-41. | Article | PubMed
9. Tiwari M. Antimetabolites: established cancer therapy. J Cancer Ther. 2012; 5:810-9. | Article | PubMed
10. Capasso C and Supuran CT. Sulfa and trimethoprim-like drugs - antimetabolites acting as carbonic anhydrase, dihydroprotease synthase and dihydrofolate reductase inhibitors. J Enzyme Inhib Med Chem. 2014; 29:379-87. | Article | PubMed
11. Eriksson S, Munch-Petersen B, Johansson K and Eklund H. Structure and function of cellular deoxyribonucleoside kinases. Cell Mol Life Sci. 2002; 59:1327-46. | Article | PubMed
12. Zhang J, Visser F, King KM, Baldwin SA, Young JD and Coss CE. The role of nucleoside transporters in cancer chemotherapy with nucleoside drugs. Cancer Metastasis Rev. 2007; 26:85-110. | Article | PubMed
13. Chen LS, Plunkett W and Gandhi V. Polyadenylation inhibition by the triphosphates of deoxyadenosine analogues. Leuk Res. 2008; 32:1573-81. | Article | PubMed Abstract | PubMed FullText
14. Kuchta RD, Ilsley D, Kravig KD, Schubert S and Harris B. Inhibition of DNA primase and polymerase alpha by arabinofuranosynucleoside triphosphates and related compounds. Biochemistry. 1992; 31:4720-8. | Article | PubMed
15. Parker WB, Shaddix SC, Chang CH, White EL, Rose LM, Brockman RW, Shortney AJ, Montgomery JA, Secrist JA, 3rd and Bennett LL, Jr. Effects of 2-chloro-9-(2-deoxy-2-fluro-beta-D-arabinofuranosyl)adenine on K562 cellular metabolism and the inhibition of human ribonucleotide reductase and DNA polymerases by its 5’-triphosphate. Cancer Res. 1991; 51:2386-94. | Article | PubMed
16. Peters GL, van der Wilt CL, van Groeningen CJ, Smid K, Meijer S and Pinedo HM. Thymidylate synthase inhibition after administration of fluorouracil with or without leucovorin in colon cancer patients: implications for treatment with fluorouracil. J Clin Oncol. 1994; 12:2035-42. | Article | PubMed
17. Hill DL and Bennett LL, Jr. Purification and properties of 5-phosphoribosyl pyrophosphate amidotransferase from adenocarcinoma 755 cells. Biochemistry. 1969; 8:122-30. | Article | PubMed
18. Genini D, Adachi S, Chao Q, Rose DW, Carrera CJ, Cottam HB, Carson DA and Leoni LM. Deoxyadenosine analogs induce programmed cell death in chronic lymphocytic leukemia cells by damaging the DNA and by directly affecting the mitochondria. Blood. 2000; 96:3537-43. | Article | PubMed
19. Fauchild CR, Maybaum J and Kenneth KA. Concurrent unilateral chromatid damage and DNA strand breakage in response to 6-thioguanine treatment. Biochem Pharmacol. 1986; 35:3533-41. | Article | PubMed
20. Marinkovic G, Kroon J, Hoogenboom M, Hoeben KA, Ruiter MS, Kurakula K, Otermin Rubin I, Vos M, de Vries CJ, van Buul JD and de Waard V. Inhibition of GTPase Rac1 in endothelium by 6-mercaptopurine results in immunosuppression in nonimmune cells: new target for an old drug. J Immunol. 2014; 192:4370-8. | Article | PubMed
21. Swann PF, Waters TR, Moutlon DC, Xu YZ, Zheng Q, Edwards M and Mace R. Role of postreplicative DNA mismatch repair in the cytotactic action of thioguanine. Science. 1996; 273:1109-11. | Article | PubMed
22. Hostetler KY, Stuhmiller LM, Lenting HB, van den Bosch H and Richman DD. Synthesis and antiretroviral activity of phospholipid analogs of azidothymidine and other antiviral nucleosides. J Biol Chem. 1990; 265:6112-7. | Article | PubMed
23. Macias J, Neukam K, Mallolas J, Lopez-Cortes LF, Carter JA, Domingo P, Moreno S, Iribarren JA, Cebotet B, Crespo M, de Los Santos I, Ortega E, et al. Use of reprogrammed cells to identify therapy for respiratory papillomatosis. N Engl J Med. 2012; 367:1220-7. | Article | PubMed

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24. Momparler RL, Bouffard DY, Momparler LF, Dionne J, Belanger K and Ayoub J. Pilot phase I-II study on 5-aza-2'-deoxycytidine (Decitabine) in patients with metastatic lung cancer. Anticancer Drugs. 1997; 8:358-68. | Article | PubMed
25. Arce C, Segura-Pacheco B, Perez-Cardenas E, Taja-Chayeb L, Candelaria M and Duennas-Gonzalez A. Hydralazine target: from blood vessels to the epigenome. J Transl Med. 2006; 4:10. | Article | PubMed Abstract | PubMed FullText
26. Dragovic G and Jevtovic D. The role of nucleoside reverse transcriptase inhibitors usage in the incidence of hyperlactatemia and lactic acidosis in HIV/AIDS patients. Biomed Pharmacother. 2012; 66:308-11. | Article | PubMed
27. Borland KM, AbdulSalam SF, Solivio MJ, Burke MP, Wolfkiel PR, Lawson SM, Stockman CA, Andersen JM, Smith S, Tolstolutskaya JN, Gurjar PN, Bercz AP, Merino EJ and Litosh VA. Base-modified thymidines capable of terminating DNA synthesis are novel bioactive compounds with activity in cancer cells. Bioorg Med Chem. 2015; 23:1869-81. | Article | PubMed Abstract | PubMed FullText
28. Litosh VA, Wu W, Stupi BP, Wang J, Morris SE, Hersh MN and Metzker ML. Improved nucleotide selectivity and termination of 3’-OH unblocked reversible terminators by molecular tuning of 2-nitrobenzyl alkylated HOMedU triphosphates. Nucleic Acids Res. 2011; 39:e39. | Article | PubMed Abstract | PubMed FullText
29. Borland KM, Lawson SM, Ventura S, Merino EJ and Litosh VA. Novel modified nucleobases that show cytotoxicity towards breast cancer cells. Abstracts of Papers, 248th ACS Nat’l Meeting Exp., San Francisco, CA, United States, August 10-14, 2014, MED1-469.
30. Broekema FI and Dikkers FG. Side-effects of cidofovir in the treatment of recurrent respiratory papillomatosis. Eur Arch Otorhinolaryngol. 2008; 265:971-9. | Article | PubMed Abstract | PubMed FullText
31. Soma MA and Albert DM. Cidofovir: to use or not to use? Curr Opin Otolaryngol Head Neck Surg. 2008; 16:86-90. | Article | PubMed

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