DOI 10.1007/s11094-016-1375-4
Pharmaceutical Chemistry Journal, Vol. 49, No. 12, March, 2016 (Russian Original Vol. 49, No. 12, December, 2015)

SEARCH FOR NEW DRUGS

SYNTHESIS, HYDROLYTIC STABILITY, AND ANTILEUKEMIC ACTIVITY OF AZACYTIDINE NUCLEOSIDE ANALOGS

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Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 49, No. 12, pp. 7 – 11, December, 2015.

Original article submitted October 28, 2014.

New azacytidine nucleoside analogs with modified carbohydrate moieties were synthesized. Screening identified a highly active 2′-fluoro-containing azacytidine analog that could potentially be of interest as an agent for treating acute myelogenous leukemia and myelodysplastic syndrome.

Keywords: myelodysplastic syndrome, azacytidine, glycosylation, fluoro-deoxynucleosides, hydrolytic stability, antileukemic activity.

Epigenetic therapy occupies a special place in myelodysplastic syndrome (MDS) therapy. The term "epigenetic" is understood to mean inherited mutations in cells that are not related to damage of gene nucleotide sequences and are reversible, e.g., DNA methylation [1]. One approach to MDS epigenetic therapy is the use of hypomethylating agents [2] such as azacytidine (5-azacitidine, Aza-C, Vidaza) and decitabine (2′-deoxy-5-azacytidine, Dacogen). These antimetabolites exert their hypomethylating effect through the N atom, which replaces the cytosine C-5 atom, makes addition of a methyl to this part of the cytidine analog impossible, and inhibits DNA methyltransferases. As a result, onco-suppressor gene functions and cell-cycle regulatory mechanisms in the hematopoietic myeloid lineage are restored [3, 4]. Currently, Aza-C is an effective therapeutic for treating MDS, acute myelogenous leukemia (AML), and chronic myelomonocytic leukemia without MDS symptoms. Furthermore, the clinical efficacy of the drug in combination with other treatments was proved against not only MDS but also solid tumors [5]. Broad use of Aza-C in medical practice is limited by the high general toxicity and hydrolytic degradation at physiological pH values [6, 7]. The goal of the present investigation was to find Aza-C nucleoside analogs with modified carbohydrates that possessed higher antileukemic activity than Aza-C, which is used in tumor chemotherapy.

Fluorine atoms in biologically active molecules are known to affect considerably their physicochemical and biological properties [8]. The increased biological activity can be explained by several factors. The affinity for lipophilic and also aqueous phases increases the permeability through cell membranes. The special biological properties of fluorinated nucleosides are also due to the ability to form F••H H-bonds, the electrostatic interaction, and the effects on the stereochemistry and conformation of the compounds. It is noteworthy that the F atom acts as a bioisostere of groups such as methyl, amine, and hydroxyl. Bioisosteric replacement is one approach used in medicinal chemistry to design more efficacious and safe drugs [9].

5′-Deoxy derivatives of pyrimidine nucleosides, which have a broad spectrum of antitumor activity and can accumulate selectively in tumor tissue (through the action of tumor angiogenesis factor thymidine phosphorylase), are especially interesting. This minimizes the systemic activity of the drug on healthy tissues [10].

We synthesized Aza-C and its nucleoside analogs that included modifications at the carbohydrate 2′-, 3′-, or 5′-positions. Condensation of 2,4-*bis*(trimethylsilyl)-5-azacytosine (**II**), which was synthesized from 5-azacytosine, with the sugars 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranoside (**Ia**); 1,2,3-tri-*O*-acetyl-5-deoxy-β-D-ribofuranoside (**Ib**); 1-*O*-acetyl-2,5-di-*O*-benzoyl-3-deoxy-3-fluoro-α/β-D-ribofu

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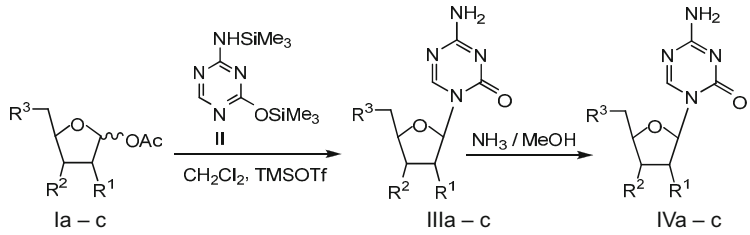
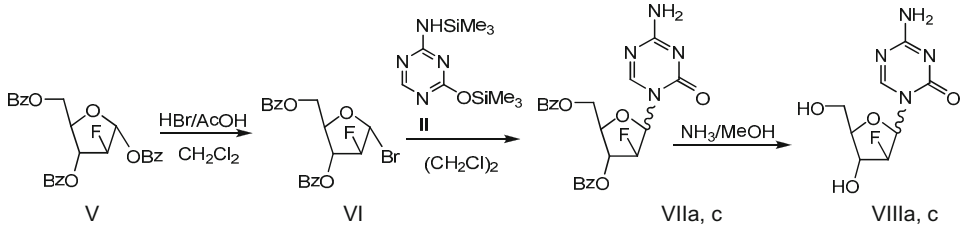
Scheme 1



Ia: $R^1 = R^2 = R^3 =$ OBz, β-isomer; **Ib**: $R^1 = R^2 =$ OAc, $R^3 =$ H, β-isomer; **Ic**: $R^1 = R^3 =$ OBz, $R^2 =$ F, α/β-isomers; **IIIa**: $R^1 = R^2 = R^3 =$ OBz; **IIIb**: $R^1 = R^2 =$ OAc, $R^3 =$ H; **IIIc**: $R^1 = R^3 =$ OBz, $R^2 =$ F; **IVa**: $R^1 = R^2 = R^3 =$ OH; **IVb**: $R^1 = R^2 =$ OH, $R^3 =$ H; **IVc**: $R^1 = R^3 =$ OH, $R^2 =$ F.

Scheme 2



VIIa, **VIIIa**: β-isomer; **VIIb**, **VIIIb**: α-isomer.

ranoside (**Ic**); or 3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranosyl bromide (**VI**) gave benzoyl derivatives **IIIa-c** and **VIIa** and **-b**. These were isolated pure via column chromatography over silica gel. Debenzoylation of intermediate nucleosides **IIIa-c** and **VIIa** and **-b** by ammonia in MeOH produced Aza-C (**IVa**) and its 5′-deoxyribo-, 3′-fluorodeoxyribo-, and 2′-fluorodeoxyarabino-analogs **IVb** and **-c** and **VIIIa** and **-b**, respectively (Schemes 1 and 2). The chemical structures of the synthesized nucleosides were elucidated using PMR and ^{19}F NMR data (Table 1) and UV and mass spectrometry. CD spectroscopy established the stereochemical features of the α/β-fluoronucleoside Aza-C analogs [11].

EXPERIMENTAL CHEMICAL PART

UV spectra in the range 200 – 300 nm were taken on a Varian Cary 100 spectrophotometer (USA). NMR spectra were obtained on a Bruker Avance-500 spectrometer (USA) at operating frequency 500 MHz for PMR and 470 MHz for ^{19}F NMR. Mass spectra were obtained in a LC-MS system using an Accela HPLC and LCQ Fleet triple quadrupole ion-trap mass detector (Thermo Electron) with electrospray ionization. CD spectra were taken on a Jasco J-20 spectropolarimeter (Japan).

General method for glycosylation of 5-azacytosine by 1-*O*-acyloxysugars Ia-c. A suspension of 5-azacytosine (0.16 g, 1.428 mmol) and ammonium sulfate (0.01 g) in hexamethyldisilazane (HMDS, 6 mL) was refluxed for 10 h. The resulting homogeneous solution was evaporated in vacuo to dryness. The residue was evaporated again with anhydrous toluene (10 mL). Solid **II** was treated with a solution of the appropriate acetate **Ia-c** (1.24 mmol) in anhydrous CH_2Cl_2 (6 mL), cooled to 0°C, treated with trimethylsilyltrifluoromethanesulfonate (0.26 mL, 1.438 mmol), stirred under Ar for 4 h at room temperature, diluted with CH_2Cl_2, and poured with stirring into cold saturated aqueous $NaHCO_3$. The organic layer and aqueous phase were extracted with CH_2Cl_2. The organic extract was dried and evaporated in vacuo to dryness. The solid was chromatographed over silica gel using first a mixture of EtOAc:hexane (1:3) and then EtOAc:hexane:MeOH (30:90:7). Fractions containing reaction products **IIIa-c** were combined and evaporated to dryness.

4-Amino-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,3,5-triazin-2(1*H*)-one (IIIa). Yield 65%, white stable foam. Mass spectrum, *m*/*z* (I_{rel}): 579.1 $[M + Na]^+$ (100), 445.1 (65), 595.1 $[M + K]^+$ (58).

4-Amino-1-(2,3-di-*O*-acetyl-5-deoxy-β-D-ribofuranosyl)-1,3,5-triazin-2(1*H*)-one (IIIb). Yield 80%, white stable foam. Mass spectrum, *m*/*z* (I_{rel}): 335.1 $[M + Na]^+$ (100), 201.1 (45), 351.1 $[M + K]^+$ (27).

4-Amino-1-(2,5-di-*O*-benzoyl-3-deoxy-3-fluoro-β-D-ribofuranosyl)-1,3,5-triazin-2(1*H*)-one (IIIc). Yield 67%, white stable foam. Mass spectrum, *m*/*z* (I_{rel}): 477.1 $[M + Na]^+$ (100), 493.1 $[M + K]^+$ (58), 343.1 (53), 150.1 (15).

Glycosylation of 5-azacytosine by bromide VI. A suspension of 5-azacytosine (0.215 g, 1.92 mmol) and ammonium sulfate (0.01 g) in HMDS (8 mL) was refluxed for 10 h. The homogeneous solution was evaporated in vacuo to

dryness. The residue was evaporated again with anhydrous toluene (10 mL). The residue of **II** was treated with a solution of bromide **VI** [prepared from 1,3,5-tri-*O*-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranoside (**V**, 0.7 g, 1.51 mmol)] in anhydrous 1,2-dichloroethane (7.5 mL), refluxed for 20 h under Ar, cooled, diluted with $CHCl_3$, and poured with stirring into ice water. The organic layer and aqueous phase were extracted with $CHCl_3$. The organic extract was dried and evaporated in vacuo to dryness until a thick stable foam formed. The residue was chromatographed over silica gel using first EtOAc and then $CHCl_3$:petroleum-ether:MeOH (15:7:1). Fractions containing reaction products **VIIa** and **-b** were combined and evaporated to dryness.

4-Amino-1-(3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-1,3,5-triazin-2(1*H*)-one (VIIa). Yield 82%, white stable foam. Mass spectrum, *m*/*z* (I_{rel}): 477.1 $[M + Na]^+$ (100), 455.1 $[M + H]^+$ (64), 493.1 $[M + K]^+$ (43), 343.1 (30), 150.1 (29).

4-Amino-1-(3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranosyl)-1,3,5-triazin-2(1*H*)-one (VIIb). Yield 5%, white amorphous powder. Mass spectrum, *m*/*z* (I_{rel}): 477.1 $[M + Na]^+$ (100), 455.1 $[M + H]^+$ (47), 493.1 $[M + K]^+$ (38), 343.1 (37), 150.1 (31).

General procedure for debenzoylation of nucleosides IIIa-c and VIIa and -b. A suspension of the appropriate nucleoside **IIIa-c** or **VIIa** or **-b** (0.318 mmol) in anhydrous MeOH (5 mL) was treated with MeOH (8 mL) saturated with dry NH_3 at 0°C, stirred for 7 h at room temperature, evaporated to dryness, and evaporated again with anhydrous MeOH. The solid was chromatographed over silica gel using a linear gradient of MeOH (0→30%, v/v) in $CHCl_3$. Fractions containing reaction product were combined and evaporated to dryness. The solid was crystallized from Et_2O.

TABLE 1. PMR and ^{19}F NMR Spectra of **III-IVa-c** and **VII-VIIIa** and **-b**

Compound	PMR spectrum,* δ, ppm, *J*, Hz	^{19}F NMR spectrum,* δ, ppm
IIIa	8.18 (c, 1H, H6), 8.08 – 7.33 (m, 15H, arom), 6.79 (br.s, 1H, NH[a]), 6.07 (d, 1H, J 4.0, H1′), 5.99 (t, 1H, H2′), 5.91 (m, 1H, J 4.0, J 5.9, H3′), 5.78 (br.s, 1H, NH[a]), 4.81 (dd, 1H, J 3.0, J 12.0, H5′), 4.77 (m, 1H, H4′), 4.71 (dd, 1H, J 4.7, H5″)	–
IIIb	8.37 (c, 1H, H6), 7.72 (d, 2H, J 22.0, NH_2), 5.60 (d, 1H, J 3.5, H1′), 5.54 (m, 1H, J 6.3, H2′), 5.17 (t, 1H, H3′), 4.06 (m, 1H, H4′), 2.06 (d, 6H, J 2.3, OAc,), 1.31 (d, 3H, J 6.2, CH3)	–
IIIc	8.08 (c, 1H, H6), 8.08 – 7.44 (m, 10H, arom), 7.02 (br.s, 1H, NH[a]), 5.96 (d, 1H, J 4.5, H1′), 5.78 (br.s, 1H, NH[a]), 5.77 (m, 1H, J 10.1, H2′), 5.67 (dt, 1H, J 53.1, H3′), 4.73 (dd, 1H, J 3.5, H5′), 4.69 (dm, 1H, J 20.8, H4′), 4.63 (dd, 1H, J 4.8, J 12.0, H5″)	– 202.38 (m, FC3′)
VIIa	8.30 (d, 1H, J 1.6, H6), 8.08 – 7.44 (m, 10H, arom), 6.53 (br.s, 1H, NH[a]), 6.37 (dd, 1H, J 2.4, J 21.5, H1′), 5.87 (br.s, 1H, NH[b]), 5.62 (dd, 1H, J 16.3, H3′), 5.42 (dd, 1H, J 49.7, H2′), 4.81 (dd, 1H, J 5.3, J 12.0, H5″), 4.76 (dd, 1H, J 3.6, H5′), 4.55 (m, 1H, H4′)	– 201.27 (m, FC2′)
VIIb	8.46 (s, 1H, H6), 8.02 – 7.51 (m, 12H, arom, NH_2), 6.11 (d, 1H, J 16.4 Hz, H1′), 5.79 – 5.67 (m, 2H, J 50.8, J 18.0, H2′, H3′), 5.22 (m, 1H, H4′), 4.61 (dd, 1H, J 3.9, J 12.0, H5′), 4.55 (dd, 1H, J 5.4, H5″)	– 186.58 (m, FC2′)
IVa	8.58 (s, 1H, H6), 7.53 (d, 2H, J 11.0, NH_2), 5.66 (d, 1H, J 3.6, H1′), 5.43 (d, 1H, J 5.0, 2′OH), 5.12 (t, 1H, J 4.9, 5′OH), 5.04 (d, 1H, J 5.8, 3′OH), 4.08 – 3.98 (m, 2H, H2′, H3′), 3.84 (m, 1H, H4′), 3.68 (m, 1H, H5′), 3.54 (m, 1H, H5″)	–
IVb	8.25 (s, 1H, H6), 7.57 (d, 2H, J 4.8, NH_2), 5.56 (d, 1H, J 3.7, H1′), 5.34 (d, 1H, J 4.7, 2′OH), 5.02 (d, 1H, J 5.9, 3′OH), 4.15 (m, 1H, J 5.2, H2′), 3.84 (m, 1H, H3′), 3.74 (m, 1H, H4′), 1.27 (d, 3H, J 6.3, $\tilde{N}H_3$)	–
IVc	8.49 (s, 1H, H6), 7.66 (d, 2H, J 11.9, NH_2), 5.83 (d, 1H, J 6.0, 2′OH), 5.79 (d, 1H, J 7.2, H1′), 5.25 (t, 1H, J 5.1, 5′OH), 4.99 (dd, 1H, J 54.3, H3′), 4.45 (dt, 1H, J 23.4, H2′), 4.18 (dm, 1H, J 25.3, H4′), 3.61 – 3.60 (m, 2H, H5′, H5″)	– 199.43 (m, FC3′)
VIIIa	8.31 (s, 1H, H6), 7.63 (d, 2H, J 3.2, NH_2,), 6.04 (dd, 1H, J 3.7, J 17.5, H1′), 5.86 (d, 1H, J 4.8, 3′OH), 5.05 (t, 1H, J 5.8, 5′OH), 4.97 (dm, 1H, J 2.3, J 52.1, H2′), 4.18 (dm, 1H, J 14.4, H3′), 3.79 (m, 1H, H4′), 3.58 (m, 1H, H5′), 3.51 (m, 1H, J 12.0, H5″)	– 197.43 (m, FC2′)
VIIIb	8.23 (s, 1H, H6), 7.61 (d, 2H, J 10.0, NH_2,), 5.84 (dd, 1H, J 1.5, J 15.8, H1′), 5.77 (d, 1H, J 4.1, 3′OH), 5.18 (dt, 1H, J 50.9, H2′), 5.01 (t, 1H, J 5.6, 5′OH), 4.33 (m, 1H, H4′), 4.28 (dm, 1H, J 19.9, H3′), 3.49 – 3.47 m (2H, H5′, H5″)	– 186.58 (m, FC2′)

* **IIIa** and **-c** and **VIIa**, $CDCl_3$; **IVa-c**, **IIIb**, **VIIb**, and **VIIIa** and **-b**, DMSO-d_6.

4-Amino-1-β-D-ribofuranosyl-1,3,5-triazin-2(1*H*)-one (IVa). Yield 60%, white amorphous powder, mp 228 – 230°C (lit. 231 – 233°C [12]). UV spectrum (H_2O), λ_{max}, nm (logε): 241 (3.94). CD spectrum (H_2O), λ, nm ($[\theta \cdot 10^{-3}]$): 215 (+ 2.04), 250 (+ 10.82), 285 (0). Mass spectrum, *m*/*z* (I_{rel}): 267.1 $[M + Na]^+$ (100), 113.0 (81), 245.1 $[M + H]^+$ (24).

4-Amino-1-(5-deoxy-β-D-ribofuranosyl)-1,3,5-triazin-2(1*H*)-one (IVb). Yield 92%, white amorphous powder, mp 212 – 214°C. UV spectrum (H_2O), λ_{max}, nm (logε): 242 (3.77). Mass spectrum, *m*/*z* (I_{rel}): 251.1 $[M + Na]^+$ (100), 113.0 (52), 229.1 $[M + H]^+$ (23).

4-Amino-1-(3-deoxy-3-fluoro-β-D-ribofuranosyl)-1,3,5-triazin-2(1*H*)-one (IVc). Yield 67%, white amorphous powder, mp 237 – 239°C. UV spectrum (H_2O), λ_{max}, nm (logε): 241 (3.94). CD spectrum (MeOH), λ, nm ($[\theta \cdot 10^{-3}]$): 215 (0), 250 (+ 5.59), 280 (0). Mass spectrum, *m*/*z* (I_{rel}): 269.1 $[M + Na]^+$ (100), 113.0 (70), 247.1 $[M + H]^+$ (23), 285.0 $[M + K]^+$ (11).

4-Amino-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-1,3,5-triazin-2(1*H*)-one (VIIIa). Yield 75%, white amorphous powder, mp 204 – 206°C. UV spectrum (H_2O), λ_{max}, nm (logε): 241 (3.94). CD spectrum (MeOH), λ, nm ($[\theta \cdot 10^{-3}]$): 215 (+ 2.15), 245 (+ 7.54), 285 (0). Mass spectrum, *m*/*z* (I_{rel}): 269.1 $[M + Na]^+$ (100), 113.0 (59), 247.1 $[M + H]^+$ (33), 285.0 $[M + K]^+$ (27).

4-Amino-1-(2-deoxy-2-fluoro-α-D-arabinofuranosyl)-1,3,5-triazin-2(1*H*)-one (VIIIb). Yield 78%, white amorphous powder. UV spectrum (H_2O), λ_{max}, nm (logε): 241 (3.94). CD spectrum (MeOH), λ, nm ($[\theta \cdot 10^{-3}]$): 215 (– 3.45), 240 (– 4.63), 285 (0). Mass spectrum, *m*/*z* (I_{rel}): 269.1 $[M + Na]^+$ (100), 113.0 (59), 247.1 $[M + H]^+$ (33), 285.0 $[M + K]^+$ (27).

The hydrolytic stability was studied using an HPLC method [13]. Solutions of **IVa-c** and **VIIIa** of concentration 0.082×10^{-4} M were prepared in water for injection (pH 6.7), phosphate buffer (pH 3.2), and borate buffer (pH 10.2). HPLC chromatograms were obtained on a Waters chromatograph with a Waters 996 spectrophotometric detector. Chromatography used an EC 250/4.6 Nucleodur 100-5 C_{18} ec column (Macherey-Nagel AG) packed with octadecylsilyl silica gel for chromatography P (5 μm) and mobile phase of KH_2PO_4 (2.72 g/L) in MeOH adjusted to pH 6.8 using Et_3N (50:950, v/v) at flow rate 1 mL/min. Chromatograms were recorded at 241 nm. The injected sample volume was 5 μL; chromatography time, 20 min; column temperature, 25°C. Peaks of impurities were identified by comparing chromatograms of the test solution with those of dissolved USP standards 5-azacytosine, Aza-C, and 1-β-D-ribofuranosyl-3-guanylurea picrate. In addition, chromatograms of the test solution and degradation products were recorded on a LC-MS.

TABLE 2. Time Required to Reduce the Concentration of Aza-C and Its Nucleoside Analogs **VIIIa** and **IVb** by 10, 20, and 30% in Water for Injection at 21°C

Residual amount, %	Time, min			
	IVa (Aza-C)	**VIIIa** (2′F-ara-Aza-C)	**IVc**, (3′F-Aza-C)	**IVb** (5′-deoxy-Aza-C)
90	25 ± 1.2	43 ± 1.7*	12 ± 0.5*	65 ± 3.1*
80	80 ± 3.9	89 ± 4.2	30 ± 1.5	175 ± 8.2
70	350 ± 14.8	360 ± 15.1	75 ± 3.4	…

* Statistically significant difference vs. reference compound ($p < 0.05$).

EXPERIMENTAL BIOLOGICAL PART

Preliminary *in vitro* screening for antileukemic activity of synthesized **IVa-c** and **VIIIa** used standard study methods [14, 15]. Human tumor cell lines from the cell-culture collection of the RAS Institute of Cytology, St. Petersburg were used to study the specific cytotoxicity of the synthesized nucleosides. These were KG-1 (acute myelogenous leukemia), TPH-1 (acute monocytic leukemia), HL-60 (acute promyelocytic leukemia), K-562 (chronic myelogenous leukemia), and MOLT-3 (acute T-lymphoblastic leukemia). Cells were cultured in RPMI 1640 medium with 20% added fetal bovine serum for HL-60, KG-1, and MOLT-3 and 10%, for THP-1 and K-562.

Cytotoxic activity was studied at concentrations 0.01 – 50.0 μM. The reference drug was Aza-C drug substance prepared by the developed method. Solutions (10 μL) of test compounds were placed in triplicate into wells of a 96-well plate with leukemia cells (1.5×10^5 cells/mL) and incubated at 37°C in a 5% CO_2 atmosphere for 48 and 72 h. When the cultivation with the test nucleosides was finished,

TABLE 3. Specific Cytotoxicity of New Aza-C Nucleoside Analogs in Human Hemoblastosis Cell Culture

Leukemia cell line	Mean effective concentration (EC_{50}, μM) of tested compounds			
	IVa (Aza-C)	**VIIIa** (2′F-ara-Aza-C)	**IVc** (3′F-Aza-C)	**IVb** (5′-deoxy-Aza-C)
KG-1	3.66 ± 0.10	0.35 ± 0.06*	> 1.0	> 10.0
TPH-1	4.25 ± 0.25	0.51 ± 0.01*	> 10.0	> 50.0
HL-60	5.37 ± 0.07	0.58 ± 0.05*	> 10.0	> 50.0
K-562	4.64 ± 0.15	> 10.0	> 50.0	> 50.0
MOLT-3	i/a#	i/a#	i/a#	i/a#

* Statistically significant difference vs. reference compound ($p < 0.05$); # i/a, inactive.

each well was treated with MTT solution in phosphate buffer and incubated for an additional 4 h. Then, cells were precipitated by centrifugation. The medium was removed from the wells. Each well was treated with DMSO (150 μL) to dissolve formazan crystals. The optical density of the dissolved formazan in the multi-well plates was measured on a Vityaz? reader (Rep. Belarus) at 570 nm.

The average of three parallel measurements was calculated for each concentration of test compound. The degree of cell growth suppression was plotted as a function of the logarithm of the concentration. The mean effective concentration for 50% suppression of the viability of the leukemia cells (EC_{50}) was determined graphically for active compounds. Table 3 presents the results for the specific *in vivo* cytotoxic activity of the Aza-C nucleoside analogs. Results were processed statistically using the Student *t*-criterion.

RESULTS AND DISCUSSION

The studies showed that nucleoside analog **VIIIa** (2′F-ara-Aza-C) exhibited substantially greater inhibition of the viability for three lines of human acute myelogenous leukemia models than analogs **IVc** (3′F-Aza-C) and **IVb** (5′-deoxy-Aza-C). The cytotoxicity of this nucleoside was also significantly greater than that of the currently widely used antileukemic drug Aza-C.

Nucleoside analog 2′F-ara-Aza-C, like reference drug Aza-C, suppressed selectively and dose-dependently the viability of cell lines KG-1 acute myelogenous leukemia, TPH-1 acute monocytic leukemia, and HL-60 acute promyelocytic leukemia. Chronic myelogenous leukemia K-562 cells exhibited low sensitivity to 2′F-ara-Aza-C whereas Aza-C in the K-562 model exhibited practically the same cytotoxicity as against the three acute myelogenous leukemia cell lines. Acute T-lymphoblastic leukemia MOLT-3 cells were completely resistant to the new nucleoside analog and Aza-C.

It is noteworthy that nucleoside analog 2′F-ara-Aza-C exhibited pronounced cytotoxicity against acute myelogenous leukemia cells at a concentration of 0.1 μM whereas the threshold concentration for Aza-C was 1.0 μM. A saturation effect was attained if the concentration of the test nucleoside was increased to 1.0 μM. The saturating concentration of Aza-C was an order of magnitude greater (10.0 μM) compared with that of 2′F-ara-Aza-C. The EC_{50} values, which represent the nucleoside concentrations that inhibit by 50% compared with a control the viability of leukemia cells, were 0.35 μM (KG-1), 0.51 μM (TPH-1), 0.58 μM (HL-60) for 2′F-ara-Aza-C and 3.66 μM (KG-1), 4.25 μM (TPH-1), and 5.37 μM (HL-60) for Aza-C. This indicated that the specific cytotoxicity of the new Aza-C analog was 9 – 10 times greater than that of the control.

Nucleoside analogs 3′F-Aza-C and 5′-deoxy-Aza-C were inactive at concentrations of 0.1 μM although they did show statistically significant cytotoxicity against highly sensitive KG-1 leukemia cells at concentrations of 1.0 and 10.0 μM, respectively. However, the cytotoxicity in the cell cultures did not strengthen but regressed as the amount of test compound increased. The low *in vitro* cytotoxicity of 5′-deoxy analog **IVb** was probably due to the lack of a 5′-OH in the carbohydrate and different metabolism than Aza-C and its fluorodeoxy analogs **IVc** and **VIIIa**.

Table 2 also shows that nucleoside analog **VIIIa** with a C-2′ F atom in the arabino configuration was 1.7 times more hydrolytically stable than Aza-C at neutral pH values. The time required to degrade 10% of **VIIIa** was 43 min whereas this limit for Aza-C was reached already after 25 min. This was an important parameter because the elimination half-life of Aza-C after s.c. injection was (41 ± 8) min. The C_{max} (750 ± 403 ng/mL) was reached 0.5 h after injection. It is noteworthy that the hydrolytic stability of 3′F-Aza-C decreased noticeably compared with the isomeric arabino analog. Obviously, conformational features caused by introducing the F atom in the carbohydrate part had a significant influence on the chemical stability of the F-containing Aza-C analogs. Apparently, the F atom in 5-Aza-C analogs **IVc** and **VIIIa**, like for 1-(3-deoxy-3-fluoro-β-D-ribofuranosyl)adenine and 1-(3-deoxy-3-fluoro-β-D-xylofuranosyl)adenine, shifted most effectively the S↔N equilibrium toward one of the conformers [16]. One of the principal structural differences of the S and N conformers was the distance between the hetero-base and exocyclic -5′CH_2OH substituent. It was substantially greater in the S conformer than in the N conformer. Intramolecular interaction between the 5′-OH and the triazine ring C-6 probably facilitated hydrolysis with ring opening. As a result, the degradation half-life was shorter for the F-containing nucleosides than for the 5′-deoxy analog. It was shown earlier that intramolecular interaction between the 5′-OH and triazine ring of decitabine facilitated its hydrolysis with ring opening, as a result of which the degradation half-life of the β-anomer was shorter than that of the α-anomer [17].

Thus, the novel 2′-F-containing Aza-C analog **VIIIa** was most promising for further studies of the acute toxicity and *in vivo* antileukemic activity.

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