Bioorganic Chemistry 38 (2010) 265–270

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

# 1,2,4-Triazoloazine derivatives as a new type of herpes simplex virus inhibitors

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ARTICLE INFO

*Article history:*
Received 23 June 2010
Available online 22 September 2010

*Keywords:*
Herpes simplex virus
Inhibitors
Nucleoside analogues
Triazoloazine derivatives

ABSTRACT

A new class of inhibitors of herpes simplex virus replication was found. The compounds under study are derived from condensed 1,2,4-triazolo[5,1-*c*][1,2,4]triazines and 1,2,4-triazolo[1,5-*a*]pyrimidines, structural analogues of natural nucleic bases. Antiherpetic activity and cytotoxicity of the compounds were studied. The corresponding triphosphates of several active compounds were prepared and tested as inhibitors of DNA synthesis catalyzed by herpes simplex virus polymerase. The potential mechanism of their action is blocking of DNA dependent DNA polymerase, a key enzyme of viral replication.



## 1. Introduction

Herpes viruses belong to widely spread and socially dangerous viral infections. Over a half of the world population are infected with the virus and up to 20% exhibit signs of the disease. In addition, herpetic infections can lead to a lethal outcome in patients with immune deficiency caused by HIV infection [1] or organ transplantation [2]. Nucleoside analogues modified at either the base or the ribose residue played an essential role for design of antiherpetic agents. Guanosine acyclic analogues, acyclovir (Zovirax) and its L-valine ester (Zelitrex®, Valtrex®), penciclovir (Denavir®, Vectavir®), ganciclovir (Cytovene®, Cymevene®), and famciclovir (Famvir®), which is an oral prodrug of penciclovir, are well known drugs used in medical practice [3–5]. Also, pyrimidine derivatives modified at the nucleic base, namely, 5-iodo-2′-deoxyuridine (Herpid®, Iodoxene®), (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (Zostex®, Helpin®), and 5-trifluoromethyl-2′-deoxyuridine (Veroptic®) have been described as antiherpetic drugs [3]. Galankevich et al. reported antiherpetic properties of tricyclic acyclovir analogues, 3-[(2-hydroxyethoxy)methyl]-9-oxyimidazolo[1,2-*a*]purine derivatives [6,7]. When penetrating into cell, nucleoside-based compounds pass three stages of phosphorylation and under catalysis of herpes virus DNA polymerase are incorporated into the 3′-terminus of viral DNA and terminate its synthesis. Disadvantages of these agents are relatively high toxicity, low bioavailability, and inevitable appearance of virus strains resistant to the drug. It is noteworthy that about 6% strains isolated from HIV-infected patients are acyclovir-resistant. In addition to the above mentioned compounds there are some publications on the wide antiviral activity of 6-nitro-1,2,4-triazolo[5,1-*c*][1,2,4]triazine-7(4H)-ones [8–10]. However the antiherpetic properties of triazoloazine derivatives were not studied.

Herein, we reported the synthesis and antiherpetic activity of 15 new derivatives of 1,2,4-triazolo[5,1-*c*][1,2,4]triazines and 1,2,4-triazolo[1,5-*a*]pyrimidines bearing an N3-alkyl fragment with a terminal hydroxyl group linked to the azine cycle (Fig. 1). We also showed that triphosphates of some triazoloazine derivatives blocked DNA synthesis catalyzed by HSV DNA polymerase.

## 2. Materials and methods

### 2.1. Physical measurement

NMR spectra were registered on a Bruker AMX III-400 spectrometer with the working frequency of 400 MHz for $^{1}$H NMR ($Me_4Si$ as the internal standard for organic solvents and sodium trimethylsilyl-1-propane sulfonate for $D_2O$), and 162 MHz for $^{31}$P NMR (with proton-phosphorus decoupling; 85% $H_3PO_4$ as the external standard). Two-dimensional correlation experiments ($^{1}$H–13C HMBC and HSQC) and $^{13}$C NMR (100 MHz) were registered on a Bruker DRX-400 spectrometer in DMSO-$d_6$. UV spectra were recorded on a Shimadzu UV-2401PC spectrophotometer (Japan). HPLC was performed on a Gilson chromatograph (France) with UV detection. The flow rate 0.5 ml/min. Radioactivity was measured on a SL-4000 Intertechnique counter (France).

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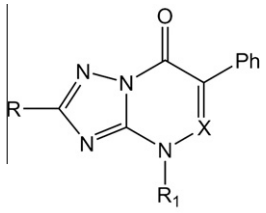
doi:10.1016/j.bioorg.2010.09.002




R: **a** = H; **b** = Me; **c** = SMe.

| Index | X | $R_1$ |
|---|---|---|
| 1 | CH | HO |
| 2 | N | HO |
| 3 | N | HO |
| 4 | N | HO |
| 5 | N | HO |

**Fig. 1.** 6-Phenyl-1,2,4-triazolo[1,5-a]pyrimidine (**1**) and 6-phenyl-1,2,4-triazolo[5,1-*c*][1,2,4]triazine-7-one (**2**–**5**) derivatives.

X-ray studies were performed on an automatic Xcalibur 3 diffractometer with a CCD detector ($\omega/2\theta$ scanning, Mo Kα irradiation, graphite monochromator) using a standard procedure. Monoclinic crystals e $a = 8.7427(6)$ Å, $b = 10.5354(6)$ Å, $c = 16.1262(15)$ Å, $\alpha = 90°$, $\beta = 103.087(7)°$, $\gamma = 90°$, $Z = 4$, $d_{sub.} = 1.370$ g/cm$^{-3}$, $\mu = 0.094$ mm$^{-1}$, $V = 1446.77(19)$ Å$^3$, a space group P2(1)/c. The structure was decoded by the direct method and specified using SHELXS-97 and SHELXL-97 programs [11] in anisotropic (isotropic for H atoms) approximation at $R = 0.0368$ ($wR = 0.0738$) for 1783 reflections with $I > 2\sigma(I_o^2)$ and GOOF 1.000. CCDC 791002 contains the supplementary crystallographic data which can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

### 2.2. Synthesis of the compounds

#### 2.2.1. Preparation of 2-R-6-pheny-1,2,4-triazolo[1,5-a]pyrimidine-7-ones (**6a**,**b**)

Synthesis of **6a**,**b** was performed similar to [12]. Briefly, ethyl α-formyl phenyl acetate (2.28 g, 11.90 mmol) was added to a solution of 2-R-5-amino-1,2,4-triazole (11.9 mmol) in acetic acid (4 ml) and the mixture was refluxed for 1 h and cooled. The precipitate was filtered and dried.

**6a** (R = H): The yield 1.76 g (8.3 mmol), 70%; mp 311 °C; $^1$H NMR (DMSO-d$_6$) $\delta$ 7.34–7.42 (m, 3H, $C_6H_5$), 7.64–7.66 (m, 2H, $C_6H_5$), 8.21 (s, 1H, CH(5)), 8.21 (s, 1H, CH(2)), 13.21–13.88 (br s, 1H, NH); $^{13}$C NMR (DMSO-d$_6$) $\delta$ 111.82 (C(6)), 127.35 (C*p*), 128.21 (C*m*), 128.67 (C*o*), 133.35 (C*i*), 138.79 (C(5)), 150.11 (C(3a)), 152.29 (C(2)), 155.74 (C(7)); Calc. (%): C, 62.26; H, 3.80; N, 26.40. $C_{11}H_8N_4O$. Found (%): C, 62.10; H, 4.00; N, 26.57%.

**6b** (R = Me): The yield 1.99 g (8.8 mmol), 74%; mp > 320 °C; $^1$H NMR (DMSO-d$_6$) $\delta$ 2.38 (s, 3H, $CH_3$), 7.33–7.42 (m, 3H, $C_6H_5$), 7.63–7.62(m, 2H, $C_6H_5$), 8.13 (s, 1H, CH(5)), 12.3–13.71 (br s, 1H, NH); $^{13}$C NMR (DMSO-d$_6$) $\delta$ 14.13 ($CH_3$), 111.88 (C(6)), 127.20 (C*p*), 128.13 (C*m*), 128.56 (C*o*), 133.51 (C*i*), 138.85 (C(5)), 150.29 (C(3a)), 155.29 (C(7)), 160.74 (C(2)); Calc. (%): C, 63.71; H, 4.46; N, 24.76%. $C_{12}H_{10}N_4O$. Found (%): C, 64.01; H, 4.24; N, 24.55.

#### 2.2.2. Preparation of 2-R-4-(4-acetoxybutyl)-6-phenyl-1,2,4-triazolo[1,5-a]pyrimidine-7-ones (**1′a**,**b**)

A suspension of 2-R-6-phenyl-1,2,4-triazolo[1,5-*a*]pyrimidine-7-one **6a**,**b** (9.4 mmol) in 17% aqueous $Na_2CO_3$ (6 ml) was stirred at room temperature for 0.5 h, the precipitate was filtered off, dried and dissolved in DMF (10 ml). 4-Bromobutyl acetate (1.79 g, 9.20 mmol) was added to the resulting solution and the reaction mixture was heated for 2 h at 100°C. The mixture was cooled, water (250 ml) was added, the residue was filtered off and crystallized from isopropanol.

**1′a** (R = H): The yield 1.838 g (5.64 mmol), 60%; mp 146 °C; $^1$H NMR (DMSO-d$_6$) $\delta$ ppm: 1.62–1.67 (m, 2H, $CH_2$), 1.82–1.93 (m, 2H, $CH_2$), 1.98 (s, 3H, $COCH_3$), 4.02 (t, 2H, $CH_2$–O), 4.28 (t, 2H, $CH_2$–N), 7.34–7.45 (m, 3H, $C_6H_5$), 7.66–7.69 (m, 2H, $C_6H_5$), 8.18 (s, 1H, CH(2)), 8.44 (s, 1H, CH(5)); $^{13}$C NMR (DMSO-d$_6$) $\delta$ ppm: 20.71 ($CH_3$), 24.84 (C(2′)), 25.03 (C(3′)), 51.05 (C(1′)), 63.34 (C(4′)), 112.09 (C(6)), 127.53 (C*p*), 128.24 (C*m*), 128.61 (C*o*), 133.04 (C*i*), 141.63 (C(5)), 150.33 (C(2)), 152.32 (C(3a)), 155.08 (C(7)), 170.42 (C=O). Calc. (%): C, 62.57; H, 5.56; N, 17.17. $C_{17}H_{18}N_4O_3$: Found (%): C, 62.55; H, 5.52; N, 17.18%.

**1′b** (R = Me): The yield 2.429 g (7.14 mmol), 76%; mp 137 °C; $^1$H NMR (DMSO-d$_6$) $\delta$ ppm: 1.62–1.65 (m, 2H, $CH_2$), 1.87–1.90 (m, 2H, $CH_2$), 2.00 (s, 3H, $COCH_3$), 2.40 (s, 3H, $CH_3$), 4.04 (t, $CH_2$), 4.23 (t, 2H, $CH_2$), 7.32–7.44 (m, 3H, $C_6H_5$), 7.65–7.66 (m, 2H, $C_6H_5$), 8.37 (s, 1H, CH); $^{13}$C NMR (DMSO-d$_6$) $\delta$ ppm: 14.81 ($CH_3$), 21.15 ($CH_3$), 25.27 (C(2′)), 25.45 (C(3′)), 51.41 (C(1′)), 63.80 (C(4′)), 112.44 (C(6)), 127.89 (C*p*), 128.65 (C*m*), 128.99 (C*o*), 133.56 (C*i*), 141.48 (C(5)), 150.94 (C(3a)), 155.17 (C(7)), 161.85 (C(2)), 170.86 (C=O); Calc. (%): C, 63.52; H, 5.92; N, 16.46. $C_{18}H_{20}N_4O_3$. Found (%): C, 63.53; H, 5.87; N, 16.55.

#### 2.2.3. Preparation of 2-R-4-(4-hydroxybutyl)-6-phenyl-1,2,4-triazolo[1,5-a]pyrimidine-7-ones (**1a**,**b**)

Compound **1′a**,**b** (3.0 mmol) was added to a solution of sodium methylate prepared from sodium (0.07 g, 3.04 mmol) and methanol (30 ml). The reaction mixture was refluxed for 1 h, cooled, neutralized with acetic acid and evaporated in vacuum. The products were isolated by column chromatography on silicagel and were eluted with ethyl acetate.

**1a** (R = H): The yield 0.511 g (1.8 mmol), 60%; mp 140 °C; $^1$H NMR (DMSO-d$_6$) $\delta$ ppm 1.47–1.53 (m, 2H, $CH_2$), 1.89–1.94 (m, 2H, $CH_2$), 3.42 (q, 2H, $CH_2$–O), 4.28 (t, 2H, $CH_2$–N), 4.48 (t, 1H, OH), 7.32–7.43 (m, 3H, $C_6H_5$), 7.64–7.66 (m, 2H, $C_6H_5$), 8.31 (s, 1H, CH(2)), 8.45 (s, 1H, CH(5)). $^{13}$C NMR (DMSO-d$_6$) $\delta$ ppm: 25.11 (C(2′)), 29.22 (C(3′)), 51.47 (C(1′)), 60.22 (C(4′)), 111.98 (C(6)), 127.52 (C-*p*), 128.25 (C-*m*), 128.62 (C-*o*), 133.03 (C-*i*), 141.71 (C(5)), 150.30 (C(3a)), 152.36 (C(2)), 155.06 (C(7)); Calc. (%): C, 63.37; H, 5.67; N, 19.71. $C_{15}H_{16}N_4O_2$ Found (%):C, 63.01; H, 5.91; N, 19.50.

**1b** (R = Me): The yield 0.447 g (1.6 mmol), 50%; mp 139 °C; $^1$H NMR (DMSO-d$_6$) $\delta$ ppm: 1.44–1.49 (m, 2H, $CH_2$), 1.86–1.94 (m, 2H, $CH_2$), 2.40 (s, 3H, $CH_3$), 3.43 (q, 2H, $CH_2$-O), 4.23 (t, 2H, $CH_2$–N), 4.48 (t, 1H, OH), 7.32–7.44 (m, 3H, $C_6H_5$), 7.65–7.67 (m, 2H, $C_6H_5$), 8.36 (s, 1H, CH). $^{13}$C NMR (DMSO-d$_6$) $\delta$ ppm: 14.39 ($CH_3$), 25.13 (C(2′)), 29.23 (C(3′)), 51.40 (C(1′)), 60.23 (C(4′)), 111.92 (C(6)), 127.44 (C*p*), 128.18 (C*m*), 128.55 (C*o*), 133.12 (C*i*), 141.08 (C(5)), 150.49 (C(3a)), 154.72 (C(7)), 161.46 (C(2)); Calc. (%): C, 64.41; H, 6.08; N, 18.78. $C_{16}H_{18}N_4O_2$: Found (%): C, 64.38; H, 5.98; N, 18.86.

#### 2.2.4. Preparation of monophosphates of **3a**,**c** (**3a**,**c-MP**) and **5b** (**5b-MP**)

Triethylamine (0.832 mmol, 0.12 ml) was added to a solution of 1,2,4-triazole (50 mg, 0.725 mmol) in $CH_3CN$ (0.9 ml). The solution was cooled and $POCl_3$ (0.028 ml, 0.302 mmol) was added. The formed precipitate was separated by centrifugation and the supernatant was added to a solution of **3a**,**c** or **5b** (0.15 mmol) in $CH_3CN$ (6 ml). In 40 min 50% aqueous pyridine (5 ml) was added to the reaction mixture. The target monophosphates were isolated on a DEAE cellulose column in a gradient of aqueous $NH_4HCO_3$ (0 → 0.3 M). Monophosphates were additionally purified by column chromatography on LiChroprep RP-18 in a gradient of methanol in water (0% → 10%).

**3a-MP**: The yield 21 mg (0.06 mmol), 42%, $^1$H NMR ($D_2O$) $\delta$ ppm: 4.25 (q, 2H, $CH_2$–O), 4.55 (m, 2H, $CH_2$–N + $D_2O$), 7.46–7.48

(m, 3H, $C_6H_5$); 7.87–7.89 (m, 2H, $C_6H_5$); 8.33 (s, 1H, CH); $^{31}$P NMR ($D_2O$) $\delta$, ppm: 3.32 s.

**3c-MP**: The yield 35 mg (0.1 mmol), 61%, $^1$H NMR ($D_2O$) $\delta$ ppm: 2.65 (s, 3H, SMe), 4.25 (q, 2H, $CH_2$–O), 4.55 (t, 2H, $CH_2$–N), 7.46–7.48 (m, 3H, $C_6H_5$); 7.87–7.89 (m, 2H, $C_6H_5$); $^{31}$P NMR ($D_2O$) $\delta$ ppm: 2.21 s.

**5b-MP**: The yield 30 mg (0.079 mmol), 52%, $^1$H NMR ($D_2O$) $\delta$ ppm: 1.59–1.63 (m, 2H, $CH_2$); 1.89–1.93 (m, 2H, $CH_2$); 2.58 (s, 3H, Me); 3.89 (q, 2H, $CH_2$–O), 4.28 (t, 2H, $CH_2$–N), 7.35–7.38 (m, 3H, $C_6H_5$); 7.75–7.77 (m, 2H, $C_6H_5$); $^{31}$P NMR ($D_2O$) $\delta$ ppm: 0.91 s.

*2.2.5. Preparation of triphosphates of **3a,c** (**3a,c-TP**) and **5b** (**5b-TP**)*

A solution of monophosphates **3a,c-MP** and **5b-MP** (0.057 mmol) in DMF (15 ml) and triethylamine (0.04 ml) was half evaporated and carbonyldiimidazole (20 mg, 0.123 mmol) was added. The mixture was stirred for 2 h and methanol (0.12 ml) was added. The solution was stirred for another 40 min and tributylammonium pyrophosphate $H_4P_2O_7 \times 1{,}5\text{-}Bu_3N$ (129 mg, 0.283 mmol) was added. In 24 h water was added. The product was isolated on a DEAE cellulose column under conditions described above. The solution was evaporated, the residue was dissolved in water and purified on a LiChroprep RP-18 column. The compounds were eluted in a gradient of methanol in water (0% → 15%).

**3a-TP**: The yield 5 mg (0.009 mmol), 17%; $^1$H NMR ($D_2O$) $\delta$ ppm: 4.38 (q, 2H, $CH_2$–O), 4.67 (t, 2H, $CH_2$–N), 7.46–7.48 (m, 3H, $C_6H_5$); 7.85–7.88 (m, 2H, $C_6H_5$); 8.27 (s, 1H, CH); $^{31}$P NMR ($D_2O$) $\delta$ ppm: −22.45 (t, 1P, $P_\beta$), −10.93 (d, 1P, $P_\gamma$), −9.73 (d, 1P, $P_\alpha$). UV ($H_2O$) $\lambda_{max}$ 320 nm, $\varepsilon$ 13,180; $\lambda_{max}$ 269 nm, $\varepsilon$ 11,220.

**3c-TP**: The yield 8 mg (0.016 mmol), 26%; $^1$H NMRP ($D_2O$) $\delta$ ppm: 2.68 (s, 3H, SMe), 4.46 (q, 2H, $CH_2$), 4.77 (m, 2H, $CH_2 + D_2O$), 7.54–7.56 (m, 3H, $C_6H_5$); 7.92–7.94 (m, 2H, $C_6H_5$); $^{31}$P NMR ($D_2O$) $\delta$ ppm: −22.44 (t, 1P, $P_\beta$), −10.90 (d, 1P, $P_\gamma$), −9.75 (d, 1P, $P_\alpha$). UV ($H_2O$) $\lambda_{max}$ nm $\lambda_{max}$ 323 nm, $\varepsilon$ 13,490; $\lambda_{max}$ 251 $\varepsilon$ 26,900.

**5b-TP**: The yield 7 mg (0.014 mmol), 23%; $^1$H NMR ($D_2O$) $\delta$ ppm: 1.72–1.77 (m, 2H, $CH_2$), 2.02–2.08 (m, 2H, $CH_2$); 2.51 (s, 3H, Me); 3.98 (q, 2H, 2H, $CH_2$–O), 4.48 (t, 2H, $CH_2$–N), 7.54–7.56 (m, 3H, $C_6H_5$); 7.88–7.90 (m, 2H, $C_6H_5$); $^{31}$P NMR ($D_2O$) $\delta$ ppm: −22.65 (t, 1P, $P_\beta$), −10.35 (d, 1P, $P_\gamma$), −10.13 (d, 1P, $P_\alpha$). UV ($H_2O$) $\lambda_{max}$ nm 321, $\varepsilon$ 12,900; $\lambda_{max}$ 263 nm, $\varepsilon$ 9,1200.

### 2.3. In vitro studies

*2.3.1. Cell lines and viruses*

*Vero* cell culture (green monkey kidney epithelial cells, ATCC No CRL-1586) was obtained from Laboratory of tissues, Ivanovskii Institute of Virology, Russian Academy of Medical Sciences (Moscow). The acyclovir-sensitive HSV-1/$L_2$ strain was obtained from the State collection of viruses of Ivanovskii Institute of Virology, Russian Academy of Medical Sciences (Moscow). The *Vero* cell culture was grown in Dulbecco's modified medium (D-MEM) with 5% fetal calf serum (PanEco, Russia) at 37 °C in a 96-well plate in the atmosphere of 5% $CO_2$.

*2.3.2. Antiherpetic activity and cytotoxicity*

The antiviral effects of the compounds were estimated from their ability to prevent the development of the virus-induced cytopathic effect (CPE) similar to the procedure described earlier [4,13]. Quantitatively the antiviral effect was expressed as $ID_{50}$ (the concentration at which CPE was reduced by 50%). Briefly, *Vero* cells placed in 96-well plates ($0.8 \times 10^6$ cell/ml) were infected with the virus with varied multiplicity of infection (0.01 or 0.1 PFU/cell) and incubated in the Eagle's medium supplied with the 199 medium (1:1) and 2% fetal calf serum. The tested compounds at concentrations up to 1000 μM were added directly before infecting. After 72 h incubation the number of living cells was measured using the trypan blue exclusion method. Cytotoxicity ($CD_{50}$) was estimated in the presence of tested compounds at the concentrations of 0–1000 μM after 72 h incubation with uninfected cells and calculated as compound concentration, at which 50% cells died [13].

*2.3.3. Inhibition of recombinant HSV DNA polymerase by triphosphates of **3a,c** and **5b***

*Recombinant HSV DNA polymerase* was prepared as described in [14]. The effect of the synthesized compounds on HSV DNA polymerase activity was measured by inhibition of [$^{32}$P]dAMP incorporation into the primer-template complex:

3′-dGGCAGTTAAGGACATCAGAGCTCGGAACTGATGCGATTGACC/[5′-32P] dCCGTCAATTCCTGTAGTC. The reaction mixture contained 50 mM buffer HEPES, pH 7.5, 100 mM $(NH_4)_2SO_4$, 6 mM $MgCl_2$, 5 μM dNTP, 1 μCi [α-$^{32}$P]dATP (6000 Ci/mM), 10 nM primer-template complex, and triphosphates of **3a,c** or **5b** at various concentrations. The reaction was started with addition of 2U HSV DNA polymerase and after incubation for 30 min at 37 °C the mixture was loaded onto DEAE filters. The filters were dried on air and the free radioactive label was washed off with 0.4 M phosphate buffer (pH 6). Radioactivity was measured on a scintillation counter by the Cherenkov method.

## 3. Results and discussion

### 3.1. Synthesis and general properties of synthesized compounds

Fig. 1 presents the structures of the synthesized triazolo-triazine and triazolo-pyrimidine derivatives.

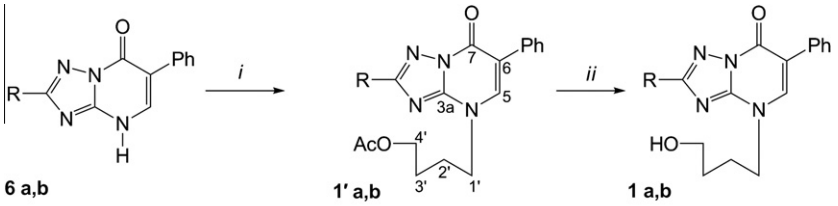
For preparation of compounds **1a,b** we used alkylation of 6-phenyl-1,2,4-triazolo[1,5-*a*]pyrimidine-7-ones **6a,b** with bromobutyl acetate under basic conditions (Scheme 1). The resulting compounds **1′a,b** were deacylated to give 1,2,4-triazolo[1,5-*a*]pyrimidine-7-one derivatives with sodium methylate.

Proton resonances in the $^1$H NMR spectra of the corresponding heterocyclic fragments and N-alkyl substituents in compounds **1′a,b** and **1a,b** were registered. The appearance of resonances at $\delta$ 4.80 ppm in the spectra of 1,2,4-triazolo[1,5-a]pyrimidine-7-ones **1a,b**, were ascribed to hydroxyl groups according to the data of NMR experiments on the deuterium exchange in the presence of $CF_3COOD$ that unambiguously confirmed the removal of an acetyl group.

The position of the N-substituent and ascription of the resonances of compounds **1′a,b** and **1a,b** in $^1$H and $^{13}$C NMR spectra were performed based on the data of two two-dimensional correlation experiments (2D HSQC и HMBC). The position of the alkyl substituent at the pyrimidine cycle is confirmed by the presence of two cross-peaks of N-$CH_2$ proton resonances and carbon C(3a) and C(5) resonances in 2D HMBC of **1′a,b** and **1a,b** spectra. These data were verified by the X-ray analysis of compound **1b** (Fig. 2), which demonstrated that azole and azine cycles were annealed at positions [1,5-*a*] and formed a nearly plane bicyclic system non-conjugated with the plane of the phenyl substituent. The torsion angle between C(4)C(3) and C(6)C(11) was 38.9°. 1,2,4-Triazolo[5,1-*c*][1,2,4]triazines (**2–5**) containing acyclic fragments were prepared similar to [15].

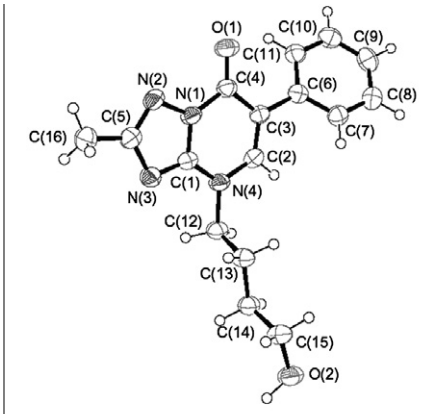
Compounds **2a-c** were synthesized by the interaction of (2-acetoxyethoxy)methyl acetate with NH-heterocycles **2′a-c** (Scheme 2).

Similarly to the synthesis of **1a,b**, the key reaction in the preparation of 1,2,4-triazolo[1,5-c][1,2,4]triazines **3a–c**, **4a–c**, and **5a–c** was alkylation of heterocycles **7a–c** with the corresponding halogen-containing compounds under basic conditions (Scheme 3). The rational for using **6a,b** and **7a–c** with "abnormal" bases for the preparation of compounds **1–5** was as follows. The structure



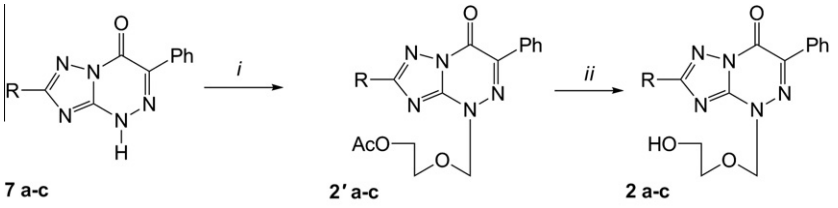

*i*: $Na_2CO_3$, Br-$CH_2$-$CH_2$-$CH_2$-$CH_2$-OAc; *ii*: MeONa

**Scheme 1.** Preparation of 4-(4-hydroxybutyl)-6-phenyl-1,2,4-triazolo[1,5-a]pyrimidine-7-ones **1a**,**b**.




| Selected bond lengths (Å) | Selected valent angles (degrees) |
|---|---|
| O(1)-C(4) 1.215(2); N(1)-C(1) 1.356 (2); N(1)-N(2) 1.380(1); N(1)-C(4) 1.414(2); N(4)-C(1) 1.358(2); N(4)-C(2) 1.365(2); N(4)-C(12) 1.480(2); C(1)-N(3)1.315(2); N(3)-C(5) 1.380(2); C(3)-C(2) 1.356(2); C(3)-C(4) 1.458(2); C(3)-C(6) 1.478(2); O(2)-C(15) 1.425(2); O(2)-H(2) 0.97(2); C(12)-C(13) 1.51(2); N(2)-C(5) 1.321(2); C(13)-C(14) 1.518(2); C(14)-C(15) 1.506(2); C(6)-C(11) 1.393(2); C(6)-C(7) 1.400(2); C(7)-C(8) 1.375(2); C(10)-C(9) 1.379(2); C(10)-C(11) 1.384(2); C(5)-C(16) 1.481(2); C(8)-C(9) 1.380(2). | C(1)-N(1)-N(2) 108.5(1); C(1)-N(1)-C(4) 127.2(1); N(2)-N(1)-C(4) 124.3(1); C(1)-N(4)-C(2) 117.7(1); C(1)-N(4)-C(12) 121.6(1); C(2)-N(4)-C(12) 120.6(1); N(3)-C(1)-N(1) 111.9(1); N(3)-C(1)-N(4) 129.2(1); N(1)-C(1)-N(4) 118.9(1); C(1)-N(3)-C(5) 101.8(1); C(2)-C(3)-C(4) 119.9(1); C(2)-C(3)-C(6) 118.9(1); C(4)-C(3)-C(6) 121.1(1); C(15)-O(2)-H(2) 109.4(1); N(4)-C(12)-C(13) 112.1(1); C(5)-N(2)-N(1) 102.3(11); C(12)-C(13)-C(14) 111.7(1); C(15)-C(14)-C(13) 113.5(1); O(1)-C(4)-N(1) 120.6(1); O(1)-C(4)-C(3) 128.2(1) N(1)-C(4)-C(3) 111.2(1); C(11)-C(6)-C(7) 117.5(1); C(11)-C(6)-C(3) 121.9(1); C(7)-C(6)-C(3) 120.4(1); C(8)-C(7)-C(6) 121.6(1); O(2)-C(15)-C(14) 112.2(1); C(9)-C(10)-C(11) 120.5(2); N(2)-C(5)-N(3) 115.5(1); N(2)-C(5)-C(16) 122.6(1); N(3)-C(5)-C(16) 121.0(1); C(10)-C(11)-C(6) 120.9(1); C(7)-C(8)-C(9) 119.8(2); C(10)-C(9)-C(8) 119.8(2). |

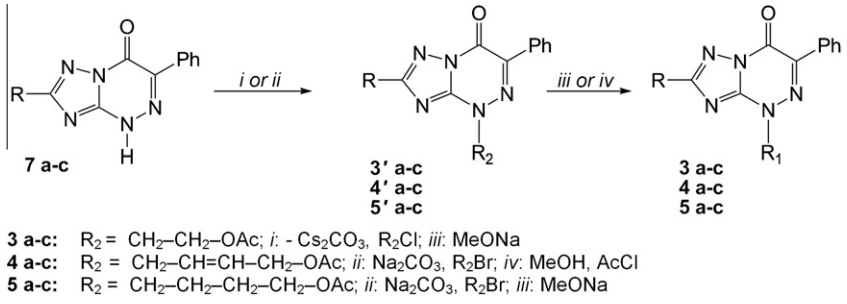
**Fig. 2.** Molecular structure of compound **1b**.




*i*: $ZnCl_2$, AcO–$CH_2$–O–$CH_2$–$CH_2$–OAc; *ii*: MeONa

**Scheme 2.** Synthesis of 4-(2-acetoxyethoxymethyl)-6-phenyl-1,2,4-triazolo[1,5-a]pyrimidine-7-ones (**2a**–**c**).

of bases in **1**–**5** is close to that of inosine analogues [16,17]. On the other hand, compounds **1**–**5** can also be considered as pyrimidine analogues with an annealed azole cycle. The antiviral activity of such bicyclic pyrimidine derivatives was shown earlier. For example, nucleoside analogues derived from imidazolo[2,1-c]pyrimidine-5-ones displayed activity against hepatitis B virus [18,19]. Among 1,2,4-triazolo[1,5-c][1,2,4]triazines some compounds were shown to be active against various strains of influenza virus A and B [10,20], and 6-arylazolo[1,5-a]pyrimidine-7-ones were patented as effective agents against hepatitis C virus [21]. We focused our interest on compounds **6b** and **7b**,**c** containing phenyl residue because introduction of the phenyl residue at position 6 of




**Scheme 3.** Synthesis of N-hydroxyalkyl-1,2,4-triazolo[1,5-c][1,2,4]triazines **3a–c**, **4a–c**, and **5a–c**.

1,2,4-triazolo[5,1-c][1,2,4]triazine-7-ones or [1,2,4-triazolo[1,5-a]pyrimidine-7-ones selectively promoted N3-alkylation of **6a**,**b** and **7a–c** at the azine cycle [22]. Another advantage of phenyl derivatives was their high chemical stability in both acid and alkaline media, which was essential for the synthesis of the corresponding triphosphates. It is noteworthy that N-alkylation of nitro-1,2,4-triazolo[5,1-c][1,2,4]triazine-7-ones and [1,2,4-triazolo[1,5-a]pyrimidine-7-ones resulted in destruction of the azine cycle [23]. Introduction of sugar residue at N-3 position of azine cycle of purine was based on earlier published data [24–26]. It was shown that 2′,3′-dideoxy-3-isoadenosine displayed anti-HIV activity and some of N3,5′-cyclo-4(β-D-ribofuranosyl-*vic*-triazolo[4,5-*b*]pyridine-5-one derivatives were active against hepatitis C virus. According to our data the highest biological activity displayed the compounds containing thiomethyl or methyl residue in azole cycle of 1,2,4-triazolo[5,1-c][1,2,4]triazine-7-ones.

### 3.2. Antiherpetic activity and cytotoxicity in cell culture

Antiherpetic properties of compounds **1–5** were studied in the *Vero* cell culture infected with herpes simplex virus type 1 (HSV-1) with varied multiplicity of infection. The results are shown in Table 1. As is seen, the structure of the compounds affected their activity. Compounds **2** containing a 2-hydroxyethoxymethyl group inhibited virus replication by 50% at concentrations exceeding 250 μM. At the same time for compounds **4** bearing a hydroxybutene fragment the 50% inhibition was observed at concentrations 15–30 μM and demonstrated the highest selectivity index at two tested PFU/cell.

1,2,4-Triazolo[1,5-c][1,2,4]triazines **5a**,**b** and 1,2,4-triazolo[1,5-a]pyrimidines **1a**,**b** containing an N-hydroxybutyl substituent showed similar activity, which implies that the presence or absence of the nitrogen atom in the six-membered ring does not significantly affect antiviral properties of these series of heterocycles. It is noteworthy that all the compounds showed low toxicity in cell experiments. Their toxicity was comparable with that of the antiherpetic drugs acyclovir or ganciclovir [27] although selectivity index was lower than that of acyclovir and ganciclovir due to less profound antiherpetic activity.

**Table 1**
Cytotoxicity and anti-HSV activity of the synthesized compounds.

| Compound | $CD_{50}$ (mM) | Multiplicity of infection | | | |
|---|---|---|---|---|---|
| | | 0.1 PFU/cell | | 0.01 PFU/cell | |
| | | $ID_{50}$ (mM)[a] | SI | $ID_{50}$ (mM)[a] | SI |
| **1a** | >0.8 | 0.06 | >13 | 0.05 | >16 |
| **1b** | >0.93 | 0.12 | >7.7 | 0.06 | >15.4 |
| **2a** | 0.5 | 0.25 | 2 | ND | ND |
| **2b** | >0.5 | 0.5 | >1 | ND | ND |
| **2c** | >0.5 | 0.25 | >2 | ND | ND |
| **3a** | >0.5 | 0.25 | >2 | ND | ND |
| **3b** | >1 | 0.12 | >8.3 | 0.06 | ⩾16.7 |
| **3c** | >0.5 | 0.12 | >4 | 0.03 | ⩾16.7 |
| **4a** | >0.5 | 0.25 | >2 | ND | ND |
| **4b** | 0.95 | 0.03 | 32 | 0.015 | 63 |
| **4c** | 0.53 | 0.015 | 35 | ⩾0.007 | ⩽76 |
| **5a** | 1.13 | 0.12 | 9.3 | 0.07 | 16 |
| **5b** | ⩾0.98 | 0.06 | 16.3 | 0.03 | ⩾32 |
| **5c** | ⩾0.5 | 0.06 | >8 | 0.03 | ⩾16 |

$CD_{50}$, cytotoxic dose, i.e., the agent concentration, at which 50% of uninfected cells die; $ID_{50}$, inhibitory dose, i.e., the agent concentration, at which the cytopathic effect is decreased by 50%; SI, selectivity index, $CD_{50}/ID_{50}$; PFU, plaque forming unit. ND – not determined.

[a] Mean values of three independent experiments are given.

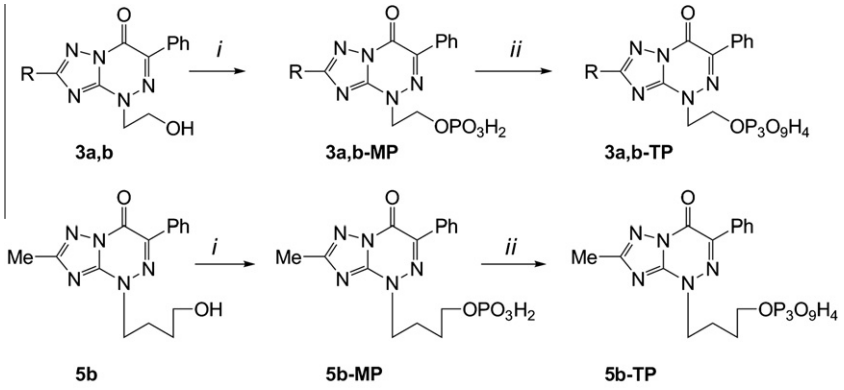
### 3.3. Inhibition of HSV-1 DNA polymerase

One of the mechanisms of antiviral activity of nucleoside analogues involves inhibition of viral DNA synthesis catalyzed by viral DNA polymerase. Therefore we prepared triphosphates of **3a**,**c** and **5b** (**3a**,**c**-**TP** and **5b**-**TP**) (Scheme 4). At the first stage the corresponding monophosphates were obtained similarly to the method described in [28]. The monophosphates were treated with N,N′-carbonyldiimidazole (CDI) followed by addition of tributylammonium pyrophosphate to give crude **3a**,**c**-**TP** and **5b**-**TP**. The compounds were purified by column chromatography on DEAE cellulose and LiChroprep RP-18 columns.

The resulting **3a**,**c**-**TP** and **5b**-**TP** were studied in cell-free experiments as inhibitors of DNA synthesis catalyzed by herpes simplex virus DNA polymerase (Table 2). As is seen, compound **3c**-**TP** bearing a methylthio group more effectively inhibited incorporation of [α-$^{32}$P]dAMP into the 3′-terminus of the primer-template complex than **3a**-**TP**. These data correlate with the results on the inhibition of virus replication in the cell culture (Table 1). Triphosphate of antiherpetic drug acyclovir or well known inhibitor of HSV DNA polymerase foscarnet used as controls suppressed the activity of polymerase more effectively if compared with synthesized compounds. It is noteworthy that compounds lacking a triphosphate or an N-acyclic fragments with a terminal hydroxyl group did not inhibit viral DNA polymerase. 50% Inhibition of the enzyme was not achieved at the concentrations exceeding 800 μM (data not shown).

## 4. Conclusion

A new type of inhibitors of herpes simplex virus based on 1,2,4-triazolo[5,1-*c*][1,2,4]triazine and 1,2,4-triazolo[1,5-*a*]pyrimidine structures was discovered. The synthesized compounds displayed antiherpetic activity and low cytotoxicity in cell cultures. Antiviral properties are strongly structure dependent. Potentially, the mechanism of their action is blocking of DNA dependent DNA polymerase, a key enzyme of viral replication.




**Scheme 4.** Synthesis of triphosphates **3a**,**c**-**TP** and **5b**-**TP**.

**Table 2**
50% Inhibition of incorporation of [α-$^{32}$P]dATP catalyzed by HSV DNA polymerase into the primer-template complex in the presence of **3a**,**c**-**TP**, **5b**-**TP**, foscarnet and acyclovir triphosphate.

| Compound | Concentration (μM)[a] |
|---|---|
| **3a-TP** | 160 ± 28 |
| **3c-TP** | 100 ± 18 |
| **5b-TP** | 150 ± 25 |
| Acyclovir-TP | 30 ± 6 |
| Foscarnet | 10 ± 2 |

[a] Data of three independent experiments.

## Acknowledgments

This work was supported by Russian Federation on Basic Research Grants 08-04-00552 and 09-04-12204, Presidium of Russian Academy of Sciences on "Molecular and Cellular Biology" and State contracts 02.740.11.0260 and 02.522.12.2011.

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