ISSN 2073-2244
www.alb-shkenca.org

AKTET
Journal of Institute Alb-Shkenca
Revistë Shkencore e Institutit Alb-Shkenca

SYNTHESIS OF SOME NEW ACYCLIC PYRIMIDINE NUCLEOSIDES
SINTEZA E DISA NUKLEOZIDEVE TË REJA ACIKLIKE TË PIRIMIDINËS

AFËRDITA NURA-LAMA[a],* MUHAMET BICAJ[b] AND RAMIZ HOTI[b],
[a]University of Prishtina, Faculty of Mining and Metallurgy, Parku industrial Trepça, Mitrovica, Kosovo
[b]University of Prishtina, Department of Chemistry, Faculty of Natural Sciences, Pristina, Kosovo
Email: dita.nura49@gmail.com

ABSTRACT

A new acyclic nucleosides substituted at 5-position of the uracil ring have been synthesized. We synthesized 5-(bromomethyl)-1-(2,3-O-acetyl-2,3-dihydroxypropyl)uracil (2) and with the nucleophilic displacement with sodium cyanide we prepared the corresponding 5-(cyanomethyl)-1-(2,3-O-acetyl-2,3-dihydroxypropyl)uracil.(3) From this compound we synthesized other new 5-substituted acyclic nucleosides. Hydrogenation of (3) in anhydrous acetic acid over Pd/C catalyst gave the 5-acetaminoethyl-substituted derivative (4), the methanolysis afforded 5-(methoxycarbonyl) methyl derivative (5) and acidic hydrolysis gave the 5-(amidomethyl)-1-(2,3-O-acetyl-2,3-dihydroxypropyl)uracil(6). All compounds were characterized by 1H and 13C NMR, IR and by elementary analysis.

PERMBLEDHJE

Janë sintetizua disa aciklo nukleozide te reja të zëvendësuara në pozicion -5. Është sintetizua 5-(bromometil)-1-(2,3-O-acetil-2,3-dihidroksipropil)uracil (2) dhe me çvendosjen nukleofile me cianur natriumi kemi përgaditë 5-(cianometil)-1-(2,3-O-acetil-2,3-dihidroksipropil)uracil-in përkatës (3). Nga ky komponim janë sintetizua nukleozidet tjera aciklike të zëvendësuara në pozicion-5. Hidrogjenimi katalitik i komponimit (3) mbi Pd/C në acid acetik anhider ka dhënë 5-acetaminoetil-derivatin e zëvendësuar (4), metanoliza jep 5-(metoksikarbonil) metil derivat (5) dhe hidroliza acide jep 5-(amidometil)-1-(2,3-O-acetil-2,3-dihidroksipropil)uracil(6). Te gjitha komponimet jane karakterizua me 1H dhe 13C NMR, IR dhe me analizën elementare.

Key Words: acyclic nucleosides, 5-substituted nucleosides, pyrimidine nucleosides

INTRODUCTION

A respectable number of pyrimidine nucleosides analogues as derivative of natural pyrimidine exhibits biological activity. Consequently, modified nucleosides are studied for their potential activity as enzyme inhibitors resulting in antiviral and antitumor[1] and antitumor[2] activity. In particular, uracil derivatives substituted at C-5 position or modified at the furanose ring present strong biological activitiy such as the well known (*E*)-5-(2-bromovinyl)-dUrd (BVDU)[3]. 5-Fluorouracil (FU) cream is in the clinical use for the treatment of the'actinic keratosis'[4]. On the other hand, 5-FU pyrimidine acyclonucleosides was analyzed to find fewer side effects and the safe limits expansion [5] and a characteristic biochemical modulator 5-cloro-2,4-dihhydroxypyridine was found as a potent inhibitor of degradation of 5-FU *in vivo* [6] which is active for the treatment of gastric, colorectal, head, neck and other solid tumors[7]. Compound 5-bromo deoxyuridine derivative shows a broad spectrum of antiherpes activity towards herpes simplex virus type-1 and type-2 (HSV-1, HSV-2), varicella zoster virus (VZV), and human cytomegalovirus (HCMV)[8] Recently 5-substituted pyrimidine nucleosides have drawn attention and were valued for their antiviral activity against poxviruses[9] A series of acyclic nucleoside analogues of 5'-O-tritylthymidine (such as 5-jodouracil, 5-ethyluracil, 5-methylcytosine, 3-N-methylthymine,) were synthesized and valued as potential human mitochondrial thymidine kinase (TK-2) inhibitors [10] and some compounds showed a marked specificity. The esterified acetic acid chain was isolated from yeast t-RNA and was identified as 2-tio-5-uridineacetic acid methyl ester [11]. 5-Methoxymethyl -2'-deoxyuridine (MMUdR) was found to have potent antiviral activity against herpes simplex virus type-1, and was not toxic to host cells with antiviral concentrations and greater[12] than 100, and N4-Butanoyl-5-methoxymethyl-2'-

deoxycytidine is a potent inhibitor of HSV-1 replication[13]. Synthesis of 5-substituted acyclic pyrimidine analogue represents a significant synthetic challenge for the discovery of a potential drug. With respect to found biological activity it appeared of interest to synthesize the present acyclic pyrimidine nucleosides specifically substituted in 5-position at pyrimidine base.

MATERIALS AND METHOD

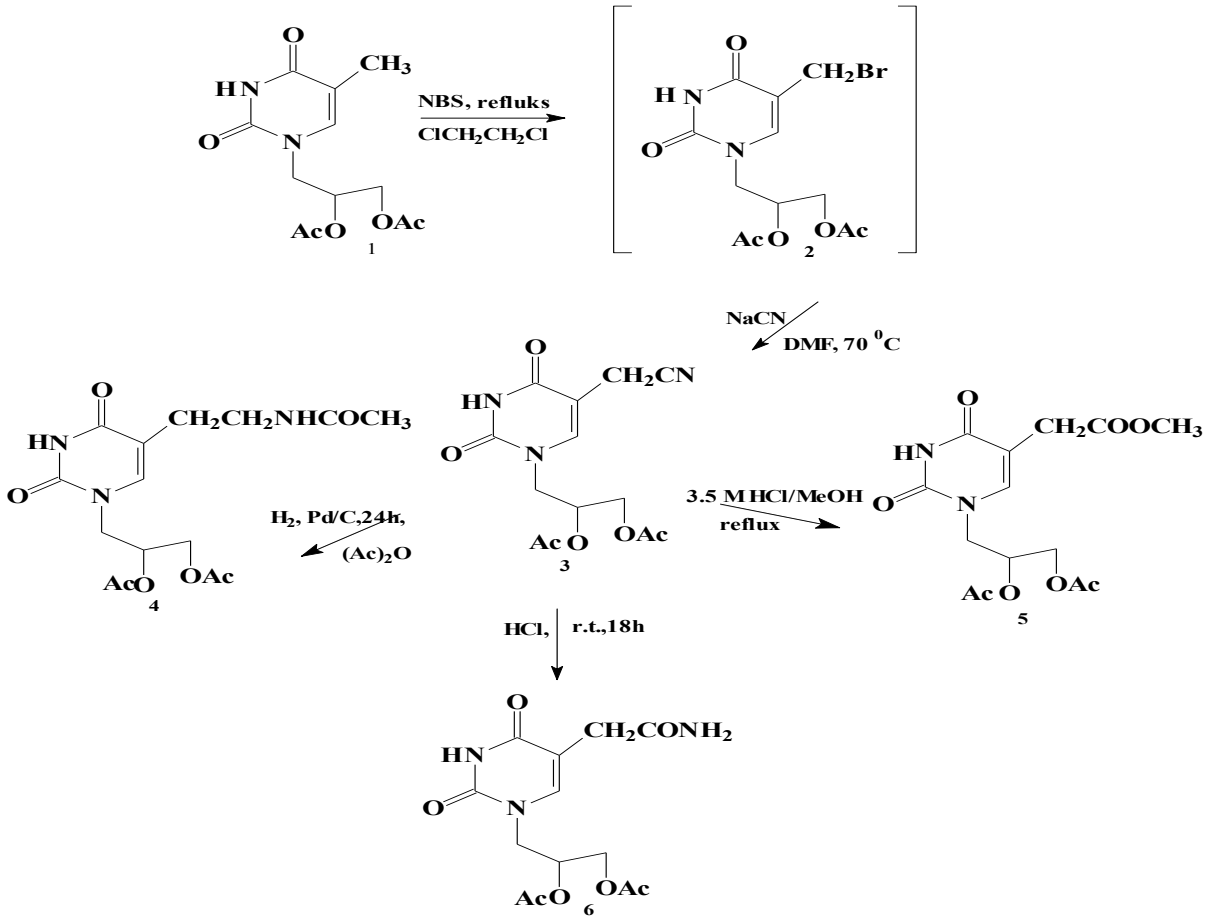
Melting points, uncorrected, were taken with a Kofler hot-stage apparatus. IR spectra were determined for potassium bromide pellets on a Perkin Elmer 297 spectrophotometer; ^{1}H-NMR and ^{13}C-NMR spectra for solutions in DMSO-d_6, unless otherwise stated, were recorded on a "JEOL FX90Q" spectrometer operating at 89.55 and 22.5 MHz, respectively with tetramethylsilane as internal standard (s, d, t and q refer to off-resonance decoupled spectra). The silica gel (Merck, HF_{254} type 60) for TLC and preparative TLC was activated at 110 °C for 60 min. The products were developed in CH_2Cl_2/MeOH (9:1) and recovered from TLC chromatographic plates with acetone, unless otherwise stated. The products were rendered visible by UV illumination or iodine vapour.

5-(Cyanomethyl)-1-(2,3-*O*-acetyl-2,3-dihydroxypropyl)uracil (3) To a solution of 1 (1.088g, 4.38 mmol) in 1,2-dichloroethane, NBS (880 mg, 496 mmol) was added. The mixture was refluxed with use of a 500-W reflecting photo-lamp under dry nitrogen atmosphere for 8 h. The solution of the 5-bromomethyl derivate 2 which has been formed was evaporated quickly to dryness under reduced pressure, and a solution of dried NaCN (234 mg, 4 mmol) in anhydrous DMF (35 mL) was added. The mixture was stirred at 70 °C for 2 h under a N_2-atmosphere and the solvent evaporated to dryness under reduced pressure. The crude product was dissolved in CH_2Cl_2 and filtered off. The filtrate was partitioned between water and a 20% solution of $Na_2S_2O_3$. The organic layer was dried (Na_2SO_4) and the solvent removed under reduced pressure. The oily residue was purified by preparative TLC (ethyl acetate) to give the product (680 mg, 55%); mp 170-171 °C (from methanol).IR: ν = 3455, 3060, 2250, 1731, 1680, 1471, 1374, 1234, 1050, 959, 764, 606, 420 cm^{-1}. ^{1}H NMR: δ = 2.01 (s, 3 H, Me), 2.08 (s, 3 H, Me), 3.48 (s, 2 H, H-7), 3.82 (dd, 1 H, H_b-1', $J_{b,2'}$ = 8.4 Hz, $J_{b,a}$ = 14.4 Hz), 4.05 (dd, 1 H, H_a-1', $J_{a,2'}$ = 3.6 Hz, $J_{a,b}$ = 14.4 Hz), 4.12 (dd, 1 H, $H_{b\text{-}3'}$, $J_{b,2'}$ = 6 Hz, $J_{b,a}$ = 12 Hz), 4.24 (dd, 1 H, H_a-3', $J_{a,2'}$ = 3.6 Hz, $J_{a,b}$ = 12.4 Hz), 5.25-5.20 (m, 1 H, H-2'), 7.72 (s, 1 H, H-6), 11.6 (s, 1 H, NH). ^{13}C NMR: δ = 20.57 (q, Me), 20.63 (q, Me), 48.08 (t, C-1'), 14.79 (t, C-7), 62.64 (t, C-3'), 68.74 (d, C-2'), 103.62 (s, CN), 144.03 (d, C-6), 150.73 (s, C-2), 162.69 (s, C-4), 118.17 (s, C-5), 169.84 (s, CO-Me), 170.12 (s, CO-Me). *Anal.* $C_{13}H_{15}N_3O_6$ (309.27), calcd. C, 50.48; 4.88; N, 13.58; found: C, 50.23; H, 5.00; N, 13.58%.

5-(Acetaminoethyl)-1-(2,3-*O*-acetyl-2,3-dihydroxypropyl)uracil (4) To a solution of 3 (100 mg, 0.32 mmol) in acetic anhydride (10 mL), 10% Pd/C (200 mg) was added. The mixture was stirred under (0.35 M Pa) of H_2 at room temp. for 24 h. The catalyst was filtered off and the filtrate evaporated to dryness. Preparative TLC (2 developments in CH_2Cl_2/ MeOH 9:1) gave the white crude product (62 mg, 54%); mp112-4 °C (from MeOH). IR: ν = 3366, 3160, 3040, 2920, 2830, 1750, 1730, 1690(s), 1680, 1650, 1550, 1475, 1410, 1373, 1345, 1275, 1255, 1235, 1175, 1070, 1048, 1022, 958, 875 cm^{-1}. ^{1}H NMR: δ = 1.77 (s, 3 H, OCH_3), 1.98 (s, 3 H, Me), 2.02 (s, 3 H, Me), 2.29 (t, 2 H, H-7, J = 6.7 Hz), 3.08-3.12 (2 H, m, H-8), 4.21-3.82 (4 H, m, H_2-1', H_2-3'), 5.18-5.30 (1 H, m, H-2'), 7.38 (1 H, s, H-6), 7.64-7.78 (1 H, m, NHCO), 11.17 (1 H, s, 3-NH). ^{13}C NMR: δ = 170.09 (s, NHCOMe), 169.75 (s, COMe), 169.07 (s, COMe), 163.88 (s, C-4), 150.95 (s, C-2), 144.07 (d, C-6), 110.27 (s, C-5), 68.96 (d, C-2'), 62.64 (t, C-3'), 47.63 (t, C-1'), 39.84 (t,C-8), 26.69 (t, C-7), 22.68 (q, NHCOCH$_3$), 20.65 (q, Me), and 20.54 (q, Me). Anal. $C_{15}H_{21}N_3O_7$ (355.34), calcd. C, 50.70; H, 5.96; N, 11.83; found: C, 50.56; H, 6.25; N, 11.64%.

5-[(Methoxycarbonyl)methyl]-1-(2,3-*O*-acetyl-2,3-dihydroxypropyl)uracil (5) A solution of 3 (60 mg, 0.19 mmol) in anhydrous methanolic HCl (40 mL, 3.5 M) was refluxed over night. The solvent was evaporated to dryness under reduced pressure and the residue dissolved in cooled (0 °C) anhydrous pyridine (3 mL). Acetic anhydride (1 mL) was added and the mixture stirred at 5 °C 16 h. and one hour in room temp. Then anhydrous MeOH (2 mL) was added, stirred for 1 h, and the solvent removed under reduced pressure. The product was isolated in 53% yield; mp 219-220.5 °C (from CH_2Cl_2). IR: ν = 3125, 3090, 3010, 2920, 2820, 1720, 1675, 1470, 1440, 1400, 1370, 1360, 1330, 1250, 1240, 1230, 1205, 1165, 1130, 1040, 980, 945, 925, 880, 790, 770, 750 cm^{-1}. ^{1}H NMR: δ = 11.43 (s, 1 H, 3-NH), 7.58 (s, 1 H, H-6), 5.23-5.17 (m, 1 H, H-2'), 4.25-4.21 (m, 1 H, H_a-3'), 4.10 (dd, 1 H, H_b-3', $J_{b,2'}$ = 5.7 Hz, $J_{b,a}$ = 12 Hz), 4.03-4.01 (m, 1 H, H_a-1'), 3.75 (dd, 1 H, H_b-1', $J_{b,2'}$ = 8.8 Hz, $J_{b,a}$ = 14 Hz), 3.58 (s, 3 H, OCH_3), 3.25 (s, 2 H, H-7), 2.02 (s, 3 H, Me), 1.98 (s, 3 H, Me). ^{13}C NMR: δ = 171.00 (s, OOMe),170.14 (s, COMe), 169.86 (s, COMe), 163.53 (C-4), 150.85 (C-2), 144.07 (C-6), 106.90 (C-5), 68.57 (C-2'), 62.67 (C-3'), 31.29 (C-7), 48.09 (C-1') 20.56 (Me), 51.75 (OCH_3). *Anal.*

$C_{14}H_{18}N_2O_8$ (342.29), calcd. C, 44.44; H, 5.86; N, 7.41;
found: C, 44.68; H, 5.62; N, 7.27%.



Scheme 1

5-(Amidomethyl)-1-(2,3-*O*-acetyl-2, 3-dihydroxypropyl) uracil (6) Compound 3 (120 mg, 0.38 mmol) was treated with conc. HCl (1 mL). The mixture was stirred at room temp. for 18 h and evaporated to dryness. The residue was dissolved in anhydrous pyridine (5 mL), cooled to 5 °C, and acetic anhydride (2 mL) was added. The suspension was then stirred at room temp. for 4 h and evaporated to dryness. Preparative TLC (3 developments in CH_2Cl_2/MeOH 95:5) gave the product (96 mg, 76%); mp 197-199 °C (from MeOH).IR: ν = 3442, 3041, 2804, 2362, 2341, 2043, 1739, 1672, 1471, 1407, 1433, 1371, 1342, 1245, 1172, 1084, 1047, 1020, 972, 953, 907, 880, 810, 786, 762, 604, 567, 467 cm^{-1}. ^{1}H NMR: δ = 11.33 (s, 1 H, 3-NH), 7.48 (s, 1 H, H-6), 7.28 (s, 1 H, $CONH_2$), 6.87 (s, 1 H, $CONH_2$), 5.21-5.23 (m, 1 H, H-2'), 1.99 (s, 3 H, Me), 2.03 (s, 3 H, Me), 3.04 (d, 1 H, H_a-7, $J_{a,b}$= 16 Hz), 2.94 (d, 1 H, H_b-7, $J_{b,a}$ = 16 Hz), 4.24 (dd,1 H, H_a-3', $J_{a,2'}$ = 3.2 Hz, $J_{a,b}$ = 12.4 Hz), 4.10 (dd, 1 H, H_b-3', $J_{b,2'}$ = 5.6 Hz, $J_{b,a}$ = 12.4 Hz), 4.03 (dd, 1 H, H_a-1', $J_{a,2'}$ = 3.2 Hz, $J_{a,b}$ = 14 Hz), 3.75 (dd, 1 H, H_b-1', $J_{b,2'}$ = 8.8 Hz; $J_{b,a}$ = 14 Hz). ^{13}C NMR: δ = 20.57 (q, Me), 32.53 (t, C-7), 47.92(t, C-1'), 62.65 (t, C-3'), 68.72 (d, C-2'), 107.99 (s, C-5), 143.80 (s, C-6), 150.92 (s, C-2), 163.79 (s, C-4), 169.96 (s, COMe), 170.14 (s, COMe), 171.42 (s, $CONH_2$).*Anal.* $C_{13}H_{17}N_3O_7$ (327.29), calcd. C, 47.71; H, 5.23; N, 12.84; found: C, 47.77; H, 5.30; N, 12.73%.

RESULTS AND DISCUSSIONS

In the course of preparing aliphatic C-5 substituted uracil derivatives we starting from thymine. With a coupling reaction of silylated base with allyl bromide we prepared the 1-allylthymine and cis-hydroxylation of this compound using $KMnO_4$ as oxidant afforded 1-(2,3-dihydroxypropyl) thymine[14]. The reaction with acetic anhydride in pyridine gave protected derivative1-(2,3-diaciloxypropyl)thymine (1). In order to convert the compound 1 into the 5-(bromomethyl)-1-(2,3-dihydroxypropyl)uracil (2), we applied selective bromination of 1, (Scheme 1), with N-bromosuccinimide (NBS) using a reflecting photo-lamp under nitrogen atmosphere. Synthesis of 5-cyanomethyl-derivative 3 was realized in situ, without purifying the compound 2. The nucleophilic displacement with sodium cyanide yielded the corresponding 5-(cyanomethyl)-1-(2,3-*O*-acetyl-2,3-dihydroxypropyl)uracil (3) in 55%. The IR-spectrum of 3 produced an expected band at 2250 cm^{-1} for CN, and the ^{13}C and ^{1}H NMR spectra gave rise to resonances at 103.62 (s) and 3.48 ppm (s, 2 H), respectively, indicating the presence of the cyanomethyl group. Catalytic hydrogenation of cyanomethyl derivative 3 in anhydrous acetic acid over 10% Pd/C catalyst gave the 5-(Acetaminoethyl)-1-(2,3-*O*-acetyl-2,3-dihydroxypropyl)uracil (4) in 54% yield. The ^{13}C and ^{1}H NMR spectra of compounds originating from NH-$COCH_3$ group were observed at 1.77 (s, 3H, $COC\underline{H}_3$), 7.64-7.78 (m, 1H, N<u>H</u>CO) and 22.68 for (-CH_3), 170.09 for (-CO-), respectively, while the signal representing cyano group was absent. Methanolysis of 3 in methanolic hydrochloric acid refluxed over night and yielded 5-[(Methoxycarbonyl) methyl]-1-(2,3-*O*-acetyl-2,3-dihydroxypropyl)uracil (5) in 53%. The ^{13}C and ^{1}H NMR spectra of compounds 5 displayed singlet for methyl protons at 3.58 ppm and at 51.75 ppm for (-OCH_3). Acidic hydrolysis of the nitrile 3 with conc. hydrochloric acid gave the 5-(Amidomethyl)-1-(2,3-*O*-acetyl-2, 3-dihydroxypropyl) uracil (6) in 76% yields. The signal belonging to amide group was observed at 3442 (NH st), 1739-1672 cm^{-1}(CO) in the IR spectra. Two displayed additional signals in 1H NMR, belonging to the -CO–NH_2 group at 7.28 and 6.87 ppm , and doublet for methylenic protons (7-CH_2) at 3.04 and 2.94 ppm with coupling constant J = 16 Hz. ^{13}C NMR specter displayed signal at 171.42 ppm belonging to amide group (-$CONH_2$) and at 32.53 ppm(t) signal for (7-CH_2 $CONH_2$). Those new 5-substituted compounds are candidate for their biological activity.

REFERENCES

1.R. K. Robins and G. R. Revankar, *Antiviral Drug Development*, E. De Clercq and R. T. Walker (Eds.), Plenum: New York, 1988, p. 11, b) E. De Clercq , Molecular targeted for antiviral Agents, J. Pharmacol. Exp. Ther. 297, 1-10, (2001)

2.M. MacCoss and M. J. Robins, Chemistry of Antitumor Agents, D. E. V. Wilman (Ed.), Blackie and Son: U. K. 1990, p. 261. b) R. K. Robins and G. D. Kinin, ibid. p. 299

3.Jan Balzarini, Christina Bohman, and Erik De Clercq, Differential Mechanism of Cytostatic Effect of (E)-5-(2-Bromovinyl)-2'-deoxyuridine, 9-(1,3-Dihydroxy-2-propoxymethyl)guanine, and Other Antiherpetic Drugs on Tumor Cells Transfected by the Thymidine Kinase Gene of Herpes Simplex Virus Type 1 or Type 2, The Journal of Biological Chemistry, Vol. 268, No. 9, pp. 6332-6337,1993

4.D. de Berker, JM McGregor and BR Hughes, Guidelines for the management of Actinic Keratoses, BJD, Vol. 156, No. 2, February 2007 (p222-230) .

5.Rosowsky A, Kim. Sh, Wick M. Synthesis and antitumor activity of an acyclonucleoside derivative of 5-fluorouracil. , J. Med. Chem. 1981, 24(10), 1177-81

6.Shirasaka T, Shimamoto Y, Kato T, Fukushima M., Invention of a tumor-selective 5-fluorouracil derivative named S-1 by biochemical modulation of 5-fluorouracil, Gan To Kagaku Ryoho, 1998, 25(3), 371-84

7.Schöfski P., The modulated oral fluoropyrimidine prodrug S-1, and its use in gastrointestinal cancer and other solid tumors, Anticancer Drugs, 2004, 15(2): 85-106

8.Rakesh Kumar, Mahendra Nath, and D. Lorne J. Tyrrell, Design and Synthesis of Novel 5-Substituted Acyclic Pyrimidine Nucleosides as Potent and Selective Inhibitors of Hepatitis B Virus, J. Med. Chem., 2002, 45 (10), pp 2032–2040.

9.Eearl R. Kern, Mark N. Prichard, Debra C. Quenelle, Kathy A. Keith, Kamal N. Tiwari, Joseph A. Maddry, John A. Secrist, Activity of pyrimidine nucleosides against poxviruses, Antimicrob. Agents Chemother. (2008) Doi; 10. 1128/AAC. 01257-08.

10. Hernandez, A. I., Balzarini, J., Karlsson, A., Camarasa, M. J. and Perez-Perez, M. J. J. Acycle nucleoside analogues as novel inhibitors of human mitohondrial thymidine kinase, Med. Chem. 45, 4254-4263 (2002).

11. Lubomir Baczynskyj, K. Biemann, Sulfur-Containing Nucleoside from Yeast Transfer Ribonucleic Acid: 2-Thio-5(or 6)-uridine Acetic Acid Methyl Ester, Science, Vol. 159, 1482, (1967)

12. Babiuk LA, Meldrum B, Gupta VS, Rouse BT., Comparison of the antiviral effects of 5-methoxymethyl-deoxyuridine with 5-iododeoxyuridine, cytosine arabinoside, and adenine arabinoside., Antimicrob Agents Chemother. 1975 Dec;8(6):643-50.

13. Zoghaib WM, Mannala S, Gupta VS, Tourigny G, Reid RS, Synthesis, conformation, and antiviral activity of 5-methoxymethyl-2'-deoxycytidine analogs, Nucleosides Nucleotides Nucleic Acids, 2003 Feb; 22(2):223-38. PMID: 12744607
.

14. Nura-Lama A., Acta Chimica Kosovica, Hidroksimetilimi në sintezën e 5-hidroksimetil-1-(2,3-dihidroksipropil)uracilës, 13 (1), 21-28 (2004)