Synthesis and antiviral activity of nucleoside analogs based on 1,2,4-triazolo[3,2-*c*][1,2,4]triazin-7-ones

V. L. Rusinov,[a,b] O. N. Chupakhin,[a,b] S. L. Deev,[a] T. S. Shestakova,[a] E. N. Ulomskii,[a] L. I. Rusinova,[a] O. I. Kiselev,[c] and E. G. Deeva[c]*

[a]*B. N. Eltsyn Ural State Technical University — UPI, 19 ul. Mira, 620002 Ekaterinburg, Russian Federation. Fax: +7 (343) 374 0458. E-mail: rusinov@mail.ustu.ru*
[b]*I. Ya. Postovsky Institute of Organic Synthesis, Ural Branch of the Russian Academy of Sciences, 22/20 ul. S. Kovalevskoi, 620041 Ekaterinburg, Russian Federation*
[c]*Influenza Research Institute, Russian Academy of Medical Sciences, 15/17 ul. Professora Popova, 197376 St.-Petersburg, Russian Federation. E-mail: deevsl@yandex.ru*

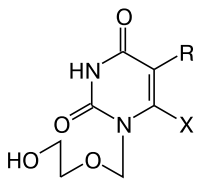
Nucleoside analogs containing hydroxybutyl, hydroxyethoxymethyl, allyloxymethyl, and propargyloxymethyl fragments were synthesized based on 1,2,4-triazolo[3,2-*c*][1,2,4]triazin-7-ones isosteric to purine bases. Some of the compounds obtained inhibit *in vitro* reproduction of influenza and respiratory syncytial virus infection.

Key words: nucleoside analogs, antiviral drugs, 1,2,4-triazolo[3,2-*c*][1,2,4]triazin-7-one derivatives.

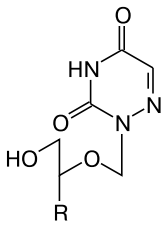
Analogs of natural nucleosides are a group of compounds widely represented in the list of substances possessing antiviral activity.[1] They are active in the steps of synthesis of viral nucleic acids, where they interfere in metabolism by replacing natural nucleosides. In this connection, the structures of such modified nucleosides should match the structure of the enzyme active site responsible for the assembly of viral nucleic acids. Hence, the antimetabolite should copy or mimic the most significant elements of the natural nucleoside structures.

Construction of a molecule of a nucleoside analog usually consists of a modification of the carbohydrate residue or a nucleic base.[1] For example, acyclic nucleosides, such as antiherpetic drugs acyclovir, 9-(2-hydroxyethoxymethyl)guanine,[2] or pencyclovir, 9-[3,3-bis(hydroxymethyl)-propyl]guanine,[3] used in the treatment of hepatitis B, are the structural analogs of guanosine where the carbohydrate residue is replaced by the aliphatic chains containing hydroxy groups. 1-(2-Hydroxyethoxymethyl)uracil derivatives **1** exhibited pronounced antihepatitis activity.[4]

One of the promising directions in the search for antiviral drugs is the synthesis of 1,2,4-triazines and azoloannulated 1,2,4-triazines, which can be considered as azaanalogs of pyrimidine and purine bases, as well as synthesis of modified nucleosides on their basis. For example, 1-(2-hydroxyethoxymethyl)- (**2**) and 1-[(1,3-dihydroxyisopropoxy)-methyl]-6-azauracil (**3**) derivatives inhibit reproduction of the type 1 and type 2 hepatitis viruses.[4,5]

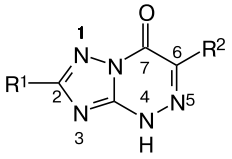


1



2, 3

R = H(**2**), $HOCH_2$ (**3**)



4a—f

R^1 = H (**a**, **d**), Me (**b**, **e**), SMe (**c**, **f**)
R^2 = NO_2 (**a**—**c**), COOEt (**d**—**f**)

In the present work, we describe the synthesis of nucleoside analogs based on 2-R^1-6-nitro- and 2-R^1-6-ethoxycarbonyl-1,2,4-triazolo[3,2-*c*][1,2,4]triazin-7-ones (**4a—f**) containing 4-hydroxybutyl, 2-hydroxyethoxymethyl, allyloxymethyl, and propargyloxymethyl groups at the N(4) atom. In other words, we consider a series of nucleosides that are modified in both the aglycon and glycoside parts. The reason for the choice of 1,2,4-triazo-

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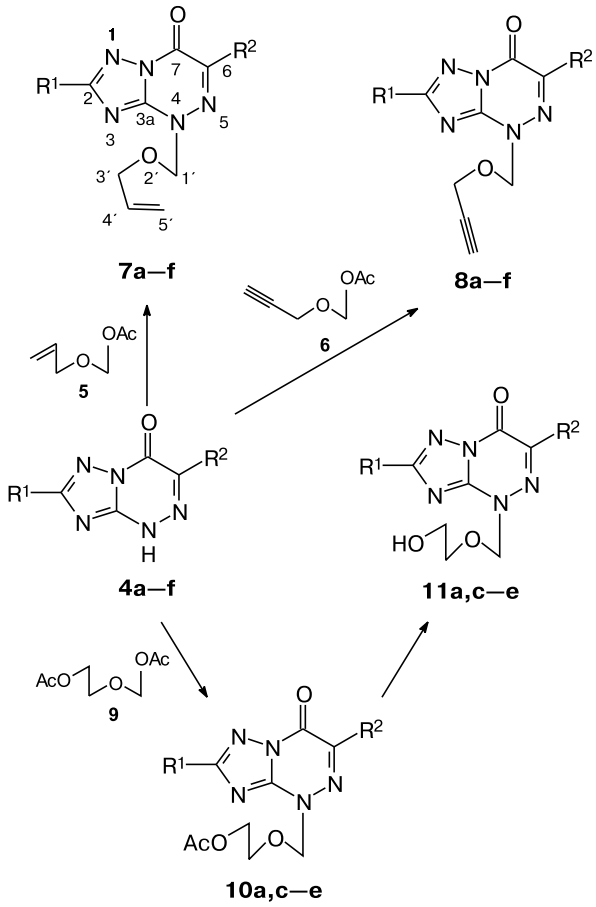
lo[3,2-*c*][1,2,4]triazin-7-ones (**4**) as the bases is, from the one hand, their structural similarities with both purines and azauracils and, from the other hand, the fact that representatives of this class of compounds have already manifested antiviral activity.[6,7] Triazavirin, an antiviral drug, was developed based on this class of compounds.[8]

We have found that the reactions of compounds **4a—f** with allyloxymethyl acetate (**5**) and propargyloxymethyl acetate (**6**) afford derivatives **7a—f** and **8a—f**, respectively (Scheme 1, Table 1).

Scheme 1



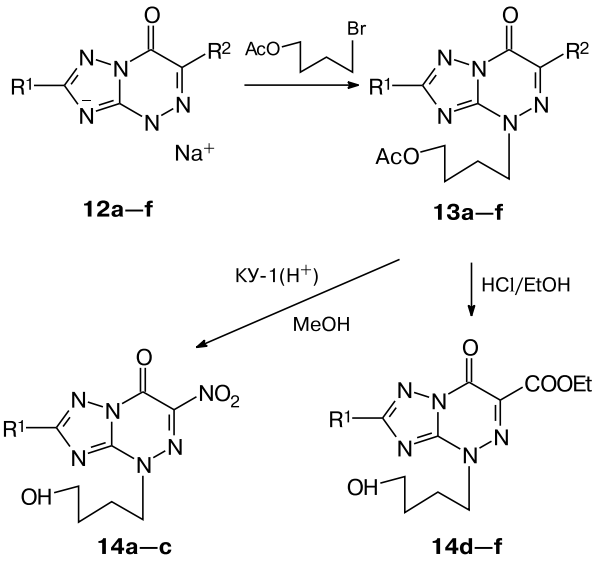
	a	b	c	d	e	f
R^1	H	Me	SMe	H	Me	SMe
R^2	NO_2	NO_2	NO_2	COOEt	COOEt	COOEt

Fusion of compounds **4a,c—e** with an excess of (2-acetoxyethoxy)methyl acetate (**9**) in the presence of $ZnCl_2$ leads to the corresponding 2-R^1-4-(2-acetoxyethoxy)-methyl-6-R^2-1,2,4-triazolo[3,2-*c*][1,2,4]triazin-7-ones (**10a,c—e**) (see Scheme 1). What is essential is that in all the cases the reactions of heterocycles **4a—f** with compounds **5**, **6**, and **9** are regioselective.

Deacylation of compounds **10a,c—e** was performed by alcoholysis in the presence of the ion-exchange resin KU-1 (H^+) in 25—48% yields. For nitro derivatives **10a,c**, deprotection lasted 12 h in methanol at room temperature giving rise to nucleoside analogs **11a,c**. For the preparation of compounds **11d,e**, it was necessary to carry out the reaction in ethanol at 0 °C for much longer time (24 h).

The ability of 1,2,4-triazolo[3,2-*c*][1,2,4]triazin-7-ones to react with iodo- and bromoalkanes under basic conditions[9,10] was used by us for the synthesis of the corresponding 4-hydroxybutyl derivatives. Treatment of sodium salts **12a—f** with 4-bromobutyl acetate in DMF at 100 °C for 3 h resulted in compounds **13a—f** (Scheme 2, Table 1).

Scheme 2



R^1 = H (**a**,**d**), Me (**b**,**e**), SMe (**c**, **f**)
R^2 = NO_2 (**a—c**), COOEt (**d—f**)

As in the case of compounds **10a,c—e**, acid-catalyzed hydrolysis was used for the removal of the acyl protecting groups in compound **13a—f**, which proceeded without formation of side products and more selectively. The use of sodium ethoxide or methoxide for nitro derivatives **13a—c** resulted in destruction of the heterocyclic system.[10] Therefore, the removal of the acyl groups from compounds **13a—c** was effected by methanolysis in the presence of the ion-exchange resin KU-1 (H^+) to give modified nucleosides **14a—c** in 40—70% yields. Acid hydrolysis has proved efficient for triazolotriazine derivatives **13d—f** as well. In this case, deacylation occurred only upon reflux in ethanol saturated with hydrogen chloride, which has been obtained by addition of acetyl chloride to ethanol. This way for the removal of acyl protection is convenient for preparative purposes, since, first, no use of anhydrous ethanol is required and, second, the saturation procedure itself requires no additional time and equipment.

The IR spectra of compounds **7a—f**, **8a—f**, **10a,c—e**, **13a—f** exhibit the absorption band at 1700—1770 cm^{-1} (Table 2) corresponding to the carbonyl group, which confirms that it is N-alkylation that occurs in reactions of heterocycles **4a—f** and their sodium salts **12a—f**. The presence of vibrational bands of the nitro group in the region 1300, 1500 cm^{-1} is characteristic of the nitro derivatives. The band in the region 2110—2130 cm^{-1} in the IR spectra of 4-propargyloxymethyl-1,2,4-triazolo[3,2-*c*][1,2,4]triazines (**8a—f**) is evidence for the presence of an acetylenic fragment in these compounds.

The ^1H NMR spectra of compounds **7a—f**, **8a—f**, **10a,c—e**, **11a,c—e**, **13a—f**, and **14a—f** exhibit signals for the protons of the heterocyclic part and *N*-alkyl substituent (see Table 2). In the case of derivatives **7a—f**, the allylic fragment is found in the form of a characteristic ABX-system.[11] The terminal protons of the $—C=CH_2$ group are found in the region δ 5.18—5.30 as two single-proton doublets of doublets. At the same time, the signal for the —CH=C— proton is a doublet of doublets of triplets. Analysis of the signal splitting in the spectrum of compounds **8a—f** at δ 3.16—3.51 (J = 2.1—2.2 Hz), represented by a triplet, allows us to undoubtedly assign it to the proton of the acetylenic group.[12]

The position of alkylation in the reactions of NH-heterocycles **4a—f** with compounds **5**, **6**, and **9** and of sodium salts **12a—f** with 4-bromobutyl acetate was determined based on the data from 2D-correlation NMR experiments (^1H, ^{13}C, g-HMBC, and g-HSQC), which were recorded for triazolotriazines **7d,e**, **8c—e**, **10e**, **13a,c—e**. Thus the presence of a cross-peak in the 2D g-HMBC spectra between the signals for the protons of the $N—CH_2$ fragment and the carbon atom C(3a) of the heterocyclic part confirms that the alkyl fragment is attached to the atom N(4). In addition, the 2D NMR experiments g-HMBC and g-HSQC confirmed assign-

Table 1. Yields, melting points, and elemental analysis data for compounds **7a—f**, **8a—f**, **10 a,c—e**, **11 a,c—e**, **13a—f**, **14 a—f**

Compound	Yield (%)	M.p. /°C	Found / Calculated (%)			Molecular formula
			C	H	N	
7a	58	45	38.14 38.10	3.17 3.20	33.30 33.32	$C_8H_8N_6O_4$
7b	68	85	40.37 40.61	3.90 3.79	31.82 31.57	$C_9H_{10}N_6O_4$
7c	59	101	36.02 36.24	3.39 3.38	28.18 28.17	$C_9H_{10}N_6O_4S$
7d	63	67	47.67 47.31	3.84 3.66	25.38 25.09	$C_{11}H_{13}N_5O_4$
7e	45	67	49.13 49.14	5.11 5.16	23.90 23.88	$C_{12}H_{15}N_5O_4$
7f	32	58	44.14 44.30	4.73 4.61	21.54 21.54	$C_{12}H_{15}N_5O_4S$
8a	56	109	38.70 38.40	2.21 2.42	33.33 33.60	$C_8H_6N_6O_4$
8b	67	115	40.73 40.92	3.21 3.05	31.85 31.81	$C_9H_8N_6O_4$
8c	19	127	36.75 36.49	2.65 2.70	28.56 28.39	$C_9H_8N_6O_4S$
8d	63	107	47.47 47.65	3.97 3.97	25.35 25.27	$C_{11}H_{11}N_5O_4$
8e	34	126	49.44 49.48	4.48 4.50	23.54 24.05	$C_{12}H_{13}N_5O_4$
8f	25	323	44.35 44.58	4.16 4.02	21.24 21.67	$C_{12}H_{13}N_5O_4S$
10a	38	68	36.52 36.25	3.51 3.38	27.83 28.18	$C_9H_{10}N_6O_6$
10c	38	75	34.69 34.88	3.23 3.49	23.90 24.42	$C_{10}H_{12}N_6O_6S$
10d	41	64	43.52 44.31	4.53 4.61	21.79 21.54	$C_{12}H_{15}N_5O_6$
10e	50	91	46.14 46.02	5.10 5.01	20.98 20.65	$C_{13}H_{17}N_5O_6$
11a	47	56	33.07 32.82	3.16 3.15	32.86 32.81	$C_7H_8N_6O_5$
11c	25	85	31.55 31.79	3.23 3.33	27.90 27.80	$C_8H_{10}N_6O_5S$
11d	37	59	42.09 42.40	4.75 4.59	24.99 24.73	$C_{10}H_{13}N_5O_5$
11e	48	61	44.47 44.44	5.15 5.09	23.73 23.56	$C_{11}H_{15}N_5O_5$
13a	62	90	40.81 40.54	4.08 4.05	28.97 28.38	$C_{10}H_{12}N_6O_5$
13b	43	85	42.67 42.58	4.31 4.55	26.98 27.09	$C_{11}H_{14}N_6O_5$
13c	70	76	38.60 38.59	4.22 4.12	24.67 24.55	$C_{11}H_{14}N_6O_5S$
13d	39	120	48.33 48.30	5.06 5.30	21.98 21.66	$C_{13}H_{17}N_5O_5$
13e	47	84	49.20 49.85	5.40 5.64	20.88 20.77	$C_{14}H_{19}N_5O_5$
13f	51	88	45.73 45.53	5.51 5.18	18.34 18.96	$C_{14}H_{19}N_5O_5S$
14a	89	71	37.57 37.80	3.90 3.94	33.10 33.07	$C_8H_{10}N_6O_4$
14b	69	72	40.00 40.30	4.71 4.48	31.43 31.34	$C_9H_{12}N_6O_4$
14c	75	99	36.45 36.00	4.09 4.00	27.69 28.00	$C_9H_{12}N_6O_4S$
14d	30	Oil	47.03 46.97	5.65 5.38	24.78 24.90	$C_{11}H_{15}N_5O_4$
14e	47	50	48.59 48.81	5.80 5.80	23.63 23.72	$C_{12}H_{17}N_5O_4$
14f	81	124	44.10 44.03	5.51 5.20	21.73 21.41	$C_{12}H_{17}N_5O_4S$

Table 2. ^{1}H NMR and IR spectra of compounds **7a—f**, **8a—f**, **10a,c—e**, **11 a,c—e**, **13a—f**, **14a—f**

Compound	^{1}H NMR, δ (*J*/Hz)	IR, ν/cm^{-1}
7a	4.22 (dt, 2 H, C(3′)H$_2$, *J* = 5.5, *J* = 1.5); 5.19 (dd, 1 H C(5′)H$\underline{H}_a$, *J* = 10.7, *J* = 1.3); 5.30 (dd, 1 H C(5′)H$\underline{H}_b$, *J* = 17.2, *J* = 1.3); 5.74 (s, 2 H, NC(1′)H$_2$); 5.87 (ddt, 1 H, C(4′)H, *J* = 17.2, *J* = 10.7, *J* = 5.5); 8.44 (s, 1 H, CH)	1730 (C=O); 1550, 1350 (NO$_2$)
7b	2.51 (s, 3 H, C—CH$_3$); 4.20 (dt, 2 H, C(3′)H$_2$, *J* = 5.4, *J* = 1.4); 5.19 (dd, 1 H C(5′)H$\underline{H}_a$, *J* = 10.3, *J* = 1.4); 5.30 (dd, 1 H, C(5′)H$\underline{H}_b$, *J* = 17.2, *J* = 1.4); 5.74 (s, 2 H, NC(1′)H$_2$); 5.87 (ddt, 1 H, C(4′)H, *J* = 17.2, *J* = 10.3, *J* = 5.4); 5.67 (s, 2 H, C(1′)H$_2$)	1760 (C=O); 1560, 1345 (NO$_2$)
7c	2.68 (s, 3 H, S—CH$_3$); 4.22 (dt, 2 H, C(3′)H$_2$, *J* = 5.3, *J* = 1.4); 5.19 (dd, 1 H, C(5′)H$\underline{H}_a$, *J* = 10.4, *J* = 1.4) 5.30 (dd, 1 H C(5′)H$\underline{H}_b$, *J* = 17.1, *J* = 1.4); 5.74 (s, 2 H, NC(1′)H$_2$); 5.87 (ddt,1 H, C(4′)H, *J* = 17.2, *J* = 10.4, *J* = 5.5); 5.67 (s, 2 H, C(1′)H$_2$)	1720 (C=O); 1550, 1350 (NO$_2$)
7d	1.32 (t, 3 H, OCH$_2\underline{CH}_3$, *J* = 7.0); 4.17 (dt, 2 H, C(2′)H$_2$, *J* = 5.5, *J* = 1.5); 4.37 (q, 2 H, O$\underline{CH}_2$CH$_3$, *J* = 7.0); 5.19 (dd, 1 H, C(5′)H$\underline{H}_a$, *J* = 10.7, *J* = 1.3); 5.30 (dd, 1 H C(5′)H$\underline{H}_b$, *J* = 17.1, *J* = 1.3); 5.74 (s, 2 H, NC(1′)H$_2$); 5.87 (ddt, 1 H, C(4′)H, *J* = 17.2, *J* = 10.4, *J* = 5.5); 5.67 (s, 2 H, C(1′)H$_2$); 8.48 (s, 1 H, CH)	1730, 1770 (C=O)
7e	1.33 (t, 3 H, OCH$_2\underline{CH}_3$, *J* = 7.0); 2.46 (s, 3 H, C—CH$_3$); 4.18 (dt, 2 H, C(2′)H$_2$, *J* = 5.4, *J* = 1.4); 4.37 (q, 2 H, O$\underline{CH}_2$CH$_3$, *J* = 7.0); 5.20 (dd, 1 H C(5′)H$\underline{H}_a$, *J* = 10.7, *J* = 1.4); 5.31 (dd, 1 H C(5′)H$\underline{H}_b$, *J* = 17.2, *J* = 1.4); 5.74 (s, 2 H, NC(1′)H$_2$); 5.87 (ddt, 1 H, C(4′)H, *J* = 17.2, *J* = 10.7, *J* = 5.5)	1700, 1725 (C=O)
7f	1.33 (t, 3 H, OCH$_2\underline{CH}_3$, *J* = 7.0); 2.66 (s, 3 H, SCH$_3$); 4.17 (dt, 2 H, C(2′)H$_2$, *J* = 5.4, *J* = 1.4); 4.37 (q, 2 H, O$\underline{CH}_2$CH$_3$, *J* = 7.0); 5.19 (dd, 1 H, C(5′)H$\underline{H}_a$, *J* = 10.3, *J* = 1.4); 5.31 (dd, 1 H, C(5′)H$\underline{H}_b$, *J* = 17.2, *J* = 1.4); 5.74 (s, 2 H, NC(1′)H$_2$); 5.69 (ddt, 1 H, C(4′)H, *J* = 17.2, *J* = 10.3, *J* = 5.5)	1720, 1750 (C=O)
8a	3.25 (t, 1 H, C(5′)H, *J* = 2.2); 4.35 (d, 2 H, C(3′)H$_2$, *J* = 2.2); 5.73 (s, 2 H, NC(1′)H$_2$); 8.45 (s, 1 H, CH)	1720 (C=O); 1550, 1360 (NO$_2$); 2130 (C≡CH)
8b	2.54 (s, 3 H, C—CH$_3$); 3.22 (t, 1 H, C(5′)H, *J* = 2.2); 4.34 (d, 2 H, C(3′)H$_2$, *J* = 2.2); 5.77 (s, 2 H, NC(1′)H$_2$)	1720 (C=O); 1560, 1360 (NO$_2$); 2130 (C≡CH)
8c	2.54 (s, 3 H, SCH$_3$); 3.22 (t, 1 H, C(5′)H, *J* = 2.3); 4.34 (d, 2 H, C(3′)H$_2$, *J* = 2.3); 5.77 (s, 2 H, NC(1′)H$_2$)	1720 (C=O); 1560, 1360 (NO$_2$); 2120 (C≡CH)
8d	1.34 (t, 3 H, OCH$_2\underline{CH}_3$, *J* = 7.2); 3.51 (t, 1 H, C(5′)H, *J* = 2.1); 4.36 (d, 2 H, C(3′)H$_2$, *J* = 2.1); 4.40 (q, 2 H, O$\underline{CH}_2$CH$_3$, *J* = 7.2); 5.79 (s, 2 H, NC(1′)H$_2$); 8.49 (s, 1 H, CH)	1710, 1760 (C=O); 2120 (C≡CH)
8e	1.34 (t, 3 H, OCH$_2\underline{CH}_3$, *J* = 7.2); 2.46 (s, 3 H, C—CH$_3$); 3.51 (t, 1 H, C(5′)H, *J* = 2.2); 4.36 (d, 2 H, C(3′)H$_2$, *J* = 2.2); 4.40 (q, 2 H, O$\underline{CH}_2$CH$_3$, *J* = 7.2); 5.73 (s, 2 H, NC(1′)H$_2$)	1720, 1740 (C=O); 2120 C≡CH)
8f	1.39 (t, 3 H, OCH$_2\underline{CH}_3$, *J* = 7.2); 2.67 (s, 3 H, S—CH$_3$); 3.16 (t, 1 H, C(5′)H, *J* = 2.2); 4.30 (d, 2 H, C(3′)H$_2$, *J* = 2.2); 4.49 (q, 2 H, O$\underline{CH}_2$CH$_3$, *J* = 7.2); 5.69 (s, 2 H, NC(1′)H$_2$)	1720, 1740 (C=O); 2110 (C≡CH)
10a	2.00 (s, 3 H, COCH$_3$); 3.89 (t, 2 H, OC(3′)H$_2$, *J* = 4.6); 4.13 (t, 2 H, OC(4′)H$_2$, *J* = 4.6); 5.78 (s, 2 H, NC(1′)H$_2$); 8.45 (s, 1 H, CH)	1730, 1710 (C=O); 1560, 1340 (NO$_2$)
10c	2.00 (s, 3 H, COCH$_3$); 2.69 (s, 3 H, SCH$_3$); 3.88 (t, 2 H, OC(3′)H$_2$, *J* = 4.6); 4.16 (t, 2 H, OC(4′)H$_2$, *J* = 4.6); 5.69 (s, 2 H, NC(1′)H$_2$)	1730, 1700 (C=O); 1550, 1350 (NO$_2$)
10d	1.32 (t, 3 H, OCH$_2\underline{CH}_3$, *J* = 7.2); 1.98 (s, 3 H, COCH$_3$); 3.87 (t, 2 H, OC(3′)H$_2$, *J* = 4.5); 4.15 (t, 2 H, OC(4′)H$_2$, *J* = 4.5); 4.37 (q, 2 H, O$\underline{CH}_2$CH$_3$, *J* = 7.2); 5.67 (s, 2 H, NC(1′)H$_2$); 8.40 (s, 1 H, CH)	1710, 1720, 1750 (C=O)
10e	1.32 (t, 3 H, OCH$_2\underline{CH}_3$, *J* = 7.0); 1.98 (s, 3 H, COCH$_3$); 2.46 (s, 3 H, C—CH$_3$); 3.85 (t, 2 H, OC(3′)H$_2$, *J* = 4.6); 4.12 (t, 2 H, OC(4′)H$_2$, *J* = 4.6); 4.37 (q, 2 H, O$\underline{CH}_2$CH$_3$, *J* = 7.0); 5.69 (s, 2 H, NC(1′)H$_2$)	1700, 1710, 1740 (C=O)
11a	3.42—3.51 (m, 2 H, OC(3′)H$_2$); 3.67—3.80 (m, 2 H, OC(4′)H$_2$,); 5.77 (s, 2 H, NC(1′)H$_2$,); 8.44 (s, 1 H, CH)	1720 (C=O); 1540, 1350 (NO$_2$)
11c	2.69 (s, 3 H, SCH$_3$); 3.50 (t, 2 H, OC(3′)H$_2$, *J* = 4.8); 3.71 (dt, 2 H, OC(3′)H$_2$, *J* = 5.4, *J* = 6.3); 5.69 (s, 2 H, NC(1′)H$_2$);	1720 (C=O); 1530, 1340 (NO$_2$)
11d	1.24 (t, 3 H, OCH$_2\underline{CH}_3$, *J* = 7.0); 3.50 (t, 2 H, OC(3′)H$_2$, *J* = 4.7); 3.65—3.77 (m, 2 H, OC(4′)H$_2$); 4.50 (q, 2 H, O$\underline{CH}_2$CH$_3$, *J* = 7.0); 5.73 (s, 2 H, NC(1′)H$_2$); 8.33 (s, 1 H, CH)	1710, 1740 (C=O)
11e	1.37 (t, 3 H, OCH$_2\underline{CH}_3$, *J*= 7.3); 2.52 (s, 3 H, C—CH$_3$); 3.51 (t, 2 H, OC(3′)H$_2$, *J* = 4.8); 3.70 (dt, 2 H, OC(4′)H$_2$, *J* = 5.5, *J* = 6.2); 5.68 (s, 2 H, NC(1′)H$_2$)	1700, 1760 (C=O)

(*to be continued*)

Table 2 (*continued*)

Compound	^{1}H NMR, δ (*J*/Hz)	IR, ν/cm^{-1}
13a	1.71 (tt, 2 H, C(3′)H$_2$, *J* = 6.5, *J* = 15.3); 1.94 (tt, 2 H, C(2′)H$_2$, *J* = 7.0, *J* = 15.0); 2.00 (s, 3 H, COCH$_3$); 4.03 (t, 2 H, OC(4′)H$_2$, *J* = 6.5); 4.43 (t, 2 H, NC(1′)H$_2$, *J* = 7.2); 8.63 (s, 1 H, CH)	1720, 1700 (C=O); 1550, 1340 (NO$_2$)
13b	1.72 (m, 2 H, C(3′)H$_2$); 1.99 (m, 2 H, C(2′)H$_2$); 2.01 (s, 3 H, COCH$_3$); 2.55 (s, 3 H, CH$_3$); 4.04 (t, 2 H, OC(4′)H$_2$, *J* = 7.2); 4.43 (t, 2 H, NC(1′)H$_2$, *J* = 7.2)	1730, 1710 (C=O); 1540, 1360 (NO$_2$)
13c	1.72 (m, 2 H, C(3′)H$_2$); 1.99 (m, 2 H, C(2′)H$_2$); 2.01 (s, 3 H, COCH$_3$); 2.69 (s, 3 H, SCH$_3$); 4.05 (t, 2 H, OC(4′)H$_2$, *J* = 6.4); 4.45 (t, 2 H, NC(1′)H$_2$, *J* = 6.9)	1720, 1710 (C=O); 1560, 1340 (NO$_2$)
13d	1.33 (t, 3 H, OCH$_2$CH$_3$, *J* = 6.9); 1.67 (tt, 2 H, C(3′)H$_2$, *J* = 6.5, *J* = 15.5); 1.91 (tt, 2 H, C(2′)H$_2$, *J* = 6.9, *J* = 15.1); 1.99 (s, 3 H, COCH$_3$); 4.03 (t, 2 H, OC(4′)H$_2$, *J* = 6.5); 4.37 (q, 2 H, OCH$_2$CH$_3$, *J* = 6.9); 4.43 (t, 2 H, NC(1′)H$_2$, *J* = 6.9); 8.48 (s, 1 H, CH)	1700, 1725, 1750 (C=O)
13e	1.31 (t, 3 H, OCH$_2$CH$_3$, *J* = 6.9); 1.68 (tt, 2 H, C(3′)H$_2$, *J* = 6.5, *J* = 15.2); 1.91 (tt, 2 H, C(2′)H$_2$, *J* = 7.3, *J* = 15.0); 1.99 (s, 3 H, COCH$_3$); 2.49 (s, 3 H, CH$_3$); 4.03 (t, 2 H, OC(4′)H$_2$, *J* = 6.5); 4.34 (t, 2 H, NC(1′)H$_2$, *J* = 7.3); 4.37 (q, 2 H, OCH$_2$CH$_3$, *J* = 6.9)	1710, 1730, 1760 (C=O)
13f	1.38 (t, 3 H, OCH$_2$CH$_3$, *J* = 6.9); 1.71 (tt, 2 H, C(3′)H$_2$, *J* = 6.5, *J* = 15.2); 1.99 (tt, 2 H, C(2′)H$_2$, *J* = 7.3, *J* = 15.0); 2.01 (s, 3 H, COCH$_3$); 2.66 (s, 3 H, SCH$_3$); 4.07 (t, 2 H, OC(4′)H$_2$, *J* = 6.5); 4.36 (t, 2 H, NC(1′)H$_2$, *J* = 7.3); 4.37 (q, 2 H, OCH$_2$CH$_3$, *J* = 6.9)	1710, 1730, 1750 (C=O)
14a	1.55 (tt, 2 H, C(3′)H$_2$, *J* = 6.5, *J* = 15.5); 1.97 (tt, 2 H, C(2′)H$_2$, *J* = 6.9, *J* = 15.1); 3.44 (dt, 2 H, OC(4′)H$_2$, *J* = 6.3, *J* = 6.6); 4.20 (t, 1 H, OH, *J* = 6.1); 4.43 (t, 2 H, NC(1′)H$_2$, *J* = 6.9); 8.54 (s, 1 H, CH)	1730 (C=O); 1520, 1360 (NO$_2$)
14b	1.53 (tt, 2 H, C(3′)H$_2$, *J* = 6.45, *J* = 15.4); 1.98 (tt, 2 H, C(2′)H$_2$, *J* = 6.9, *J* = 15.1); 2.52 (s, 3 H, C—CH$_3$); 3.41 (dt, 2 H, OC(4′)H$_2$, *J* = 6.1, *J* = 6.4); 4.20 (t, 1 H, OH, *J* = 6.1); 4.41 (t, 2 H, NC(1′)H$_2$, *J* = 6.9)	1720 (C=O); 1530, 1350 (NO$_2$)
14c	1.53 (tt, 2 H, C(3′)H$_2$, *J* = 6.5, *J* = 15.4); 1.97 (tt, 2 H, C(2′)H$_2$, *J* = 6.9, *J* = 15.1); 2.68 (s, 3 H, S—CH$_3$); 3.43 (dt, 2 H, OC(4′)H$_2$, *J* = 6.6, *J* = 7.0); 4.20 (br.s, 1 H, OH); 4.41 (t, 2 H, NC(1′)H$_2$, *J* = 6.9)	1720 (C=O); 1540, 1350 (NO$_2$)
14d	1.39 (t, 3 H, OCH$_2$CH$_3$, *J* = 7.2); 1.55 (tt, 2 H, C(3′)H$_2$, *J* = 6.4, *J* = 15.4); 2.00 (tt, C(2′)H$_2$, *J* = 6.9, *J* = 15.1); 3.22 (q, 2 H, OCH$_2$CH$_3$, *J* = 7.2); 4.10 (t, 1 H, OH, *J* = 5.1); 4.20 (m, 4 H, OC(4′)H$_2$ + NC(1′)H$_2$); 8.31 (s, 1 H, CH)	1700, 1740 (C=O)
14e	1.39 (t, 3 H, OCH$_2$CH$_3$, *J* = 7.0); 1.54 (tt, 2 H, C(3′)H$_2$, *J* = 6.4, *J* = 15.4); 1.98 (tt, 2 H, C(2′)H$_2$, *J* = 6.8, *J* = 15.2); 2.52 (s, 3 H, C—CH$_3$); 3.40 (q, 2 H, OCH$_2$CH$_3$, *J* = 7.0); 4.20 (t, 1 H, OH, *J* = 5.1); 4.35 (m, 4 H, OC(4′)H$_2$ + NC(1′)H$_2$)	1700, 1750 (C=O)
14f	1.39 (t, 3H, OCH$_2$CH$_3$, *J* = 7.0); 1.54 (tt, 2 H, C(3′)H$_2$, *J* = 6.4, *J* = 15.4); 1.98 (tt, 2 H, C(2′)H$_2$, *J* = 6.8, *J* = 15.2); 2.67 (s, 3 H, S—CH$_3$); 3.45 (q, 2 H, OCH$_2$CH$_3$, *J* = 7.0); 4.20 (t, 1 H, OH, *J* = 4.9); 4.35 (m, 4 H, OC(4′)H$_2$ + NC(1′)H$_2$)	1690, 1740 (C=O)

ment of the signals in the ^{1}H and ^{13}C NMR spectra (see Table 2 and 3).

Study of the antiviral effect against influenza viruses of the types A(H3N2), A(H5N1), and A(H0N1) (remantadin-resistant strains), as well as of the type B, showed that compounds **7a—c** and **8a—c** possess activity comparable with that of Remantadin with respect to the influenza A and exceed its effect with respect to the influenza virus B.[13] As to the modified nucleosides **7a,b** (especially, **7b**), further study revealed their activities with respect to the remantadin-resistant strains A(H0N1) and considerable reduction in reproduction of the type B influenza virus with respect to which Remantadin is inactive (Table 4).

2-R^1-4-(2-Hydroxyethoxymethyl)-6-nitro-1,2,4-triazolo[3,2-*c*][1,2,4]triazin-7-ones (**11**) and 2-R^1-4-(4-hydroxybutyl)-6-nitro-1,2,4-triazolo[3,2-*c*][1,2,4]triazin-7-ones (**14**) exhibited no antiviral effect with respect to influenza viruses.

Determination of the antiviral action of compounds synthesized with respect to the respiratory syncytial virus demonstrated the inhibitory effect of 4-allyloxymethyl-6-ethoxycarbonyl- (**7d**) and 6-nitro-4-propargyloxymethyl-1,2,4-triazolo[3,2-*c*][1,2,4]triazin-7-ones (**8a—c**). These drugs reduce the infectious activity of the virus by no less than 1.85 log ID$_{50}$ (67%) (compound **7d**), 1.7 log ID$_{50}$ (56%) (compound **8a**), 1.1 log ID$_{50}$ (34%) (compound **8b**), and 1.0 log ID$_{50}$ (22%) (compound **8c**). Other compounds studied in this work, like remantadin, display no antiviral activity with respect to respiratory syncytial virus in the *in vitro* system.

Table 3. ^{13}C NMR spectra of compounds **7d, 7e**, **8c—e, 10e**, **13a,c—e**

Compound	δ									Other signals
	C(2)	C(3a)	C(6)	C(7)	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	
7d	153.1	151.4	132.3	146.9	82.3		70.1	133.7	117.7	14.0 ($OCH_2\underline{C}H_3$); 61.9 ($O\underline{C}H_2CH_3$); 160.4 (C=O)
7e	162.7	151.5	132.3	146.5	82.3		70.1	133.7	117.7	160.5 (C=O); 61.9 ($O\underline{C}H_2CH_3$); 14.3 (CH_3); 14.0 ($OCH_2\underline{C}H_3$)
8c	167.1	152.1	141.9	141.9*	81.9		56.9	78.6**	78.6	13.7($S—CH_3$)
8d	153.1	151.4	132.5	146.9	81.5		56.8	79.0	78.0	160.4 (C=O); 62.1 ($O\underline{C}H_2CH_3$); 14.0 ($OCH_2\underline{C}H_3$)
8e	162.7	151.5	132.6	146.5	81.6		56.7	79.0	78.0	160.4 (C=O); 62.0 ($O\underline{C}H_2CH_3$); 14.3 ($C—\underline{C}H_3$); 14.0 ($OCH_2\underline{C}H_3$)
10e	162.7	151.5	132.5	146.5	83.0		67.7	62.7		170.3 ($\underline{C}OCH_3$); 160.5 (C=O); 61.9 ($O\underline{C}H_2CH_3$); 20.6 ($CO\underline{C}H_3$); 14.3 ($C—\underline{C}H_3$); 14.0 ($OCH_2\underline{C}H_3$)
13a	154.2	151.7	140.9	143.3	54.9	24.2	24.8	63.2		170.4 ($\underline{C}OCH_3$); 20.7 ($CO\underline{C}H_3$);
13c	166.9	152.2	141.2	142.0	63.2	24.1	24.8	54.9		170.4 ($\underline{C}OCH_3$); 20.6 ($CO\underline{C}H_3$); 13.6 ($S—CH_3$)
13d	153.1	151.2	131.2	147.1	54.3	24.2	24.9	63.2		170.4 ($\underline{C}OCH_3$); 160.6 (C=O); 61.7 ($O\underline{C}H_2CH_3$); 20.7 ($CO\underline{C}H_3$); 14.0 ($OCH_2\underline{C}H_3$)
13e	162.7	160.7	131.3	146.6	61.7	24.2	24.9	63.2		170.4 ($\underline{C}OCH_3$); 151.3 (C=O); 54.2 ($O\underline{C}H_2CH_3$); 20.7 ($CO\underline{C}H_3$); 14.2 ($C—\underline{C}H_3$); 14.0 ($OCH_2\underline{C}H_3$)

* Overlapped with the signal for C(6).
** Overlapped with the signal for C(5′).

Table 4. Reduction of different influenza viruses reproduction ($\Delta/\log ID_{50}$) by compounds **7a—c**, **8a—c**

Drug	C^a /μg mL^{-1}	$\Delta^b/\log ID_{50}$					
		B /Samara/ 253/99	A/H3N2 A/Hong Kong/ 1/68	A/H3N2 A/St.-Petersburg/ 22/99	A/H5N1 A/Duck/ Singapore R/F 119-3/97	AH0N1 A/Mongolia/ 56/87	AH0N1 A/PR/8/34
7a	20	2.5**	0.5*	—[c]	1.5**	2.5*	2.5*
	40	2.5**	2.0**	—[c]	3.0***	3.0***	4.0***
7b	20	1.0*	0.5*	—[c]	1.5*	2.5**	2.5**
	40	2.5**	2.0**	—[c]	3.0***	3.0***	4.0***
7c	20	2.5**	2.0**	—[c]	2.5**	4.0***	2.5**
	40	3.0***	2.5**	—[c]	3.0***	4.0***	4.0***
8a	20	—[c]	—[c]	3.0	—[c]	—[c]	—[c]
	40	—[c]	—[c]	4.0	—[c]	—[c]	—[c]
8b	20	—[c]	—[c]	2.9	—[c]	—[c]	—[c]
	40	—[c]	—[c]	4.0***	—[c]	—[c]	—[c]
8c	20	—[c]	—[c]	2.6**	—[c]	—[c]	—[c]
	40	—[c]	—[c]	4.0***	—[c]	—[c]	—[c]
Remantadin	20	0	2.5**	2.9**	3.0***	0	1.0*
	40	0.5**	2.5**	3.3***	3.0***	1.5*	1.5*
The starting virus titre		4.5	3.5	5.1	4.01	5.01	5.5

[a] *C* is the drug concentration.
[b] Δ is the difference between the virus titre in the absence of the drug (control experiment) and after action of the drug: * weak efficiency; ** moderate efficiency; *** high efficiency.
[c] No testing was made.

Experimental

^{1}H NMR spectra were recorded on a Bruker Avance II 400 spectrometer in DMSO-d_6 with Me_4Si as an internal standard. IR spectra were recorded on a Perkin Elmer Spectrum One B IR Fourier-spectrometer using a diffuse reflection adapter.

Alfa Aesar silica gel (Avocado Research Chemical Ltd, silica gel 60, 0.035—0.070 mm (220—440 mesh)) was used for column chromatography.

Melting points were measured on a Boetius heating stage.

1,2,4-Triazolo[3,2-*c*][1,2,4]triazin-7-ones (**4a**—**f**) were obtained according to the procedures described earlier.[7,12]

4-Bromobutyl acetate,[14] allyloxymethyl acetate (**5**),[15] and (2-acetoxyethoxy)methyl acetate (**9**)[16] were obtained using procedures described earlier.

Propargyloxymethyl acetate (6). A suspension of propargyloxymethyl chloride[17] (5 g, 0.048 mol) and potassium acetate (9.4 g, 0.095 mol) in dichloromethane (40 mL) was stirred for 24 h. Solids were filtered off and the solvent was evaporated *in vacuo*. Compound **6** obtained was used without additional purification.

2-R^1-4-Allyloxymethyl-6-R^2-1,2,4-triazolo[3,2-*c*][1,2,4]-triazin-7-ones 7a—f (general procedure). A suspension of 1,2,4-triazolo[3,2-c][1,2,4]triazin-7-one **4a—f** (0.001 mol) and allyloxymethyl acetate (**5**) (1.35 mL, 0.001 mol) in dichloromethane was refluxed for 5 min. After cooling, a precipitate that formed was recrystallized from propan-2-ol. Physicochemical characteristics and spectral data are given in Tables 1 and 2.

2-R^1-4-Propargyloxymethyl-6-R^2-1,2,4-triazolo[3,2-*c*]-[1,2,4]triazin-7-ones 8a—f (general procedure). A suspension of 1,2,4-triazolo[3,2-*c*][1,2,4]triazin-7-one **4a—f** (0.001 mol) and propargyloxymethyl acetate **(6)** (1.30 mL, 0.001 mol) in dichloromethane was refluxed for 5 min. After cooling, a precipitate that formed was recrystallized from ethanol.

2-R^1-4-(2-Acetoxyethoxy)methyl-6-R^2-1,2,4-triazolo[3,2-*c*]-[1,2,4]triazin-7-ones 10a,c—e (general procedure). 1,2,4-Triazolo[3,2-*c*][1,2,4]triazinone (**4a,c—e)** (0.001 mol) was fused with (2-acetoxyethoxy)methyl acetate (**9**) (0.53 g, 0.003 mol) at 150 °C for 30 min in the presence of catalytic amounts of $ZnCl_2$. The reaction mixture was cooled and the residue obtained was recrystallized from propan-2-ol. Characteristics of compounds **10a,c—e** are given in Tables 1 and 2.

2-R^1-4-(2-Hydroxyethoxy)methyl-6-nitro-1,2,4-triazolo-[3,2-*c*][1,2,4]triazin-7-ones 11a,c (general procedure). The ion-exchange resin KU-1 (3 g, H^+) was added to a solution of compound **10a,c** (0.002 mol) in methanol (15 mL) and the reaction mixture was stirred for 12 h at room temperature. The resin was filtered off and methanol was evaporated. The residue was purified by flash chromatography on a dry column using ethyl acetate as an eluent. Characteristics of compounds **11a,c** are given in Tables 1 and 2.

2-R^1-6-Ethoxycarbonyl-4-(2-hydroxyethoxy)methyl-1,2,4-triazolo[3,2-*c*][1,2,4]triazin-7-ones 11d,e (general procedure). A ion-exchange resin KU-1 (5 g, H^+) was added to a cooled to 0 °C solution of compound **10d,e** (0.002 mol) in anhydrous ethanol (20 mL) and the reaction mixture was stirred for 24 h at 0 °C. The resin was filtered off and ethanol was evaporated. The residue was purified by column chromatography using ethyl acetate as an eluent. Characteristics of compounds **11d,e** are given in Tables 1 and 2.

2-R^1-4-(4-Acetoxybutyl)-6-R^2-1,2,4-triazolo[3,2-*c*][1,2,4]-triazin-7-ones 13a—f (general procedure). 4-Bromobutyl acetate (0.58 mL, 0.003 mol) was added to a solution of sodium salt of triazolo-1,2,4-triazinone (**12a—f**)[18,19] (0.003 mol) in DMF (7 mL) and the mixture was heated for 3.5 h at 100 °C. Water (30 mL) was added to the cooled reaction mixture and the suspension obtained was shaken with ethyl acetate (3×15 mL), the organic layer was separated and concentrated. The residue obtained was recrystallized from propan-2-ol. Characteristics of compounds **13a—f** are given in Tables 1—3.

2-R^1-4-(4-Hydroxybutyl)-6-nitro-1,2,4-triazolo[3,2-*c*][1,2,4]-triazin-7-ones 14a—c (general procedure). The ion-exchange resin KU-1 (3 g, H^+) was added to a solution of compound **13a—c** (0.002 mol) in methanol (7 mL) and the reaction mixture was refluxed for 2 h. The resin was filtered off and methanol was evaporated. The residue was purified by flash chromatography on a dry column with ethyl acetate as an eluent. Characteristics of compounds **14a—c** are given in Tables 1—3.

2-R^1-6-Ethoxycarbonyl-4-(4-hydroxybutyl)-1,2,4-triazolo[3,2-*c*][1,2,4]triazin-7-ones 14d—f (general procedure). Acetyl chloride (1 mL) and ethanol (5 mL) were added to a solution of compound **13d—f** (0.002 mol) in anhydrous ethanol (10 mL), and the reaction mixture was refluxed for 1 h. After cooling the mixture, sodium acetate (3 g) and ethanol (15 mL) were added, solids were filtered off, and the filtrate was concentrated. The residue was purified by column chromatography with ethyl acetate as an eluent. Characteristics of compounds **14d—f** are given in Tables 1—3.

Determination of antiviral activity of compounds against influenza viruses. In this work, we used influenza viruses of the types A(H3N2) (A/Hong Kong/1/68 and A/St.-Petersburg/22/99), A(H5N1) (A/Duck/Singapore/R/F119-3/97) and remantadin-resistant strains A(H0N1) (A/Mongolia/56/87 and A(PR/8/34), as well as that of the type B (B/Samara/253/99). Determination of antiviral activity of compounds against influenza viruses was performed on the CAM (chick embryo chorioallantoic membrane) model on 71-well polystyrene plates. Compounds in different concentrations were dissolved in the medium for CAM and placed into the wells with CAM fragments, to which the viruses in appropriate dilutions were then added, and the plate was incubated at 33—34 °C for 48 h (for the type A influenza) and 72 h (for the type B influenza). The results were determined based on the hemagglutination reaction by addition of 1% chick erythrocytes to the culture liquid. The virus titre was calculated by the Reed and Muench method.[20] The virus-inhibitory activity of the drugs was estimated based on the decrease in the virus infectious titre (log ID_{50}) in the experiments *vs.* the control. For the control, CAM in the absence of the drug and virus, CAM with added virus, and CAM with added drug **7a—c** and **8a—c** were used. Remantadin served as the antiviral reference drug.

Determination of antiviral activity of compounds against respiratory syncytial infection. The respiratory sincitial virus (the Long strain) and the MA-104 cell culture were used. The assay of the inhibitory activity of drugs against respiratory syncytial infection included the following steps: 1) introduction of drugs in definite concentrations in a supporting medium into test tubes with the cell culture; 2) incubation of the drug with the tissue cell cultures for 1 h at room temperature; 3) after the contact, introduction of the virus into the test tubes. The observation lasted for 5—7 days depending on the development of cytopatic activity of the virus in the control test tubes. When the observation was

stopped, samples of the virus-containing cultural liquid were titrated to determine infectious activity in the trial and control samples. Activity of the viral reproduction was evaluated based on the infectious titre values calculated by the Reed and Muench method.[20] Cell cultures without additives, cell cultures with added virus, cell cultures with added drug **7a—c** and **8a—c** were used for the control in the performed experiments.

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