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Synthesis and Antiviral Activity of New 5-Substituted 2'-Deoxyuridine Derivatives

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Abstract—New 5-azole- and 5-oxime-substituted analogues of 2'-deoxyuridine are synthesized. The analogues with azole ring manifest low toxicities and antiherpetic activities on *Vero* cell culture, the imidazole derivative being the most active. The inhibitory effects of oximes of 5-formyl-deoxyuridine are comparable with those of the azole-containing nucleoside analogues, although their cytotoxicities are found to be higher; oxime of 5-formyldeoxyuridine is particularly toxic. The nucleoside analogues synthesized exhibit no marked activity on cell cultures infected with various variants of poxvirus.

Key words: *herpes simplex virus, nucleoside analogues, poxvirus*

INTRODUCTION

Many 5-substituted derivatives of 2'-deoxyuridine[1] inhibit the reproduction of herpes viruses (e.g., HSV) and VZV. 5-Iodo-, (*E*)-5-(2-bromovinyl)-, and 5-trifluoromethyl-2'-deoxyuridines are used in the therapy of herpes virus-induced infections [1]. The mechanisms of their action differ. For example, 5-iodo-2'-deoxyuridine is subjected to intracellular phosphorylation, and the resulting nucleoside 5'-triphosphate acts as a substrate of herpes virus DNA polymerases and terminates the viral DNA synthesis. On the other hand, (*E*)-5-(2-bromovinyl)-2'-deoxyuridine 5'-triphosphate can act not only as a terminating substrate, but also as a competitive inhibitor of herpes virus DNA polymerases. 5-Trifluoromethyl-2'-deoxyuridine is phosphorylated in cells to 5'-monophosphate, which inhibits thymidylate synthases [1].

Some 5-substituted 2'-deoxyuridines inhibit reproduction of the Poxviridae family viruses [2]. The replacements of proton in the uracil position 5 by halogen, nitro, formyl, trifluoromethyl, and vinyl groups are regarded as the most successful [2]. The action mechanism of these compounds involves their phosphorylation to the corresponding 5'-monophosphates that inhibit the dUMP to dTMP transformation catalyzed by thymidylate synthase. Therefore, the 5-modified analogues of 2'-deoxyuridine are promising compounds for the design of antiviral agents.

The goal of this work was the synthesis and study of antiviral properties of new 2'-deoxyuridine analogues with azole or alkyloxime substituents in position 5.

RESULTS AND DISCUSSION

5-Bromomethyl-3',5'-di-*O*-acetyl-2'-deoxyuridine (**II**) was the key compound in the synthesis of azoles (**IVa**)–(**IVd**). It was prepared by the known procedure of radical bromination of 3',5'-di-*O*-acetylthymidine (**I**) (Scheme 1) [3]. The nucleophylic substitution of the bromine atom by the imidazole, 1,2,4-triazole, or tetrazole residues in DMF yielded the corresponding azole derivatives (**IIIa**)–(**IIId**). Note that the products of bromide (**II**) interaction with tetrazole were N^1- and N^2-substituted tetrazoles (**IIIc**) and (**IIId**) at a 3 : 1 ratio.

Nucleoside azoles (**IIIa**)–(**IIId**) were isolated by column chromatography on silica gel using a gradient elution with methanol in chloroform. The target nucleosides (**IVa**)–(**IVd**) were prepared by the treatment of the corresponding diacetates (**IIIa**)–(**IIId**) with ammonia and purified by reversed-phase chromatography on a LiChroprep RP-18 column.

The reaction of the starting 5-formyl-3',5'-di-*O*-acetyl-2'-deoxyuridine (**VII**) with the corresponding *O*-alkylhydroxylamines in pyridine resulted in alkyloximes (**Xa**)–(**Xd**) (Scheme 2). Several procedures have been suggested for the synthesis of (**VII**) [3, 4]. First, we tried to prepare it by hydrolysis of 5-dibromomethyl-3',5'-di-*O*-acetyl-2'-deoxyuridine (**VI**). It is

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[1] Abbreviations: ANV, acyclovir; CD_{50}, a minimal concentration that causes the death of 50% cells; HSV-l/L_2, herpes simplex virus, type 1, strain L_2; IC_{50}, the concentration of compounds that causes inhibition of viral reproduction bo 50%; IS, selectivity index (CD_{50}/IC_{50}); TCD_{50}, tissue cytotoxic dose that causes the change of 50% of cellular monolayer; and VZV, varicella zoster virus.

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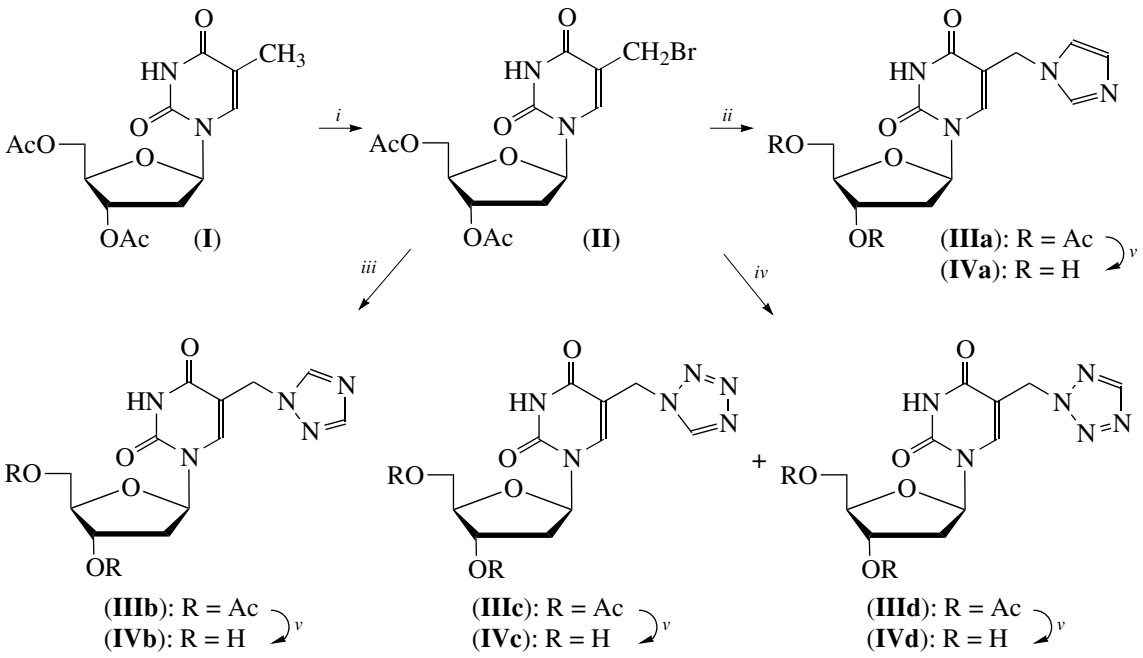


Reagents: (*i*) $Br_2/C_2H_4Cl_2$, (*ii*) imidazole/DMF, (*iii*) triazole/DMF, (*iv*) tetrazole/DMF, (*v*) ammonia/dioxane/water.

Scheme 1.

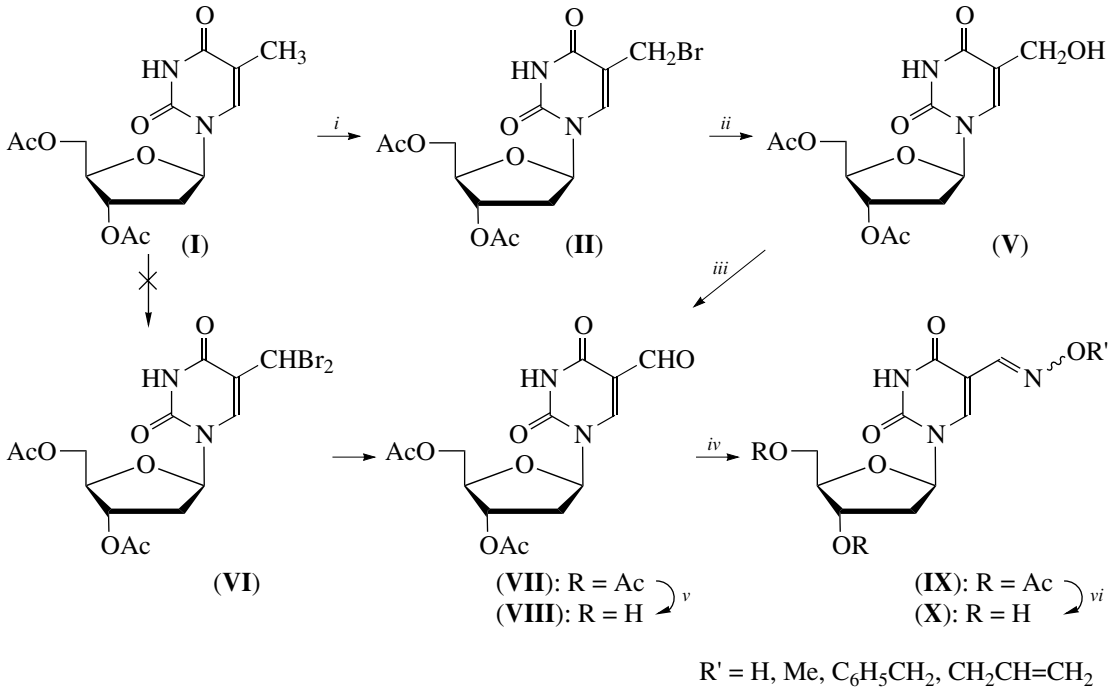
worth mentioning that the bromination of 3',5'-di-*O*-acetylthymidine (**I**) proposed for the preparation of dibromide (**VI**) [3] failed to give the target (**VII**). An analysis of the reaction mixture showed that monobromide (**II**) is initially formed and an increase in the reaction time leads to the formation of a number of byproducts; the method is therefore inapplicable. We prepared the 5-formylderivative (**VII**) by oxidation of 5-hydroxymethyl-3',5'-di-*O*-acetyl-2'-deoxyuridine (**V**) as described in [3]. Nucleoside (**V**) was obtained by the hydrolysis of 5-bromomethyl-3',5'-di-*O*-acetyl-2'-deoxyuridine (**II**) by an aqueous sodium bicarbonate solution [3]. Pyridinium chlorochromate (the Corey reagent) [5] adsorbed on aluminum oxide was used as an oxidant. Its application to the oxidation of primary alcohols enables higher yields of the target products in comparison with the use of CrO_3–pyridine complex (the Collins reagent) [5]. The reaction mixture was purified by filtration of the chromium-containing compounds adsorbed on Al_2O_3 followed by silica gel chromatography of the filtrate in a gradient of methanol in chloroform. The deblocked 5-formyl-2'-deoxyuridine (**VIII**) was obtained by the treatment of the corresponding diacetate (**VII**) with a triethylamine in aqueous methanol.

The reaction of 5-formyl derivative (**VII**) with the corresponding hydrochlorides of hydroxylamine and its *O*-alkylated derivatives in pyridine proceeded in nearly quantitative yields and led to a mixture of *syn*- and *anti*-conformers of alkyloximes (**IXa**)–(**IXd**). The isomer ratio was 1 : 1 in the case of unprotected hydroxylamine (**IXa**), and it was up to 1 : 2 in favor of the *anti*-isomer for *O*-benzylhydroxylamine (**IXc**) (Table 1). The subsequent deprotection yielded an increased portion of *anti*-conformer. The reaction products (**IXa**)–(**IXd**) were isolated as mixtures of isomers by the chromatography on silica gel in a gradient of methanol in chloroform. The target (**Xa**)–(**Xd**) were obtained by the treatment of the corresponding diacetates (**IXa**)–(**IXd**) with ammonia and purified by reversed-phase chromatography on a LiChroprep RP-8 column.

The structures of the compounds synthesized were confirmed by UV and ^1H and ^{13}C NMR spectra. The UV spectra of the 5-azole-containing nucleosides negligibly differed from the thymidine spectrum, whereas the introduction of formyl group in position 5 of uridine and the the formation of alkyloxime derivatives caused

Table 1. Ratios of *syn*- and *anti*-isomers of oximes and alkyloximes **(IXa)–(IXd)** and **(Xa)–(Xd)**

		Acetyl derivatives (**IX**)		Unprotected nucleosides (**X**)	
	R'	*syn*-	*anti*-	*syn*-	*anti*-
a	H	50	50	37	63
b	Me	41	59	35	65
c	Bn	35	65	33	67
d	$CH_2CH{=}CH_2$	38	62	26	74



Reagents: (*i*) $Br_2/C_2H_4Cl_2$, (*ii*) $NaHCO_3/H_2O$, (*iii*) $CrO_3 \cdot Py \cdot HCl/Al_2O_3/CH_2Cl_2$, (*iv*) $R'ONH_2/Py$, (*v*) aqueous $Et_3N/MeOH/H_2O$, (*vi*) ammonia/dioxane/water.

Scheme 2.

a shift in the absorption maximum to long wavelengths (λ_{max} 276 nm for 5-formyl-2'-deoxyuridine and 290–294 nm, for alkyloximes). The configurations of N^1 and N^2-substituted tetrazole derivatives were confirmed by ^{13}C NMR spectra. For example, the chemical shift of the azole CH was 143 ppm for the N^1-substituted tetrazole (**IVc**) and 153 ppm for the N^2-substituted tetrazole (**IVd**), which is consistent with the published data for N^1- and N^2-substituted tetrazoles [6]. In addition, the chemical shifts of azole protons in the ^1H NMR spectra were 8.92 and 8.56 ppm for (**IVc**) and (**IVd**), respectively. Note that the chemical shift of this proton in the N^2-isomer (**IVd**) is close to that of the corresponding proton of triazole derivative (**IVb**).

Table 2. Cytotoxicities and antiviral effects of compounds (**IVa**)–(**IVd**) and (**Xa**)–(**Xd**) on *Vero* cell culture using the HSV-1 model

Compound	TCD_{50}	ID_{50}, μM	ID_{95}, μM	SI
(**IVa**)	>1000	31.25	1000	>32
(**IVb**)	260	65	260	4
(**IVc**)	>1000	250	>1000	4
(**IVd**)	231	58	463	4
(**VIII**)	0.12	n.a.	n.a.	–
(**Xa**)	19.5	19.5	n.a.	1
(**Xb**)	180	45	180	4
(**Xc**)	194	194	n.a.	1
(**Xd**)	225	112.5	n.a.	2
Acyclovir	2222	1.7	3.56	1310

The ratios of *syn*- and *anti*-isomers of alkyloximes (**IXa**)–(**IXd**) and (**Xa**)–(**Xd**) were determined by ^1H NMR spectroscopy. For *syn*-isomers, chemical shifts of C*H*NO and H6 protons were 8.9–9.2 and 7.3–7.6 ppm, respectively; and they were practically the same (7.8–8.2 ppm) for all *anti*-isomers. UV spectra of alkyloximes (**Xa**)–(**Xd**) showed a shift of absorption maximum to long wavelengths by 14–18 nm.

The antiviral activity was tested in the *Vero* cell culture infected with herpes and pox family viruses (Table 2). The antiherpetic activity of the studied compounds was determined according to their ability to inhibit the development of the virus-induced cytopathogenic effect by 50% using the earlier described methods [7]. Our compounds manifested a moderate antiherpetic activity. Among azole-containing nucleosides (**IVa**)–(**IVd**), the imidazole derivative (**IVa**) was the most active. Its IS was 32, which is 40 times lower than that of acyclovir. The activities of 1,2,4-triazole (**IVb**) and N^2-substituted tetrazole (**IVd**) were two times and N^1-isomer (**IVc**) eight times lower than the activity of (**IVa**). Alkyloximes of 5-formyl-2'-deoxyuridine (**Xa**)–(**Xd**) also displayed the ability to inhibit the herpes

virus reproduction comparable with those of azoles (their IS were 1 to 4). However, their cytotoxicities were higher, particularly, for 5-formyl-2'-deoxyuridine (**Xa**). No antiherpetic activity was revealed in 5-formyl-2'-deoxyuridine (**VIII**), whereas its toxicity was rather high. This may be ascribed to its mutagenic effect [8]. Thus, some of the nucleoside analogues described in this work are low toxic for *Vero* cells and low effective antiherpetic agents. All the compounds studied did not exhibit a noticeable activity in the cells infected with the viruses of pox family (smallpox, monkeypox, cowpox, and vaccinia viruses).

EXPERIMENTAL

Thymidine, imidazole, triazole, tetrazole, and bromine were from Fluka (Switzerland); *O*-methylhydroxylamine hydrochloride, pyridine, and DMF, from Aldrich (United States); and hydroxylamine hydrochloride, from Reakhim (Russia).

O-Benzyl- and *O*-(2-allyl)hydroxylamine hydrochlorides were obtained by the methods described in [9] and [10], respectively.

5-Bromomethyl-3',5'-di-*O*-acetyl-2'-deoxyuridine (**II**) and 5-hydroxymethyl-3',5'-di-*O*-acetyl-2'-deoxyuridine (**V**) were prepared by the method [3].

Column chromatography was carried out on Kieselgel (40–63 μm, direct phase) and Lichroprep RP-8 (25–40 μm, reversed phase) (Merck, Germany). Reaction mixtures were monitored by TLC on precoated Kieselgel 60 F_{254} plates (Merck, Germany) developed with (A) chloroform–ethanol 9 : 1 or (B) 4 : 1 dioxane–25% ammonia.

NMR spectra (chemical shifts are given in δ scale, ppm, and spin coupling constants in Hz) were registered on an AMX III-400 (Bruker, United States) with a working frequency of 400 MHz for ^{1}H NMR [Me_4Si and sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS) were internal standards for $CDCl_3$ or DMSO-d_6 and D_2O solutions, respectively] and 100 MHz for ^{13}C NMR (methanol as an internal standard). UV spectra were recorded on a UV-2401 spectrophotometer (Shimadzu, Japan).

A general procedure for the synthesis of (5-azolylmethyl)-3',5'-di-*O*-acetyl-2'-deoxyuridines (IIIa)–(IIId). The corresponding azole (5 mmol) was added to a solution of (5-bromomethyl)-3',5'-di-*O*-acetyl-2'-deoxyuridine (**I**) (1 mmol) in dry DMF (10 ml). The mixture was kept for 48 h at 37°C, evaporated in a vacuum, and the residue was dissolved in chloroform (20 ml). The solution was washed with water (2 × 15 ml), dried with Na_2SO_4, and evaporated in a vacuum. The residue was dissolved in chloroform (0.5 ml), applied onto a silica gel column, and eluted with a gradient of methanol in chloroform (0 to 5%). The target fractions were evaporated in a vacuum.

5-Imidazolylmethyl-3',5'-di-*O*-acetyl-2'-deoxyuridine (IIIa); yield 39%; UV (H_2O): λ_{max} 267 nm; ^{1}H NMR ($CDCl_3$): 9.78 (1 H, s, 3-N*H*), 7.75 (1 H, s, H6), 7.55 (1 H, s, H2''), 7.04 (1 H, s, H5''), 7.03 (1 H, s, H4''), 6.17 (1 H, dd, $^3J_{1'2'a}$ 8.7, $^3J_{1'2'b}$ 5.3, H1'), 5.13 (1 H, d, $^3J_{2'a,3'}$ 6.0, H3'), 4.85 (2 H, s, 5-CH_2), 4.41 (1 H, m, H5'a), 4.32 (1 H, m, H4'), 4.22 (1 H, m, H5'b), 2.53 (1 H, ddd, $^2J_{2'a2'b}$ 14.33, $^3J_{1'2'b}$ 5.3, $^3J_{2'b,3'}$ 1.2, H2'b), 2.11–2.02 (1 H, m, H2'a), 2.08 (3 H, s, CH_3), and 2.06 (3H, s, CH_3).

5-Triazolylmethyl-3',5'-di-*O*-acetyl-2'-deoxyuridine (IIIb); yield 34%; UV (H_2O): λ_{max} 267 nm; ^{1}H NMR ($CDCl_3$): 9.69 (1 H, s, 3-N*H*), 8.31 (1 H, s, H3''), 7.88 (1 H, s, H6), 7.81 (1 H, s, H5''), 6.24 (1 H, dd, $^3J_{1'2'a}$ 8.7, $^3J_{1'2'b}$ 5.6 5.6, H1'), 5.20 (1 H, m, H3'), 5.12 (1 H, s, $^2J_{ab}$ 14.8, 5-CH_2a), 4.97 $^2J_{ab}$ 14.8, 5-CH_2b), 4.38 (1 H, m, H5'a), 4.31 (2 H, m, H4', H5'b), 2.53 (1 H, ddd, $^2J_{2'a2'b}$ 14.3, $^3J_{1'2'b}$ 5.6, $^3J_{2'b,3'}$ 1.6, H2'b), 2.17–2.01 (1 H, m, H2'a), 2.16 (3H, s, CH_3), and 2.10 (3H, s, CH_3).

5-(1-Tetrazolylmethyl)-3',5'-di-*O*-acetyl-2'-deoxyuridine (IIIc); yield 31%; UV (H_2O): λ_{max} 267 nm; ^{1}H NMR ($CDCl_3$): 9.75 (1 H, s, 3-N*H*), 8.92 (1 H, s, H5''), 7.96 (1 H, s, H6), 6.22 (1 H, dd, $^3J_{1'2'a}$ 8.4, $^3J_{1'2'b}$ 5.3, H1'), 5.34 (1 H, d, $^2J_{ab}$ 14.7, 5-CH_2a), 5.24 $^2J_{ab}$ 14.7, 5-CH_2b), 5.20 (1 H, m, H3'), 4.46 (1 H, dd, $^2J_{5'a,5'b}$ 12.8, $^3J_{5'a,4'}$ 5.6, H5'a), 4.30–4.27 (2 H, m, H5'b, H4'), 2.55 (1 H, ddd, $^2J_{2'a2'b}$ 14.3, $^3J_{1'2'b}$ 5.3, $^3J_{2'a,3'}$ 1.6, H2'b), 2.18 (3H, s, CH_3), 2.10 (3H, s, CH_3), and 2.14–2.01 (1 H, m, H2'a); ^{13}C NMR ($CDCl_3$): 170.67 (*C*OAc), 170.32 (*C*OAc), 162.67 (C4), 149.74 (C2), 143.53 (C5''), 140.61 (C6), 107.72 (C5), 86.02 (C1'), 82.90 (C4'), 74.07 (C3'), 63.60 (C5'), 44.44 (5-*C*H_2), 37.91 (C2'), 20.86 (*C*H_3), and 20.78 (*C*H_3).

5-(2-Tetrazolylmethyl)-3',5'-di-*O*-acetyl-2'-deoxyuridine (IIId); yield 12%; UV (H_2O): λ_{max} 267 nm; ^{1}H NMR ($CDCl_3$): 9.42 (1 H, br. s, 3-N*H*), 8.48 (1 H, s, H5''), 7.77 (1 H, s, H6), 6.21 (1 H, dd, $^3J_{1'2'a}$ 8.4, $J_{1'2'b}$ 5.6, H1'), 5.61 (1 H, d, $^2J_{ab}$ 14.6, 5-CH_2a), 5.48 (1 H, d, $^2J_{ab}$ 14.6, 5-CH_2b), 5.19 (1 H, m, H3'), 4.48 (1 H, m, H5'a), 4.28–4.23 (2 H, m, H4', H5'b), 2.57 (1 H, ddd, $^2J_{2'a2'b}$ 14.3, $^3J_{1'2'b}$ 5.6, $^3J_{2'b,3'}$ 1.6, H2'b), 2.19–2.10 (1 H, m, H2'a), 2.09 (3H, s, CH_3), and 2.07 (3H, s, CH_3 + 5'Ac); ^{13}C NMR ($CDCl_3$): 170.41 (*C*OAc), 170.27 (*C*OAc), 161.60 (C4), 153.03 (C5''), 149.65 (C2), 139.97 (C6), 107.50 (C5), 85.93 (C1'), 82.82 (C4'), 73.97 (C3'), 63.64 (C5'), 48.41 (5-CH_2), 37.95 (C2'), and 20.77 (2 CH_3).

A general procedure for the synthesis of 5-azolylmethyl-2'-deoxyuridines (IVa)–(IVd) and alkyloximes of 5-formyl-2'-deoxyuridine (Xa)–(Xd). Ammonia (24%, 1 ml) was added to a solution of the corresponding 5-azolylmethyl-3',5'-di-*O*-acetyl-2'-deoxyuridine (**IIIa**)–(**IIId**) or alkyloximes of

5-formyl-3',5'-di-*O*-acetyl-2'-deoxyuridines (**IXa**)–(**IXd**) (0.2 mmol) in dioxane (2 ml), and the mixture was kept for 20 h at 20°C. The mixture was evaporated in a vacuum; the residue was dissolved in water (1 ml) and applied onto a LiChroprep column RP-8 (2 × 18 cm). The products were eluted in a gradient of methanol (0 to 7%) in 0.01 M NH_4HCO_3. The target fractions were evaporated in a vacuum, and the residue was coevaporated with water (2 × 15 ml) and lyophilized from water.

5-Imidazolylmethyl-2'-deoxyuridine (IVa); yield 88%; UV (H_2O): λ_{max} 267 nm; ^{1}H NMR (D_2O): 7.66 (1 H, s, H6), 7.61 (1 H, s, H2''), 7.01 (1 H, s, H4''), 6.87 (1 H, s, H5''), 6.10 (1 H, t, $^3J_{1'2'}$ 6.5, H1'), 4.27 (1 H, m, H3'), 3.88 (1 H, ddd, $^3J_{4'5'a}$ 4.7, $^3J_{4'3'}$ 4.1, $^3J_{4'5'b}$ 3.4, H4'), 3.66 (1 H, dd, $^2J_{5'a5'b}$ 12.5, H5'b), 3.55 (1 H, dd, $^2J_{5'a5'b}$ 12.5, $^3J_{4'5'a}$ 4.7, H5'a), 2.27 (1 I, ddd, $^2J_{2'a2'b}$ 14.3, $^3J_{1'2'}$ 6.5, $^3J_{2'a,3'}$ 4.7, H2'a), and 2.18 (1 H, ddd, $^2J_{2'a2'b}$ 14.3, $^3J_{1'2'}$ 6.5, $^3J_{2'b,3'}$ 6.3, H2'b).

5-Triazolylmethyl-2'-deoxyuridine (IVb); yield 85%; UV (H_2O): λ_{max} 267 nm; ^{1}H NMR (D_2O): 8.39 (1 H, s, H3''), 7.92 (1 H, s, H5''), 7.91 (1 H, s, H6), 6.15 (1 H, t, $^3J_{1'2'}$ 6.5, H1'), 5.04 (2 H, s, 5-CH_2), 4.34 (1 H, m, H3'), 3.92 (1 H, m, H4'), 3.71 (1 H, dd, $^2J_{5'a5'b}$ 12.8, $^3J_{5'a,4'}$ 3.4, H5'a), 3.62 (1 H, dd, $^2J_{5'a5'b}$ 12.8, $^3J_{5'b,4'}$ 5.0, H5'b), and 2.35–2.22 (2 H, m, H2').

5-(1-Tetrazolylmethyl)-2'-deoxyuridine (IVc); yield 82%; UV (H_2O): λ_{max} 267 nm; ^{1}H NMR (D_2O): 9.04 (1 H, s, H5''), 8.03 (1 H, s, H6), 6.10 (1 H, t, $^3J_{1'2'}$ 6.5, H1'), 5.26 (2 H, s, 5-CH_2), 4.30 (1 H, m, H3'), 3.89 (1 H, m, H4'), 3.70 (1 H, dd, $^2J_{5'a,5'b}$ 12.6, $^3J_{5'a,4'}$ 3.3, H5'a), 3.60 (1 H, dd, $^2J_{5'a,5'b}$ 12.6, $^3J_{5'b,4'}$ 4.8, H5'b), and 2.35–2.15 (2 H, m, H2').

5-(2-Tetrazolylmethyl)-2'-deoxyuridine (IVd); yield 86%; UV (H_2O): λ_{max} 267 nm; ^{1}H NMR (D_2O): 8.59 (1 H, s, H5''), 8.06 (1 H, s, H6), 6.13 (1 H, t, $J_{1'2'}$ 6.5, H1'), 5.47 (2 H, s, 5-CH_2), 4.32 (1 H, m, H3'), 3.90 (1 H, m, H4'), 3.70 (1 H, dd, $^2J_{5'a,5'b}$ 12.8, $^3J_{5'a,4'}$ 3.4, H5'a), 3.60 (1 H, dd, $^2J_{5'a,5'b}$ 12.8, $^3J_{5'b,4'}$ 4.7, H5'b), and 2.32–2.27 (2 H, m, H2').

5-Formyl-3',5'-di-*O*-acetyl-2'-deoxyuridine (VII). An oxidizing agent $CrO_3 \cdot Py \cdot HCl/Al_2O_3$ (12.8 g, 12.8 mmol) was added in four portions to a solution of 5-hydroxy-3',5'-di-*O*-acetyl-2'-deoxyuridine (**V**) (6 g, 4.6 mmol) in dry dichloroethane (50 ml). The mixture was kept for 18 h at 25°C, filtered, and the filtrate was evaporated in a vacuum. The residue was dissolved in chloroform (2.5 ml), applied onto a silica gel column (2.1 × 22 cm), and eluted with a gradient of methanol in chloroform (0 to 3%). The fractions containing the target product (**VII**) were evaporated in a vacuum; yield 1.17 g (72%); UV (H_2O): λ_{max} 275.8 nm; ^{1}H NMR ($CDCl_3$): 9.97 (1 H, s, C*H*O), 8.45 (1 H, s, H6), 6.29 (1 H, dd, $^3J_{1',2'a}$ 7.8, $^3J_{1',2'b}$ 5.9, H1'), 5.22 (1 H, m, H3'), 4.38–4.27 (3 H, m, H4' and H5'), 2.58 (1 H, ddd, $^2J_{2'a2'b}$ 14.3, $^3J_{1',2'b}$ 5.9, $^3J_{2'b,3'}$ 2.2, H2'b), 2.25 (1 H, ddd, $^2J_{2'a2'b}$ 14.3, $^3J_{1',2'a}$ 7.8, $^2J_{2'a,3'}$ 6.8, H2'a), 2.17 (3H, s, CH_3), and 2.08 (3H, s, CH_3).

5-Formyl-2'-deoxyuridine (VIII). A solution of 5-formyl-3',5'-di-*O*-acetyl-2'-deoxyuridine (**VII**) (30 mg, 0.1 mmol) in 0.9 M Et_3N in 50% MeOH (5 ml) was incubated at 20°C for 2 h and evaporated in a vacuum. The residue was dissolved in water (1 ml), applied onto a LiChroprep d_6RP-8 column (2 × 18 cm), and eluted with a 0 to 7% gradient of MeOH in 0.01 M $NaHCO_3$. The fractions containing the target (**VIII**) were evaporated in a vacuum, and the residue was coevaporated with water (15 ml × 2) and lyophilized from water; yield 21 mg (93%); UV (H_2O): λ_{max} 275.8 nm; ^{1}H NMR (DMSO-d_6): 9.97 (1 H, s, C*H*O), 8.39 (1 H, s, H6), 6.18 (1 H, dd, $^3J_{1',2'a}$ 7.8, $^3J_{1',2'b}$ 5.9, H1'), 5.22 (1 H, m, H3'), 4.38–4.27 (3 H, m, H4', H5'), 2.58 (1 H, ddd, $^2J_{2'a2'b}$ 14.3, $^3J_{1',2'b}$ 5.9, $^3J_{2'b,3'}$ 2.2, H2'b), and 2.25 (1 H, ddd, $^2J_{2'a2'b}$ 14.3, $^3J_{1',2'a}$ 7.8, $^2J_{2'a,3'}$ 6.8, H2'a).

A general procedure for the synthesis of 5-formyl-3',5'-di-*O*-acetyl-2'-deoxyuridine alkyloximes (IXa)–(IXd). A solution of hydroxylamine hydrochloride or its corresponding *O*-alkylated derivative (0.1 mmol) in pyridine (5 ml) was added to a solution of 5-formyl-3',5'-di-*O*-acetyl-2'-deoxyuridine (**VII**) (0.1 mmol) in pyridine (2 ml). The mixture was kept for 20 h at 20°C and evaporated in a vacuum; the residue was dissolved in chloroform (20 ml), washed with water (2 × 15 ml), dried with Na_2SO_4, and evaporated in a vacuum. The residue was dissolved in chloroform (0.5 ml), applied onto a silica gel column (2 × 17 cm), eluted with a gradient of methanol in chloroform (0 to 10%), and the target fractions were evaporated in a vacuum.

Oxime of 5-formyl-3',5'-di-*O*-acetyl-2'-deoxyuridine (IXa); yield 90%; UV (H_2): λ_{max} 290.5 nm; ^{1}H NMR ($CDCl_3$): 9.89 (1 H, br. s, 3-N*H*), 9.86 (1 H, br. s, 3-N*H*), 9.20 (0.5 H, s, 5-C*H syn*), 8.08 (1 H, s, H6 *anti* and 5C*H anti*), 7.59 (0.5 H, s, H6 *syn*), 6.32 (1 H, m, H1'), 5.28 (1 H, m, H3'), 4.40–4.25 (3 H, m, H5, H4'), 2.65–2.48 (1 H, m, H2'a), 2.38–2.17 (1 H, m, H2'b), 2.15 (3H, s, CH_3), and 2.05 (3H, s, CH_3).

***O*-Methyloxime of 5-formyl-3',5'-di-*O*-acetyl-2'-deoxyuridine (IXb);** yield 94%; UV (H_2O): λ_{max} 293.8 nm; ^{1}H NMR ($CDCl_3$): 9.56–9.53 (1 H, br. s, 3-N*H*), 9.03 (0.41 H, br. s, 5-CH=*syn*), 8.08 (0.59 H, s, H6, *anti*), 8.04 (0.59 H, s, H6, CH=, *anti*), 7.50 (0.41 H, s, H6 *syn*), 6.35 (0.59 H, dd, $^3J_{1',2'a}$ 8.7, $^3J_{1',2'b}$ 5.6, H1' *anti*), 6.25 (0.41 H, dd, $^3J_{1',2'a}$ 7.5, $^3J_{1',2'b}$ 5.9, H1' *syn*), 5.25 (1 H, m, H3'), 4.35–4.28 (3 H, m, H5', H4'), 3.92 (3 × 0.41 H, s, CH_3ON *syn*), 3.82 (3 × 0.59 H, s, CH_3ON *anti*), 2.65–2.45 (1 H, m, H2'a), 2.26–2.11 (1 H, m, H2'b),and 2.18 and 2.13 (2 × 3 H, 2 s, 3'Ac and 5'Ac).

***O*-Benzyloxime of 5-formyl-3',5'-di-*O*-acetyl-2'-deoxyuridine (IXc);** yield 92%; UV (H_2O): λ_{max} 293.9 nm; ^1H NMR ($CDCl_3$): 9.85 (1 H, br. s, 3-N*H*), 8.99 (0.35 H, s, 5-CH=, *syn*), 8.15 (0.65 H, s, H6, *anti*), 8.07 [0.65 H, s, 5-CH=, *anti*), 7.50 (0.35 H, s, H6, *syn*), 7.42–7.25 (5 H, m, C_6H_5), 6.30 (0.65 H, dd, $^3J_{1'2'}$ 8.4, $^3J_{1'2'}$ 6.2, H1' *anti*), 6.19 (0.35 H, dd, $^3J_{1'2'}$ 7.8, $^3J_{1'2'}$ 5.9, H1' *syn*), 5.24 (0.35 H, m, H3' *syn*), 5.18 (0.65 H, m, H3' *anti*), 5.10 (2 × 0.65 H, s, C*H*$_2$C$_6$H$_5$ *anti*), 4.95 (2 × 0.35 H, s, C*H*$_2$C$_6$H$_5$ *syn*), 4.39–4.28 (3 × 0.65 H, m, H5', H4' *anti*), 4.13 (0.35 H, m, H4' *syn*), 3.88 (0.35 H, dd, $^2J_{5'a,5'b}$ 12.1, $^3J_{5'a,4'}$ 4.7, H5'a *syn*), 3.73 (0.35 H, dd, $^2J_{5'a,5'b}$ 12.1, $^3J_{5'a,4'}$ 4.1, H5'b, *syn*), 2.58–2.45 (1 H, m, H2'b), 2.25–2.18 (1 H, m, H2'a), 2.10 (3H, s, C*H*$_3$), and 1.98 (3H, s, C*H*$_3$).

***O*-Allyloxime of 5-formyl-3',5'-di-*O*-acetyl-2'-deoxyuridine (IXd);** yield 88%; UV (H_2): λ_{max} 293.7 nm; ^1H NMR ($CDCl_3$): 9.69 (1 H, br. s, 3-N*H*), 9.03 (0.38 H, s, 5-CH=, *syn*), 8.10 (0.62 H, s, H6 *anti*), 8.07 [0.62 H, s, 5-CH=, *anti*), 7.54 (0.38 H, s, H6, *syn*), 6.32 (0.62 H, dd, $J_{1'2'a}$ 8.4, $J_{1'2'b}$ 5.6, H1' *anti*), 6.24 (0.38 H, dd, $J_{1'2'a}$ 7.2, $J_{1'2'b}$ 6.2, H1' *syn*), 6.02–5.88 (1 H, m, H2"], 5.38–5.10 (3 H, m, H3', H3"), 4.65 (0.38 H, d, $^3J_{1",2"}$ 5.6, H1" *syn*), 4.56 (0.62 H, d, $^3J_{1",2"}$ 5.6, H1" *anti*), 4.33 (2 × 0.62 H, m, H5' *anti*), 4.29 (2 × 0.38 H, m, H5' *syn*, 0.62 I, m, H4' *anti*), 4.24 (0.38 H, m, H4' *syn*), 2.58–2.45 (1 H, m, H2'a), 2.25–2.18 (1 H, m, H2'b), 2.12 (3 H, s, C*H*$_3$), and 1.97 (3 H, s, C*H*$_3$).

Oxime of 5-formyl-2'-deoxyuridine (Xa); yield 83%; UV (H_2O): λ_{max} 289.5 nm; ^1H NMR (D_2O): 9.12 (0.37 H, s, 5-CH=, *syn*), 8.07 [0.63 H, s, 5-CH=, *anti*), 7.86 (0.63 H, s, H6, *anti*), 7.31 (0.37 H, s, H6, *syn*), 6.16 (0.37 H, t, $J_{1'2'}$ 6.5, H1' *syn*), 6.11 (0.63 H, t, $J_{1'2'}$ 6.2, H1' *anti*), 4.33–4.22 (1 H, m, H3'), 3.94–3.85 (1 H, m, H4'), 3.73–3.55 (2 H, m, H5'), and 2.35–2.15 (2 H, m, H2').

***O*-Methyloxime of 5-formyl-2'-deoxyuridine (Xb);** yield 86%; UV (H_2O): λ_{max} 293.1 nm; ^1H NMR (D_2O): 9.08 (0.35 H, s, 5-CH= *syn*), 8.24 (0.65 H, s, H6 *anti*), 8.00 (0.65 H, s, CH= *anti*), 7.42 (0.35 H, s, H6 *syn*), 6.28–6.20 (1 H, s, H1'), 4.47–4.41 (1 H, m, H3'), 4.06–4.01 (1 H, m, H4'), 3.82 (3 H, m, CH_3), 3.77–3.73 (2 H, m, H5'), and 2.46–2.33 (2 H, m, H2').

***O*-Benzyloxime of 5-formyl-2'-deoxyuridine (Xc);** yield 78%; UV (H_2O): λ_{max} 293.6 nm; ^1H NMR (D_2O): 9.17 (0.33 H, s, 5-CH= *syn*), 8.44 (0.67 H, s, H6, *anti*), 8.01 (0.67 H, s, 5-CH= *anti*), 7.45–7.32 (0.35 H + 5 H, m, H6 *syn* and C_6H_5), 6.30–6.22 (1 H, m, H1'), 5.23 (2 × 0.33 H, s, C*H*$_2$C$_6$H$_5$ *syn*), 5.13 (2 × 0.67 H, s, C*H*$_2$C$_6$H$_5$ *anti*), 4.40 (0.67 H, m, H3' *anti*), 4.19 (0.33 H, m, H3' *syn*), 3.95 (1 H, m, H4' *anti*), 3.88 (0.33 H, m, H4' *syn*), 3.80 (0.67 H, dd, $^2J_{5'a,5'b}$ 11.8, $^3J_{5'a,4'}$ 3.4, H5'a *anti*), 3.74 (0.67 H, dd, $^2J_{5'a,5'b}$ 11.8, $^3J_{5'a,4'}$ 3.8, H5'b *anti*), 3.46 (2 × 0.33 H, m, H5'a *syn*), 2.38–2.25 (2 × 0.67 H + 0.33 H, m, H2' *anti*, 0.33 H, m, H2'a *syn*), 2.18–2.02 (0.33 H, m, H2'a *syn*).

***O*-Allyloxime of 5-formyl-2'-deoxyuridine (Xd);** yield: 80%; UV (H_2O): λ_{max} 293.5 nm; ^1H NMR (D_2O): 9.03 (0.26 H, s, 5-CH= *syn*), 8.11 (0.74 H, s, H6 *anti*), 7.89 (0.74 H, s, 5-CH= *anti*), 7.28 (0.26 H, s, H6 *syn*), 6.13 (0.26 H, t, $J_{1'2'}$ 7.1, H1' *syn*), 6.09 (0.74 H, t, $J_{1'2'}$ 7.0, H1' *anti*), 5.93–5.84 (2 H, m, H2'), 5.23 (0.74 H, d, $^2J_{2",3"a}$ 1.6, H3"a *anti*), 5.19 (0.26 H, d, $^2J_{2",3"a}$ 1.6, H3"a *syn*), 5.16 (0.74 H, d, $^2J_{2",3"a}$ 8.6, H3"b *anti*), 5.14 (0.26 H, d, $^2J_{2",3"a}$ 8.5, H3"b *syn*), 4.54 (0.26 H, d, $^3J_{1",2"}$ 5.7, H1" *syn*), 4.47 (0.74 H, d, $^3J_{1",2"}$ 5.9, H1" *anti*), 4.31 [0.74 H, m, H3' (*anti*), 4.27 [0.26 H, m, H3' *syn*), 3.89 (1 H, m, H4'), 3.70 [0.74 H, dd, $^2J_{5'a,5'b}$ 11.5, $^3J_{5'a,4'}$ 3.1, H5'a *anti*), 3.65 [0.74 H, dd, $^3J_{5'a,4'}$ 4.7, H5'b *anti*), 3.63 [0.26 H, m, H5'a *syn*), 3.54 (0.26 H, dd, $^2J_{5'a,5'b}$ 11.2, $^3J_{5'a,4'}$ 4.2, H5'b *syn*), and 2.38–2.13 (2 H, m, H2').

Experiments on Cell Cultures

A reference strain HSV-1/L_2 was obtained from the State Collection of Viruses of the Ivanovsky Institute of Virology, Russian Academy of Medical Sciences, Russia. The strains were passaged in 1 : 1 mixture of the Eagle and 199 media with the addition of 2% fetal calf serum. The *Vero* cell culture (kidney cells of African green marmosets) clone E6 was used in the work. The cells were cultured in the Eagle's MEM medium (the Institute of Poliomyelitis and Viral Encephalitides, Russian Academy of Medical Sciences, Moscow) containing 7% fetal calf serum (PanEco, Moscow).

The antiherpetic activity was determined according to the ability of compounds tested to inhibit the growth of the virus-induced cytopathogenic effect by 50% using the procedure described in [7]. Acyclovir [9-(2-hydroxyethoxymethyl)guanine, Glaxo Wellcome, UK] was used as a reference compound.

Cytotoxicity of the compounds was determined on uninfected cultures by the procedure based on the ability of dead cells to be stained with Trypan Blue dye [7]. CD_{50} was taken as a concentration of compound, at which no more than 50% cells died after 72-h incubation with the compound tested [11].

The antipox effect of the compounds synthesized was studied with five viral strains: human smallpox, monkeypox, cowpox, mousepox, and vaccinia viruses. They were obtained from the State Collection of Viruses of the Vector State Center of Virology and Biotechnology. *Vero* and MK2 cell cultures were used for the activity and toxicity studies. The antiviral effect was evaluated as the viability of the virus-infected cells incubated with the compounds studied.

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REFERENCES

1. De Clercq, E., *Nat. Rev. Drug Discovery*, 2002, vol. 1, no. 1, pp. 13–25.
2. De Clercq, E., *Clinical Microbiology Reviews,* 2001, vol. 14, pp. 382–397.
3. Barwolff, D and Langen, P, in *Nucleic Acid Chemistry*, Townsend, L.B.. and Tipson, R.S., Eds., New York: Wiley, 1978, vol. 1, pp. 359–366.
4. Szmanda, A., Markiewicz, M., Godzina, P., and Markiewicz, W.T., *Collect. Czech. Chemical Commun.*, 2002, vol. 5, pp. 16–26.
5. Tietze, L.F. and Eicher, T., *Reaktionen und Synthesen im organische-chemischen Praktikum und Forschungslaboratorium*, New York: Georg Thieme Verlag Stutgart, 1991. Translated under the title *Preparativnaya organicheskaya khimiya*, Moscow: Mir, 1999.
6. Ostrovsky, V.A., Studentsov, E.P., Poplavsky, V.S., Ivanova, N.V., Gurskaya, G.V., Zavodnik, Y.E., Jasko, M.V., Semizarov, D.G., and Krayevsky, A.A., *Nucleosides Nucleotides,* 1995, vol. 14, pp. 1289–1300.
7. De Clercq, E., Descamps, J., Verheist, G., Walker, R.T., Jones, A.S., Torrence, P.F., and Shugar, D., *J. Infect. Dis.*, 1980, vol. 141, pp. 563–573.
8. Klungland, A., Paulsen, R., Rolseth, V., Yamada, Y., Ueno, Y., *et al.*, *Toxicol. Lett.*, 2001, vol. 119, pp. 71–78.
9. Khomutov, R.M., Severin, E.S., Gnuchev, N.V., and Derevyanko, T.Ya., *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1967, no. 8, pp. 1820–1823.
10. Khomutov, A.R., Khurs, E.N., and Khomutov, R.M., *Bioorg. Khim.*, 1988, vol. 14, pp. 385–391.
11. Aleksandrova, L.A., Andronova, V.L., Karpenko, I.L., Skoblov, Yu.S., Adani, A., and Galegov, G.A., *Bioorg. Khim.*, 2002, vol. 28, pp. 455–461.