Reviews

Biologically active azolo-1,2,4-triazines and azolopyrimidines

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The review presents current data on the methods of synthesis and biological activity of purine-isosteric azolo-annulated 1,2,4-triazines and pyrimidines with a bridgehead nitrogen atom, which are among the most promising classes of biologically active compounds.

Key words: heterocyclic polynitrogen compounds, azolo[5,1-*c*]-1,2,4-triazines, azolo[1,5-*a*]-pyrimidines, biological activity, antiviral activity, antiglycation activity, adenosine receptor agonists.

Introduction

Azolo-annulated pyrimidines and 1,2,4-triazines have attracted continuing interest due primarily to their structural similarity to heterocyclic bases of DNA and RNA. As a consequence, these compounds can act as antimetabolites, being effective biologically active compounds. So, it was not surprising that the targeted synthesis of structural analogues of DNA and RNA bases and their nucleosides has received considerable attention, resulting in the design of a series of antiviral agents, such as acyclovir and ribavirin.[1,2]

Adenosine analogues with a close structural similarity to biogenic purines are antagonists of adenosine receptors, which regulate various physiological processes. It is quite natural that in recent years these compounds were considered as promising materials for the design of drugs for Parkinson′s and Alzheimer′s diseases and cardiac ischemia.[3] Fundamental research on the role of adenosine receptors has demonstrated that they regulate the development of pathological states, including those related to pulmonary diseases and sepsis, through cellular signaling systems.[4—6]

One of the lines of research in this area is the synthesis of azolo[1,5-*a*]pyrimidines and azolo[5,1-*c*]-1,2,4-triazines of general formulas **1** and **2**, which can be considered as isosteres (structural analogues) of purine bases of DNA and RNA, such as hypoxanthine, adenine, and guanine.

This review is concerned with methods of synthesis and properties of pyrazolo- or 1,2,4-triazolo[5,1-*c*]-1,2,4-triazines and pyrazolo- or 1,2,4-triazolo[1,5-*a*]pyrimi-

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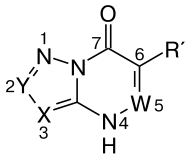
Azoloazines



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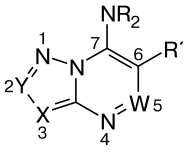
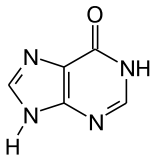


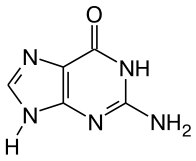
2

R = H, Alk, Ar; R′ = F, Cl, Br, I, NO_2, CF_3, CN, CO_2Et, Ar, Het;
X, Y = N, CH, CAlk, CAr, CCO_2Et, CSH, CSAlk;
W = N, CH, CAlk, CAr, COH, CNH_2

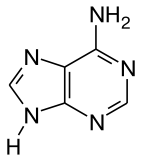
Biogenic purines



Hypoxanthine



Guanine



Adenine

dines, including their antiviral and antidiabetic activity and effects on adenosine receptors, thereby uncovering opportunities to use such heterocyclic systems in the design of new biologically active compounds.

In the literature on the methods of synthesis and chemical properties of azolo[1,5-*a*]pyrimidines and azolo-[5,1-*c*]-1,2,4-triazines, different atomic numbering schemes are employed for heterocyclic systems. In the present review, the atoms in compounds **1** and **2** are numbered starting at the azole nitrogen atom.

1. Azolo[5,1-*c*]-1,2,4-triazines

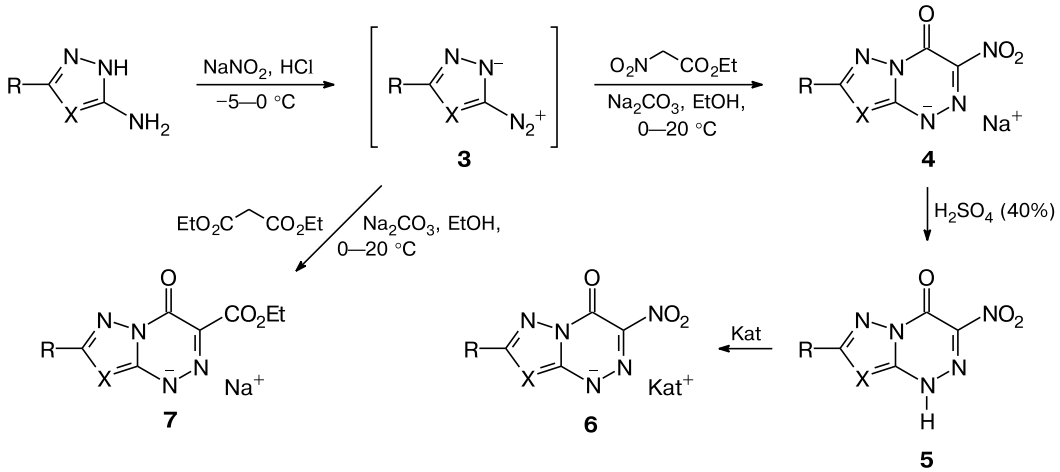
1.1. Synthesis of azolo[5,1-c]-1,2,4-triazin-7-ones

Two fundamentally different approaches are used to construct the azolo[5,1-*c*]-1,2,4-triazine system: the fusion of the azine ring to the azole moiety and the annulation of the azole ring to the azine one.[7] In our studies, we focused on the annulation of the azine ring to the azole moiety in preparing pyrazolo- or 1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones.

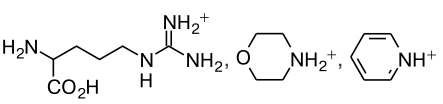
In the series of the compounds under consideration, nitro derivatives belonging to a new family of antiviral agents[7—14] are of most interest. The synthesis of 6-nitro derivatives was accomplished by the reaction of diazopyrazoles or diazo-1,2,4-triazoles **3**, which were prepared by diazotization of the appropriate aminoazoles, with ethyl nitroacetate in an alkaline medium. Under these conditions, 6-nitropyrazolo[5,1-*c*]-1,2,4-triazin-7-ones and 6-nitro-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones were isolated in the form of sodium salts **4** (Scheme 1).[7—15] The formation of sodium salts is attributed to the fact that azolo[5,1-*c*]-1,2,4-triazin-7-ones are fairly strong NH-acids by themselves.[16]

The treatment of sodium salts **4** with dilute sulfuric acid afforded 6-nitroazolo[5,1-*c*]-1,2,4-triazin-7-ones **5**, and the reaction of the latter with ammonia, aliphatic amines, or amino acids gave the corresponding ammonium salts **6**. This method was applied to prepare 6-carbethoxy derivatives **7** (see Scheme 1).

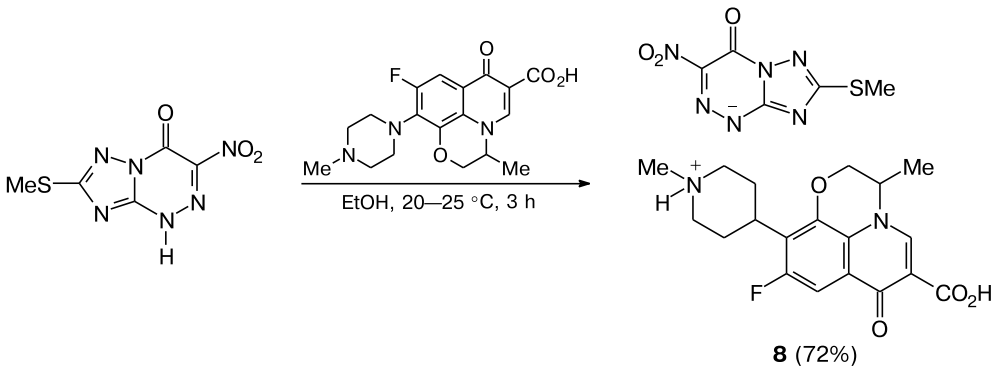
Scheme 1



R = H, Me, SMe, SEt, SPr, SBu, Ph, 4-pyridyl; X = N, CH, CCN, CCO_2Et, CCO_2H, CBr;

Kat^+ = NH_4^+, HO_2C⁄⁄NH_3^+, 

Scheme 2



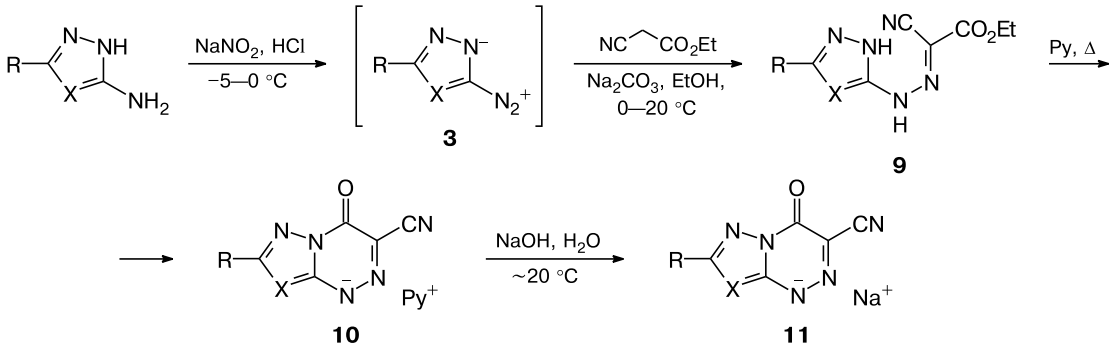
This approach was employed in the synthesis of salts of 2-methylthio-6-nitroazolo[5,1-*c*]-1,2,4-triazin-7-one with levofloxacin **8** and some other fluoroquinolones (Scheme 2).[17,18]

The azo coupling of diazoazoles **3** with ethyl cyanoacetate in the presence of sodium carbonate stops at intermediate hydrazones **9**.[7,19—21] The heating of the latter in pyridine resulted in the 1,2,4-triazine ring closure giving 6-cyanopyrazolo[5,1-*c*]-1,2,4-triazin-7-ones as pyridinium salts **10**. Sodium salts **11** were prepared by the treatment of compounds **10** with a NaOH solution (Scheme 3) in order to increase solubility in water and improve bioavailability.[15]

However, not all acetate derivatives smoothly react with azolyldiazonium salts. It was shown[22] that the reactivity of the synthon supplying two C atoms to the 1,2,4-triazine ring of azolo[5,1-*c*]-1,2,4-triazin-7-ones can be increased by the introduction of the electron-withdrawing formyl moiety. These CH-activated esters are easily coupled with azolyldiazonium salts, which is accompanied by the loss of the activating group. This procedure was applied to prepare hydrazones **12** and azolo-1,2,4-triazines **13** in high yields (Scheme 4). 6-Phenyl- and 6-pentafluorophenyl-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones **13** were isolated as sodium salts.

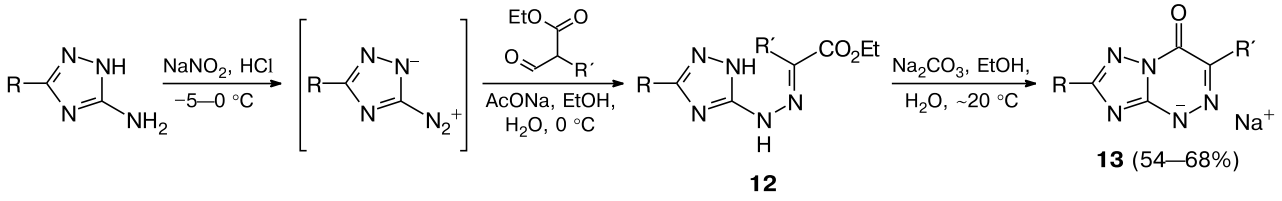
This approach was also used in the synthesis of 6-fluoro-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones **16** (Scheme 5) through the azo coupling of 1,2,4-triazolyl-5-diazonium salts with ethyl 2-fluoroacetoacetate (**14**) followed by deacetylation and cyclization.[23,24]

Scheme 3



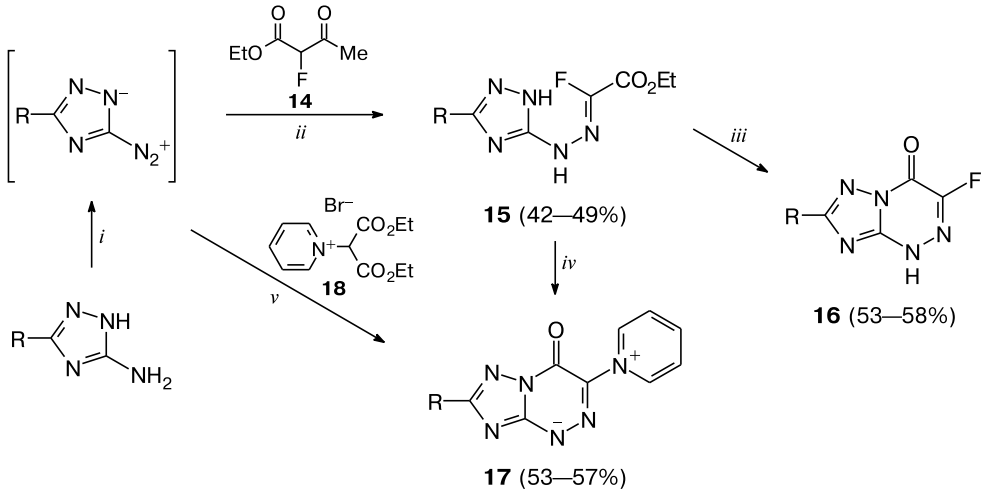
R = H, Me, SMe; X = N, CH, CCN, CCO_2Et, CCO_2H, CBr

Scheme 4



R = H, Me, SMe; R′ = Ph, C_6F_5

Scheme 5



R = H, Me, SMe

Reagents and conditions: *i*. $NaNO_2$, HCl, −5—0 °C; *ii*. AcONa, EtOH, 0—5 °C, 1 h; *iii*. AcONa, 80% EtOH, reflux, 5 h; *iv*. Py, reflux, 2 h; *v*. Py, EtOH, ~20 °C, 12 h.

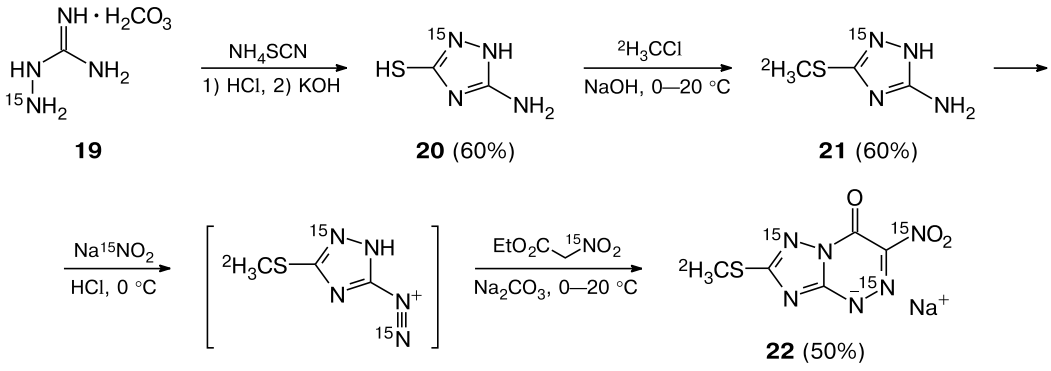
The azo coupling proceeds in the presence of sodium acetate to form 1,2,4-triazolyl hydrazones of ethyl fluoroglyoxylate **15**. It was found that the cyclization of hydrazones **15** does not occur during storage of the reactants in a sodium carbonate solution, *i.e.*, under the conditions typical of the transformations of triazolyl hydrazones, which were prepared with the use of acetoacetic and malonic esters.[7] Hydrazones **15** remain almost intact even under more severe conditions, for example, during heating in an ethanolic solution of sodium hydroxide, acetic acid, DMF, and DMSO. The heating of fluorine-containing compounds **15** in an aqueous ethanolic solution of sodium acetate under reflux proved to be a convenient procedure for the transformation of **15** into 6-fluoro-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones **16** (see Scheme 5).[23,24] An attempt to prepare fluorine-containing triazolo-1,2,4-triazines **16** by heating under reflux in pyridine led to the formation of product **17** *via* two processes — the nucleophilic substitution of fluorine and cyclization. 2-R-6-Pyridinium 1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-onates **17** were also synthesized by the independent synthesis based on the reaction of triazolyldiazonium salts with diethyl pyridinium malonate bromide (**18**) in the presence of pyridine (see Scheme 5).[23]

6-Nitro-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones **5** are generated according to a similar scheme *via* the coupling of diazo-1,2,4-triazoles with nitromalonic ester.[11,25]

Various procedures for the incorporation of stable isotopes into the 1,2,4-triazolo[5,1-*c*]-1,2,4-triazinone structure were developed[26—30] in order to study metabolism and pharmacokinetics of these compounds.

To introduce the ^{15}N isotope into the azole ring of the diazonium salt, a method was developed based on the use of aminoguanidine **19** (^{15}N (86%)), the treatment of which with ammonium thiocyanate produced thiourazole **20** (Scheme 6). In the next step, the selective incorporation

Scheme 6



of deuterium atoms was accomplished (compound **21**) by alkylation with deuterated methyl chloride (^{2}H (98%)). The subsequent diazotization with nitrous acid generated from $Na^{15}NO_2$ (^{15}N (98%)) gave an additional label, and the coupling with the deliberately synthesized nitro-^{15}N-acetate produced sodium salt of 2-methyl[$^2H_3$]thio-6-nitro[^{15}N]-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin[1,5-^{15}N]-7-one (**22**) containing three ^{15}N isotope labels and three deuterium labels. The positions of isotopes are determined by the scheme of the synthesis and specific characteristics in the ^{15}N and ^{13}C NMR spectra.

1.2. Alkylation and synthesis of natural nucleoside analogues

The reactivity of the synthesized compounds was studied in order to extend the range of azolo[5,1-*c*]-1,2,4-triazin-7-one derivatives. A convenient method for the modification of azolo[5,1-*c*]-1,2,4-triazin-7-ones is based on the alkylation, which allows the introduction of various substituents at the nitrogen atoms of the heterocyclic system. This reaction, apart from investigation of the reactivity of the molecular multicenter structure, can be used as a convenient model to assess the possibility of synthesis of natural nucleoside analogues. The ratio of the resulting isomers can easily be determined a nd diagnostic features for the determination of isomer structures, which are produced when using complex alkylating agents, can be revealed.

The in-depth study of the alkylation of 2-R-6-nitropyrazolo- and 2-R-6-nitro-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones, which are of most interest as biologically active compounds, demonstrated that the reactions of their sodium salts **23** with alkyl halides or dimethyl sulfate afford a mixture of *N*-alkyl derivatives **24**—**26** (Scheme 7, Table 1).[31—34] The *O*-alkylation products were not detected.

The isomer ratio depends on the nature of the alkylating agent and reaction conditions. It was found that the alkylation with methyl iodide in DMF at 60 °C affords alkylation products at the N(4) atom **24** in 80—90% yield (see Table 1). The employment of methanol as the solvent in this reaction leads to a substantial increase in the percentage of N(3)-isomer **26**. An increase in the chain length in the alkylating agent results in a decrease in the selectivity.

The alkylation of 6-carbethoxy-, 6-aryl-, and 6-(benzimidazol-2-yl)-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones gives N(4)-isomers as the major products.[33] The alkylation of azolo[5,1-*c*]-1,2,4-triazin-7-ones unsubstituted at the 6 position with methyl iodide or dimethyl sulfate affords a mixture of N(4)-methyl derivatives and N(5)-isomers having a betaine structure.[35]

Of special notice is the alkylation with sterically hindered reagents. Thus, the introduction of an adamantyl moiety into azolo[5,1-*c*]-1,2,4-triazin-7-ones **27** is a promising approach to the production of antiviral agents. In the series of azolo[5,1-*c*]-1,2,4-triazines under consideration, this reaction easily proceeds with adamantanol in sulfuric acid (Scheme 8, Table 2).[7,36,37]

The reaction affords isomers **28** and **29**. The yield of adamantylation products and the isomer ratio depend on the nature of the substituents and the reaction temperature (see Table 2). At low temperature (−15 °C), the **28** : **29** ratio is 1 : 3; an increase in the temperature to +20 °C leads to an increase in the fraction of compound **28** (**28** : **29** ≈ 2 : 1). Interestingly, the storage of any isomer in sulfuric acid for 12 h gave an equilibrium mixture of compounds **28** and **29**.[37]

The adamantylation of sodium salts of 1,2,4-triazolo-[5,1-*c*]-1,2,4-triazin-7-ones with bromoadamantane is a much more complex process compared to the conventional alkylation with primary or secondary alkyl halides and occurs only under heating in sulfolane.[36,37]

The formation of N(4)-isomer **28** as the major product is indicative of a higher thermodynamic stability of the alkylation product at the triazine ring.

Since azolo[5,1-*c*]-1,2,4-triazines are considered as isosteres of biogenic purines,[7] the synthesis of natural nucleoside analogues based on these compounds is of interest.

One of the most commonly used approaches to the glycosylation of NH-heterocycles is based on the reactions of silyl derivatives of nitrogen heterocycles with tetraacetyl-(tetrabenzoyl)ribose in the presence of Lewis acids.[38] Anomalous nucleosides based on 1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones **27** were synthesized by the one-step version of this reaction.[39] Thus, the reaction of compounds **27** with ribose tetraacetate in the presence of *N*,*O*-bis-(trimethylsilyl)acetamide (BSA) and trimethylsilyl triflate (TMSOTf) produces acyl derivatives **30** (Scheme 9).

The reaction is regioselective and proceeds exclusively at the N(4) atom.[40] The acyl deprotection is accomplished in sodium methoxide (for R′ = Ph) or *via* acid hydrolysis (for R′ = CO_2Et) to form natural nucleoside analogues **31**.

Under similar conditions, 6-phenyl-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones **27** react with glucose pentaacetate to give compounds **32** (Scheme 10).[40] This reaction, like that with ribose derivatives, is regioselective and occurs exclusively at the N(4) atom, which was confirmed by X-ray diffraction (Figs 1 and 2).

A standard approach in the nucleoside synthesis is based on the use of sodium salts of NH-heterocycles **27** and halogenated sugars. The alkylation conditions for sodium salts of azolo[5,1-*c*][1,2,4]triazin-7-ones[31] proved to be suitable for the synthesis of compounds **32**, the deacylation of which gave compounds **33** (see Scheme 10).

The synthesis of nitrogen heterocycles containing an acyclic group that mimics the ribofuranoside moiety is a promising route to biologically active structural analogues of natural nucleosides. Effective antiviral agents, such as acyclovir, penciclovir, ganciclovir, and famciclovir,

Scheme 7

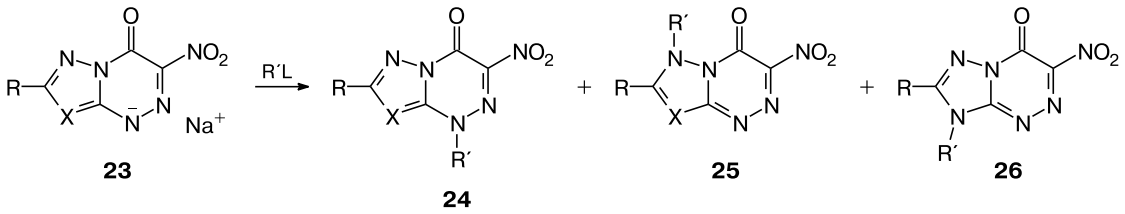


Table 1. Total yields and isomer ratios for **24**—**26** in the alkylation of sodium salts of 2-R-6-nitropyrazolo- and 2-R-6-nitro-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones **23** (see Scheme 7)

R	X	R′L	Solvent	Isomer ratio (%)*			Total yield (%)*
				24	**25**	**26**	
H	N	MeI	MeOH	80	0	20	70
H	N	MeI	DMF	85	1	14	95
H	N	MeI	MeCN	78	0	22	80
H	N	Me_2SO_4	MeOH	63	5	32	75
Me	N	MeI	MeOH	84	0	16	75
Me	N	MeI	DMF	89	2	9	97
Me	N	Me_2SO_4	MeOH	73	2	25	75
Me	N	MeI	MeCN	79	0	21	85
SMe	N	MeI	MeOH	55	0	45	85
SMe	N	MeI	DMF	90	0	10	95
SMe	N	Me_2SO_4	MeOH	80	5	20	75
H	CH	MeI	MeOH	88	0	12	85
H	CH	MeI	DMF	90	0	10	95
H	CH	MeI	MeCN	85	0	15	80
H	CMe	MeI	MeOH	100	0	0	100
H	CMe	MeI	DMF	100	0	0	100
Me	CH	MeI	MeOH	100	0	0	100
Me	CH	MeI	DMF	100	0	0	100
H	CCO_2Et	MeI	MeOH	100	0	0	95
H	CCO_2Et	MeI	DMF	98	0	2	100
H	CCO_2Et	Me_2SO_4	MeOH	97	0	3	85
Me	N	EtI	DMF	82	16	2	60
Me	CH	EtI	DMF	100	0	0	80
Me	CCO_2Et	EtI	DMF	98	0	2	90
H	N	$C_6H_{13}I$	DMF	80	17	3	60
H	N	EtO_2CH_2Br	DMF	30	60	10	70
H	CH	EtO_2CH_2Br	DMF	88	0	12	65
SMe	CH	Bu^tBr	DMF	96	0	4	67

* NMR data.

belong to acyclonucleosides used in clinical practice.[1,2] Pyrimidine derivatives modified at the heterocyclic base include 5-iodo-2′-deoxyuridine, (*E*)-5-(2-bromovinyl)-2′-deoxyiridine, and 5-trifluoromethyl-2′-deoxyuridine.[41]

Acylated acyclonucleosides **35** based on azolo[5,1-*c*]-1,2,4-triazin-7-ones were synthesized by heating sodium salts **34** in DMF with 4-bromobutyl acetate (Scheme 11). The reaction was performed under conditions characteristic of the alkylation of azolo-1,2,4-triazine salts.

The deacylation that was performed, depending on the nature of the substituent R′, with sodium methoxide, acetyl chloride, or ion-exchange resin, produced compounds **36**, which can be considered as natural nucleoside analogues.[42,43]

1,2,4-Triazolo[5,1-*c*]-1,2,4-triazin-7-ones were used as the starting compounds for the synthesis of acyclic nucleosides containing the (*Z*)-hydroxybutenyl group in the alkyl moiety, which can be considered as analogues of the antibiotic neplanocin A (S-adenosylhomocysteine hydrolase inhibitor).[44] The heating of sodium salts of 6-phenyl-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones with 4-bromobut-2-enyl acetate in DMF affords the corre-

Scheme 8

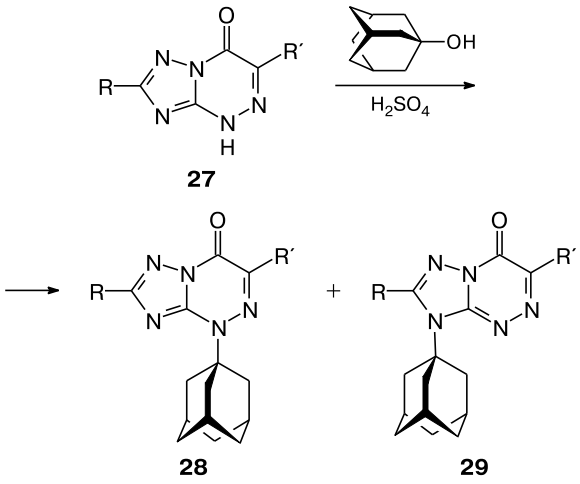
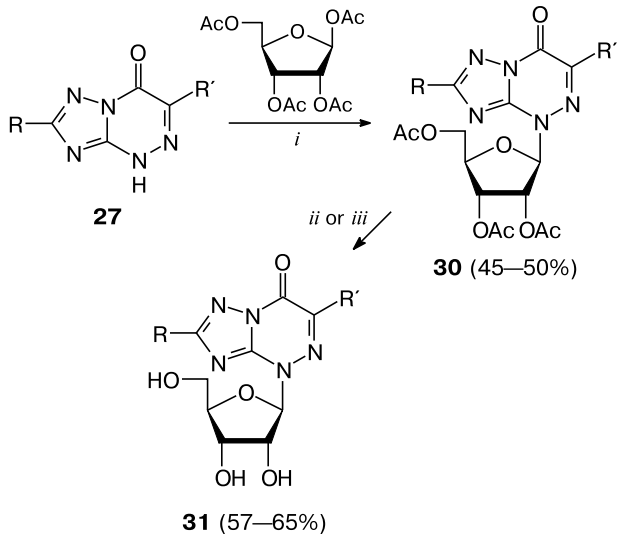


Table 2. Product yields in the reaction of 1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones **27** with adamantanol in sulfuric acid at −15 and 20 °C (see Scheme 8)

R	R′	Yield (%)*			
		28		**29**	
		−15 °C	20 °C	−15 °C	20 °C
H	CO_2Et	—	—	47	62
Me	CO_2Et	22	40	—	—
SMe	CO_2Et	20	22	—	—
SMe	CO_2Et	20	22	—	—
H	NO_2	18	51	45	29
Me	NO_2	18	52	—	—
SMe	NO_2	40	60	—	—

* NMR data.

Scheme 9



R = H, Me, SMe; R′ = Ph, CO_2Et

Reagents and conditions: *i*. BSA, TMSOTf, MeCN, ~20 °C, 3 h; *ii*. MeONa, MeOH, reflux, 2 h; *iii*. AcCl, EtOH, ~20 °C, 96 h.

sponding 4-(4-acetoxybut-2-enyl)-2-R-6-phenyl-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones **37** (Scheme 12).[45] The first alkylation of 1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones with chloroalkanes giving 4-acyloxyethyl-6-phenyl-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones **39** was performed in the presence of cesium carbonate (see Scheme 12). The removal of the acyl protecting group and the isolation of hydroxy derivatives **38** and **40** was accomplished *via* acidic methanolysis.[45]

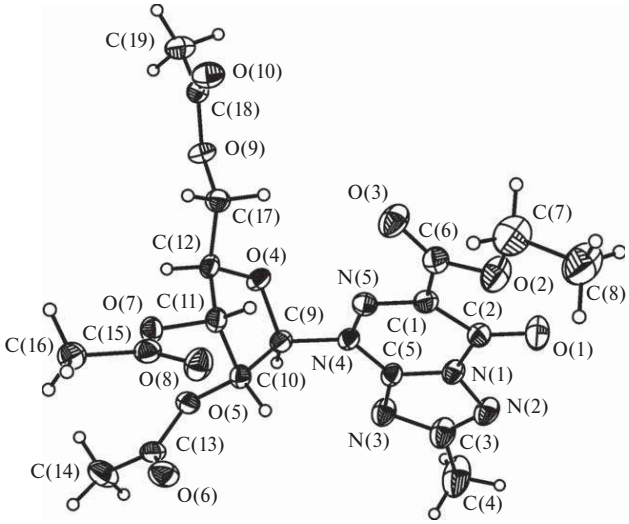


Fig. 1. Molecular structure of compound **30** (R = Me, R′ = CO_2Et).

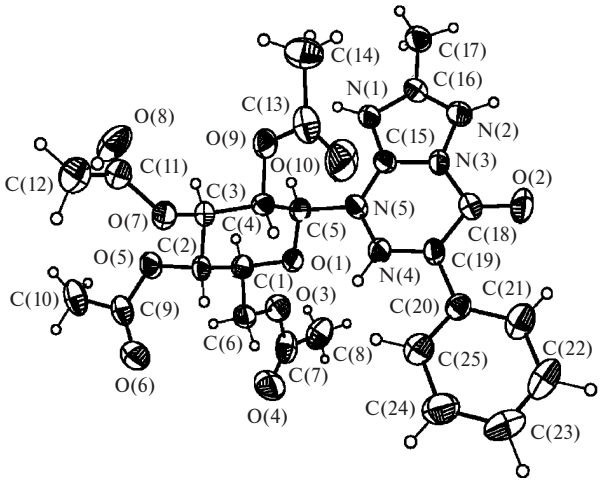
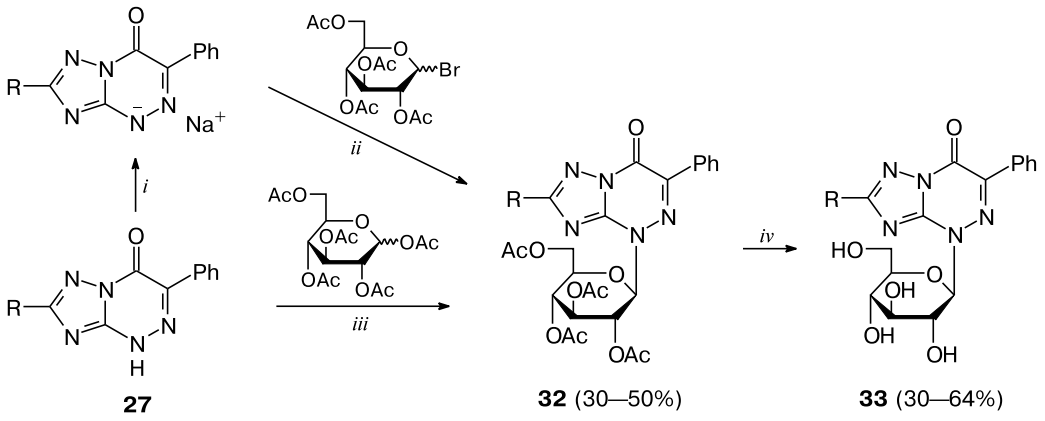


Fig. 2. Molecular structure of compound **32** (R = Me).

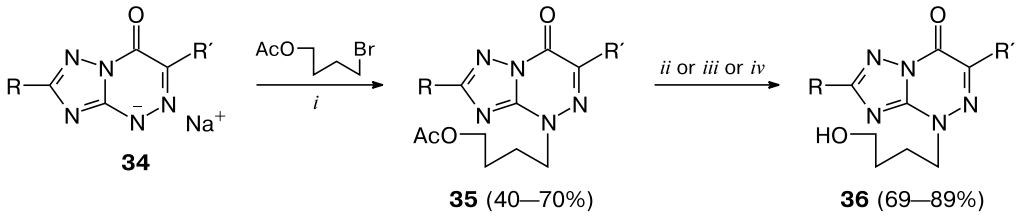
Scheme 10



R = H, Me, SMe

Reagents and conditions: *i*. Na_2CO_3, H_2O, ~20 °C, 4 h; *ii*. DMF, 100 °C, 2 h; *iii*. BSA, TMSOTf, MeCN, ~20 °C, 3 h; *iv*. MeONa, MeOH, reflux, 2 h.

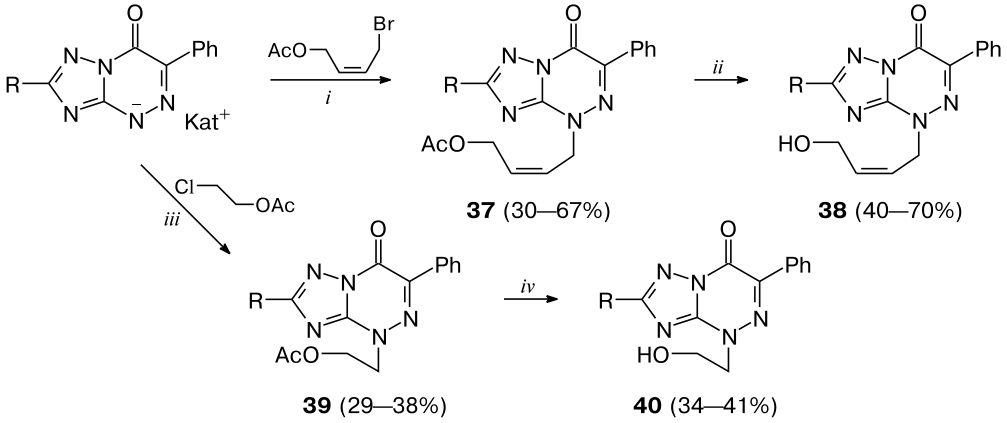
Scheme 11



R = H, Me, SMe; R′ = NO_2, CO_2Et, Ph

Reagents and conditions: *i*. DMF, 100 °C, 3 h; *ii*. MeONa, MeOH, reflux, 1 h; *iii*. AcCl, EtOH, reflux, 1 h; *iv*. QU-1 resin, MeOH, reflux, 1 h.

Scheme 12



R = H, Me, SMe; Kat = Na, Cs

Reagents and conditions: *i*. DMF, 100 °C, 2 h; *ii*. AcCl, MeOH, ~20 °C, 4 h; *iii*. DMF, $CsCO_3$, 100 °C, 2 h; *iv*. MeONa, MeOH, reflux, 1 h.

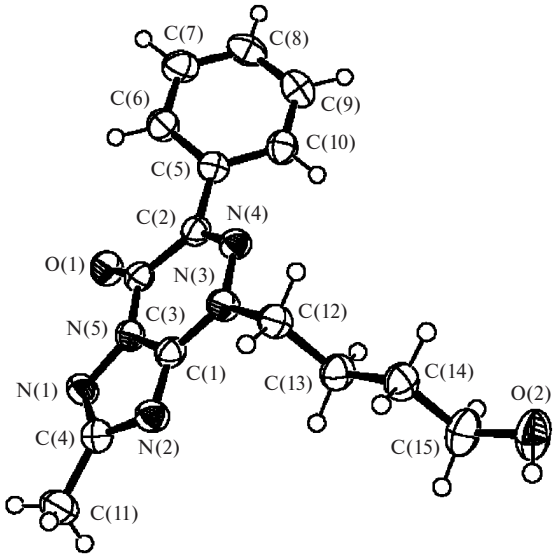


Fig. 3. Molecular structure of 4-(4-hydroxybutyl)-2-methyl-6-phenyl-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-one **36** (R = Me, R′ = Ph).

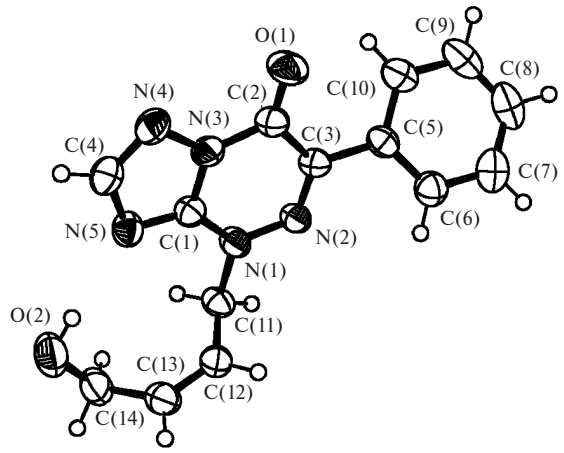


Fig. 4. Molecular structure of 4-(4-hydroxybut-2-enyl)-6-phenyl-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-one **38** (R = H).

The reaction proceeds regioselectively and produces the N(4)-isomer (Figs 3 and 4).

Relying on the fact that one of the mechanisms of antiviral activity of nucleosides is the inhibition of viral DNA synthesis catalyzed by viral DNA polymerase, a procedure was developed for the synthesis of 2-R-4-hydroxybutyl-6-phenyl-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-one triphosphates (Scheme 13). The synthesis included two steps. The first step produced monophosphates of compounds **36** in a way similar to that described previously.[46] In the next step, the monophosphates were transformed into triphosphates by the treatment with carbonyldiimidazole (CDI) and tributylammonium pyrophosphate.[42]

2-R-4-Hydroxyethyl-6-phenyl-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-one triphosphates were synthesized by a similar procedure.[42]

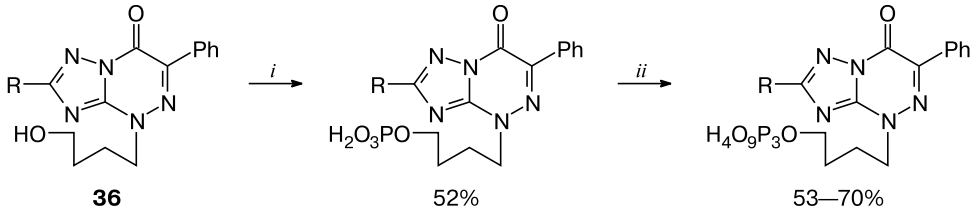
The direct alkylation of heterocyclic salts is not applicable to the preparation of acyclic nucleosides containing the 2-hydroxyethyloxymethyl group (the moiety that mimics the riboside residue in the commonly used drug acyclovir) because of instability of the corresponding halogen derivative. Therefore, the synthesis was performed using more stable 1,4-diacetoxy-2-oxabutane. The fusion of compounds **41** with an excess of (2-acetoxyethoxy) methyl acetate (**42**) in the presence of $ZnCl_2$ produces the corresponding 4-(2-acetoxyethoxy)methyl-6-R′-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones **43** (Scheme 14).[43,47]

The deacetylation of compounds **43** proceeds in the presence of the ion-exchange resin KU-1 (the H^+ form) giving natural nucleoside analogues **44**.

6-R′-1,2,4-Triazolo[5,1-*c*]-1,2,4-triazin-7-ones bearing an allyloxymethyl (**46**) or propargyloxymethyl (**48**) moiety at the 4 position were synthesized by short-term heating of triazolo-1,2,4-triazines **41** with allyloxymethyl acetate (**45**) or propargyloxymethyl acetate (**47**), respectively (see Scheme 14).[42,43]

An efficient method was developed for the introduction of the pivaloyloxymethyl moiety into the azolo-1,2,4-triazine molecule. The fusion of 1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-one **49** with pivalic anhydride, paraformaldehyde, and zinc chloride affords compound **50** (Scheme 15).[34,48]

Scheme 13

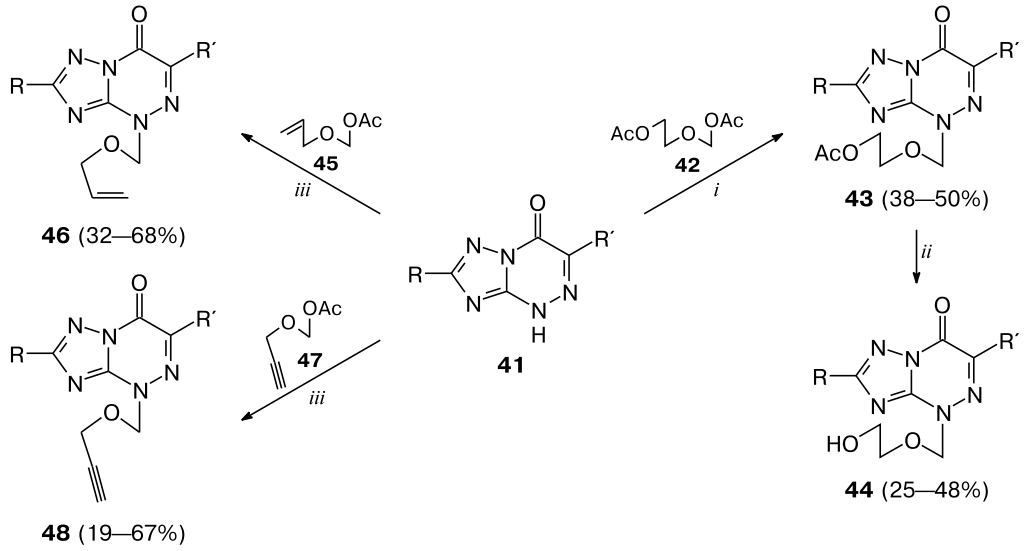


36 52% 53—70%

R = H, SMe

Reagents and conditions: *i*. $POCl_3$, 1,2,4-triazole, Et_3N; *ii*. 1) CDI, 2) $H_4P_2O_7 \cdot 1.5Bu_3N$.

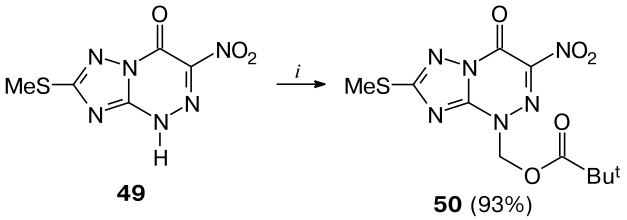
Scheme 14



R = H, Me, SMe; R′ = NO_2, CO_2Et, Ph

Reagents and conditions: *i*. $ZnCl_2$, 150 °C; *ii*. KU-1 (H^+), MeOH, ~20 °C, 12 h; *iii*. Reflux, 5 min.

Scheme 15

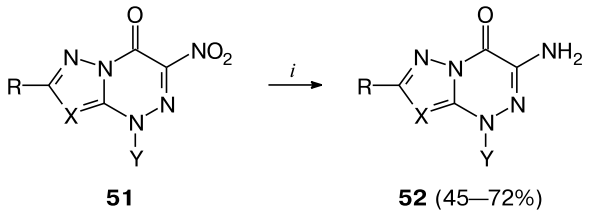


Reagents and conditions: *i*. $(Bu^tC{=}O)_2O$, CH_2O, $ZnCl_2$, 140 °C, 3 h.

1.3. Reduction of the nitro group

The nitro group in 6-nitroazolo[5,1-*c*]-1,2,4-triazin-7-ones **51** is easily reduced to the amino group with sodium dithionate giving 6-aminoazolo[5,1-*c*]-1,2,4-triazin-7-ones **52** (Scheme 16).[15,49]

Scheme 16



R = H, Me, SMe, SEt; X = N, CH, CCO_2Et; Y = H, Me, Na

Reagents and conditions: *i*. $Na_2S_2O_4$, H_2O, ~20 °C, 10 min.

The formation of 6-amino-2-methylthio-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-one was observed in the reduction of the corresponding nitro derivative catalyzed by reductases from laboratory animal liver.[50]

1.4. Nucleophilic substitution of the nitro group

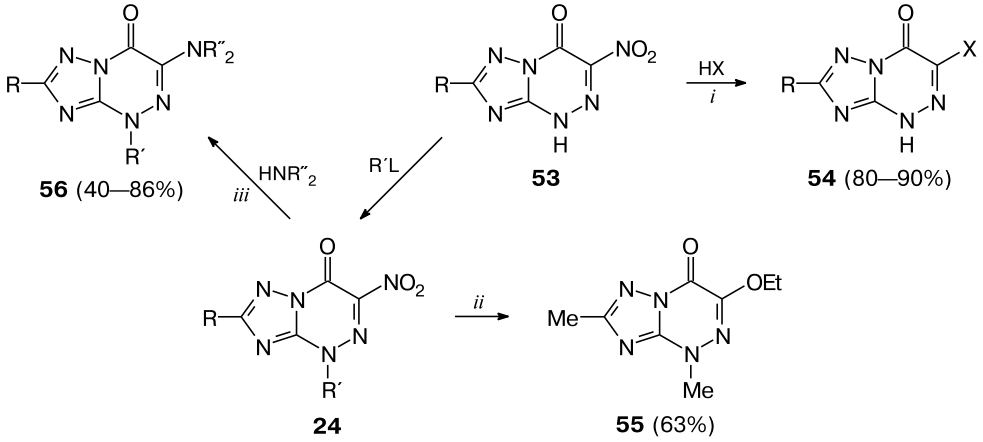
The possibility of replacing the nitro group in 6-nitroazolo[5,1-*c*]-1,2,4-triazin-7-ones **53** opens up a route to the synthesis of the previously inaccessible derivatives of this series.

The replacement of the nitro group by halogen is used to synthesize 6-bromo- and 6-chloro-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones **54**. Heating of appropriate 6-nitro-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones in an alcoholic solution of hydrogen halides affords[7,51,52] compounds **54** (Scheme 17). Compounds **54** are also produced on heating nitroazolo-1,2,4-triazines with acyl halides and phosphorus acid halides ($POCl_3$, PCl_3, PBr_3), the reaction with the latter being most facile and convenient.

The reactions of 6-nitroazolo[5,1-*c*]-1,2,4-triazin-7-ones **53** with amines and alkalis most often result in the formation of salts, which are not susceptible to subsequent nucleophilic attack. 4-Alkyl derivatives of 6-nitroazolo-[5,1-*c*]-1,2,4-triazin-7-ones **24** are unable to form an anion, which is even more favorable for their interaction with nucleophiles.[7,34,48,52] The nitro groups is smoothly replaced upon heating with sodium ethoxide to give compound **55**. 6-Amino derivatives **56** were synthesized by heating under reflux in DMF.

The nitro group can be replaced by heating compounds **24** (R = SMe) with butanethiol. The reaction with

Scheme 17



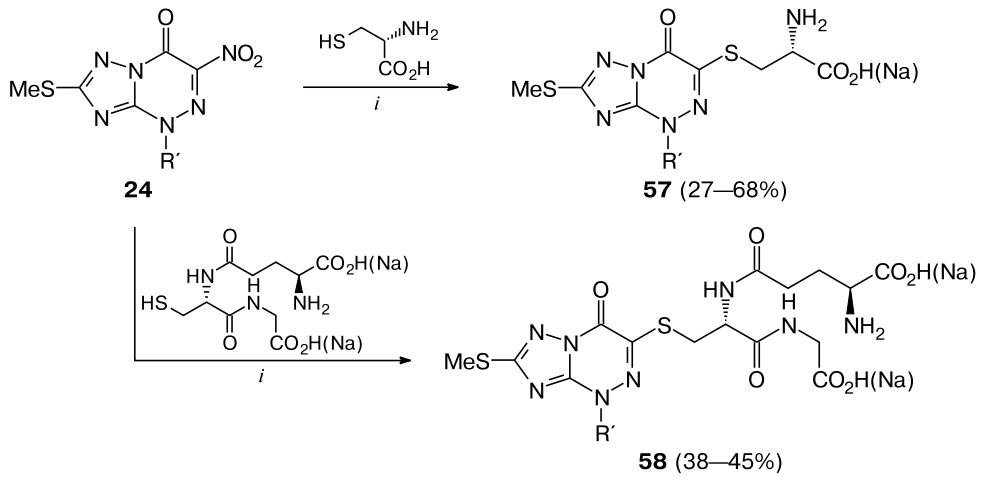
L-cysteine, which simulates the reaction with SH-containing proteins, proceeds even at room temperature both in the presence and in the absence of sodium hydrogen carbonate giving 1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones **57** (Scheme 18).[48] The nitro group is also easily replaced in compounds **24** to form compounds **58** on treatment with the tripeptide L-glutathione that more closely resembles cysteine-containing proteins.

The pivaloyloxymethyl group is widely used as a protecting group that can be removed under basic conditions.[53] The removal of the pivaloyloxymethyl group in compounds of types **57** and **58** with R′ = $CH_2O(CO)Bu^t$ gives rise to nitrogen-unsubstituted cysteine and glutathione derivatives **59** and **60**, respectively (Scheme 19).[48]

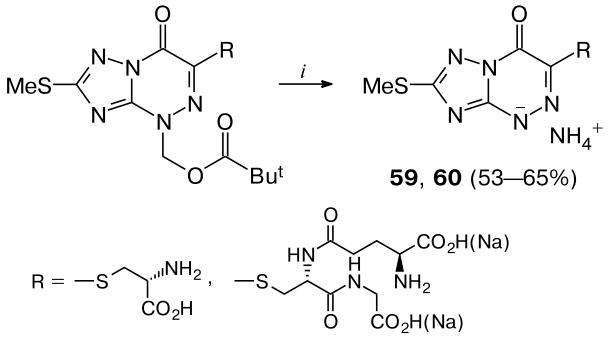
1.5. Synthesis of 7-aminoazolo[5,1-c]-1,2,4-triazines

Until recently, the preparation of 7-amino-6-nitroazolo[5,1-*c*][1,2,4]triazines **2**, which are of most interest for the synthesis of biologically active compounds, based on the fusion of the azine ring to the azole moiety with the use of nitroacetonitrile as the nitro synthon[54] has been a difficult task because the latter is an unstable and hardly accessible reactant.

Scheme 18

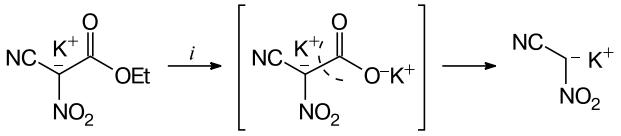


Scheme 19



Reagents and conditions: *i*. NH_3, MeOH, ~20 °C, 3 h.

Scheme 20



Reagents and conditions: *i*. KOH, H_2O, ~20 °C, 5 h.

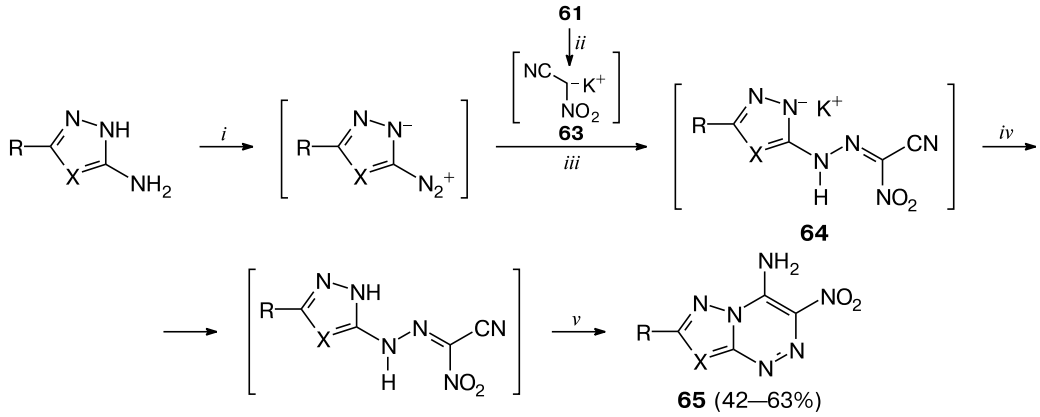
Recently, it was found[55] that the storage of available ethyl nitrocyanoacetate potassium salt **61** in a 5—10% solution of potassium hydroxide equivalent for several hours at room temperature leads to the hydrolysis of the ester group giving unstable nitrocyanoacetic acid dipotassium salt **62**, which is readily transformed into nitroacetonitrile potassium salt **63** (Scheme 20).

An evident advantage of the employment of nitro synthon **63** in the azo coupling with diazoazole salts is that there is no need to isolate and purify this compound after the decomposition of the starting salt **61** (Scheme 21).

The acidification and thermal cyclization of the resulting salts of cyanonitromethanal azolylhydrazones **64** give 7-amino-6-nitroazolo[5,1-*c*]-1,2,4-triazines **65** in good yields (see Scheme 21).[56]

7-Amino-6-nitroazolo[5,1-*c*]-1,2,4-triazines **65** are of interest by themselves and also serve as key intermediates in the synthesis of imidazo[4,5-*e*]azolo[5,1-*c*]-1,2,4-triazines that are structural analogues of adenosine receptor inhibitors.[57] The reduction of 7-amino-6-nitro-1,2,4-triazolo[5,1-*c*]-1,2,4-triazines **65** affords 6,7-diamino products **66**, which are cyclized under reflux in triethyl orthoformate to form new heterocyclic 1,2,4-triazolo-6-azapurine system **67** (Scheme 22).[56]

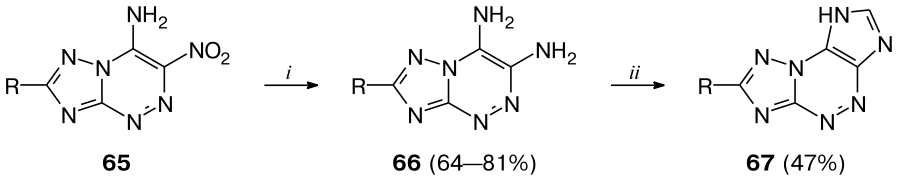
Scheme 21



R = H, Me, SMe, CO_2Et, 3-pyridyl; X = N, CCN, CCO_2Et

Reagents and conditions: *i*. $NaNO_2$, HCl, −5 °C, 10 min; *ii*. KOH, H_2O, ~20 °C, 20 h; *iii*. 1) KOAc, 5 °C, 2) ~20 °C, 1 h; *iv*. 40% H_2SO_4; *v*. DMF, reflux, 3 h.

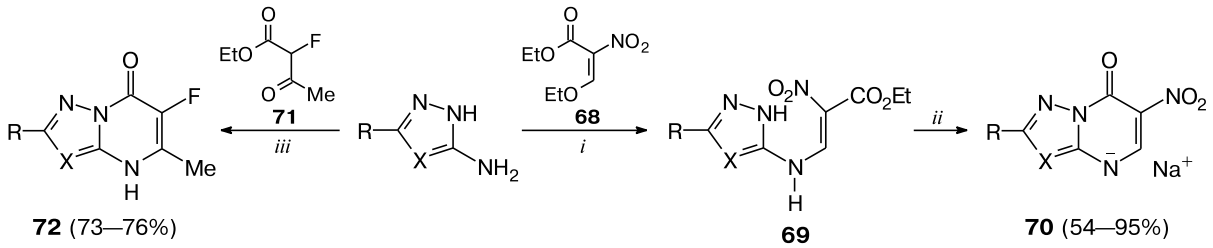
Scheme 22



R = Me, 3-pyridyl

Reagents and conditions: *i*. H_2, DMF, Pd, 45 °C, 4 h; *ii*. $HC(OEt)_3$, reflux, 2 h.

Scheme 23



R = H, Me, Et, SMe, 3-$NO_2C_6H_4$, 4-$NO_2C_6H_4$, Ph, 2-furyl, 3-pyridyl, 4-pyridyl; X = N, CH, CCO_2Et

Reagents and conditions: *i*. 70 °C, 0.5 h; *ii*. 20% Na_2CO_3 solution, 50—60 °C, 0.5 h; *iii*. AcOH, reflux, 12 h.

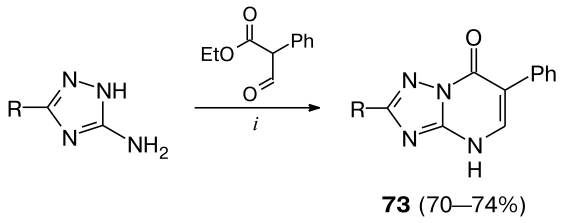
2. Azolo[1,5-*a*]pyrimidines

2.1. Synthesis of azolo[1,5-a]pyrimidin-7-ones

A general procedure for the synthesis of 6-nitro-1,2,4-triazolo[1,5-*a*]pyrimidin-7-ones is based on the condensation of aminoazoles with β-ketocarboxylic acids and their derivatives. Thus, heating of 5-aminopyrazoles or 5-amino-1,2,4-triazoles with ethyl 3-ethoxy-2-nitroacrylate **68** in the absence of a solvent produced ethyl 3-(azol-3-yl)amino-2-nitroacrylates **69**, and their treatment with a 20% sodium carbonate solution yields 2-R-6-nitroazolo[1,5-*a*]pyrimidin-7-ones, which were isolated as sodium salts **70** (Scheme 23).[58,59]

Fluorine-containing 1,2,4-triazolo[1,5-*a*]pyrimidin-7-ones were synthesized using readily available and safe ethyl 2-fluoroacetoacetate (**71**) as the key starting compound, which was heated with 3-R-5-amino-1,2,4-triazoles in acetic acid to prepare target compounds **72** (see Scheme 23).[23]

Scheme 24



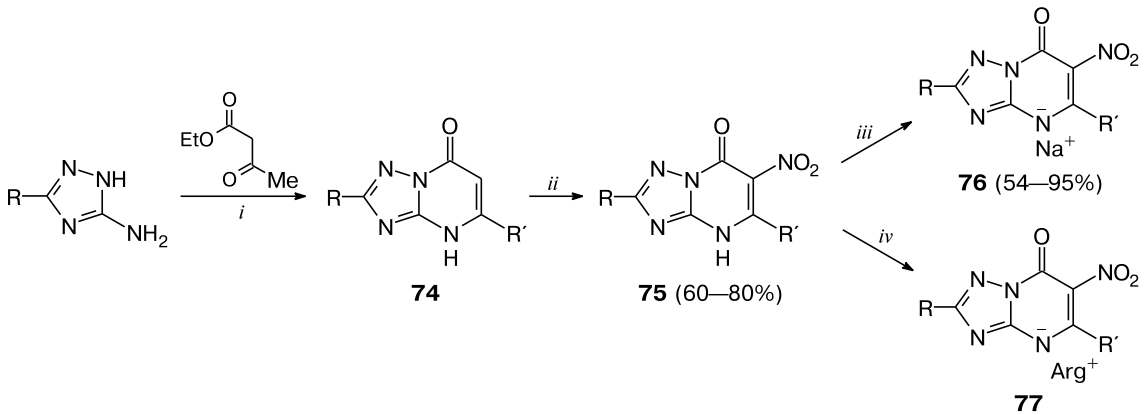
R = H, Me, SMe

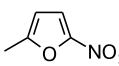
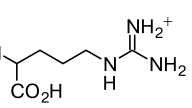
Reagents and conditions: *i*. AcOH, reflux, 1 h.

6-Phenyl-1,2,4-triazolo[1,5-*a*]pyrimidin-7-ones **73** were prepared by the condensation of 5-amino-1,2,4-triazole with α-formylphenyl acetate (Scheme 24).[42,60]

If α-nitro-β-keto ester is not available because of instability, as in the case of ethyl 2-nitroacetoacetate,[61] one can employ an alternative synthetic scheme.[59,62,63] The first step of this scheme involves the condensation of 3-R-5-

Scheme 25



R = H, Me, Et, SMe, CF_3,  , 3-pyridyl; R′ = Me, CF_3; Arg^+ = 

Reagents and conditions: *i*. AcOH, reflux, 1 h; *ii*. HNO_3, H_2SO_4, ~20 °C, 2 h; *iii*. 20% Na_2CO_3 solution; *iv*. Arg.

amino-1,2,4-triazoles with ethyl acetoacetate or ethyl trifluoroacetoacetate in acetic acid giving 1,2,4-triazolo-[1,5-*a*]pyrimidin-7-ones **74** (Scheme 25).

The subsequent nitration with a mixture of nitric and sulfuric acids affords 6-nitro-1,2,4-triazolo[1,5-*a*]pyrimidin-7-ones **75**. In general, the method is versatile in both steps; however, there are exceptions. For instance, the nitration of 2-furyl-5-methyl-1,2,4-triazolo[1,5-*a*]pyrimidin-7-one **72** (R = 2-furyl) gives the dinitration product — 5-methyl-2-(5-nitro-2-furyl)-6-nitro-1,2,4-triazolo[1,5-*a*]-pyrimidin-7-one. The treatment of the compounds with bases produces water-soluble salts **76** and **77** (see Scheme 25).[64,65]

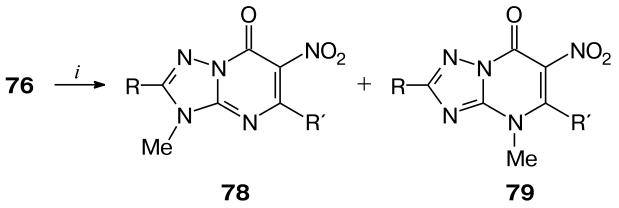
2.2. Alkylation and synthesis of natural nucleoside analogues

The treatment of compounds **75** in an alkaline medium and also of sodium salts **70** and **76** with an excess of methyl iodide in DMSO was found to give two *N*-methyl derivatives (**78** and **79**) in different ratios (Scheme 26).[62] The use of salts **76** proved to be more convenient because the reaction with the latter produces isomers in the same ratio but in quantitative yield. The isomer ratio was established by NMR spectroscopy.

The condensation of 3-R-5-amino-1,2,4-triazoles with ethyl α-formylphenylacetate produced 6-phenyl-1,2,4-triazolo[1,5-*a*]pyrimidin-7-ones **73**, which were subjected to alkylation with 4-bromobutyl acetate giving 2-R-4-(4-acetoxybutyl)-6-phenyl-1,2,4-triazolo[1,5-*a*]pyrimidin-7-ones **80** (Scheme 27).[42,60] The reaction with (*Z*)-4-bromobut-2-en-1-yl acetate yielded 4-acetoxybut-2-enyl derivatives **81**. The deacylation proceeds smoothly when using sodium methoxide or acetyl chloride.

The reaction of compounds **73** with (2-acetoxyethoxy) methyl acetate under Vorbrüggen glycosylation conditions affords a mixture of isomers **82** and **83** (see Scheme 27), their ratio being dependent on the duration of the reaction.

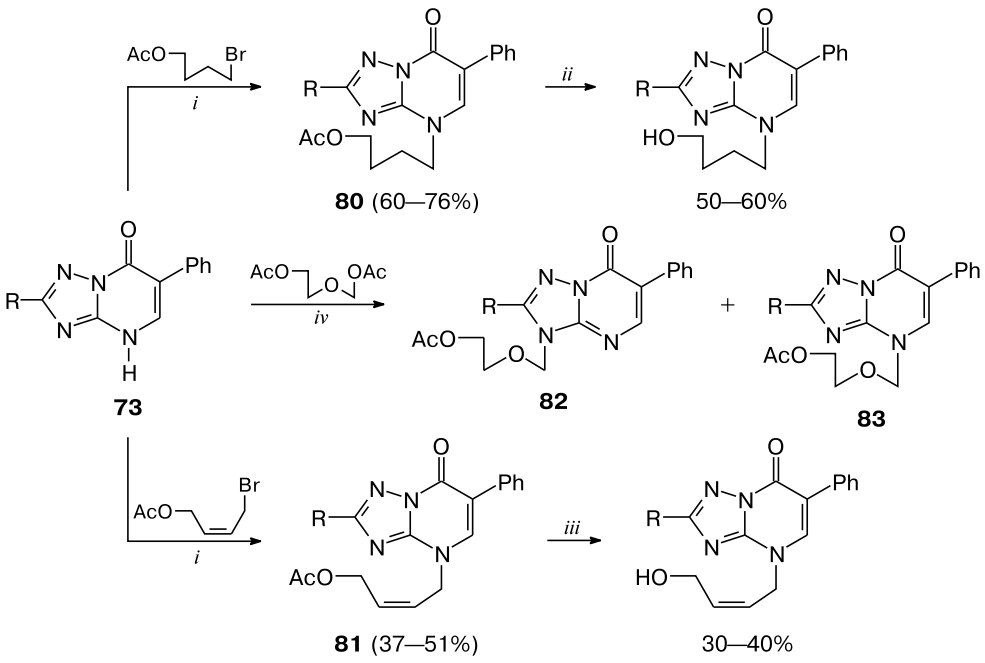
Scheme 26



76, **78**, **79**		Yield (%)		**76**, **78**, **79**		Yield (%)	
R	R′	**78**	**79**	R	R′	**78**	**79**
H	H	35	65	Et	Me	65	35
Me	H	35	65	SMe	Me	20	80
H	Me	70	30	CF_3	Me	15	85
Me	Me	65	35	H	CF_3	100	0

Reagents and conditions: *i*. MeI, DMF, reflux, 1 h.

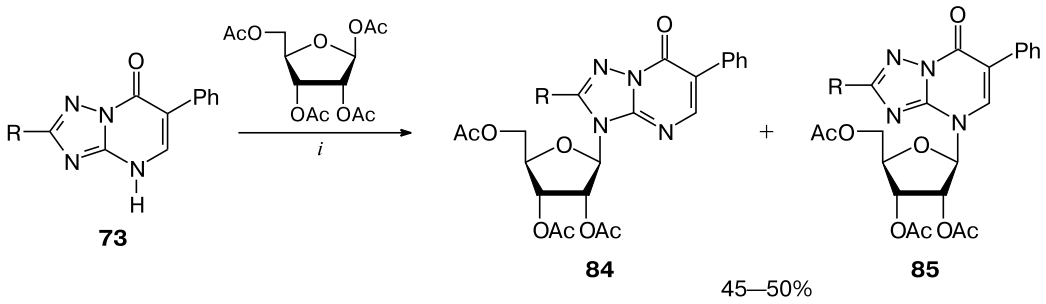
Scheme 27



R = H, Me, SMe

Reagents and conditions: *i*. 17% Na_2CO_3 solution, ~20 °C, 0.5 h; *ii*. MeONa, MeOH, reflux, 1 h; *iii*. AcCl, MeOH, ~20 °C, 4 h; *iv*. MeCN, TMSOTf, 20 °C.

Scheme 28



R = H, Me, SMe

Reagents and conditions: *i*. BSA, TMSOTf, MeCN, ~20 °C, 3 h.

Initially, the mixture consists mainly of N(3)-isomer **82**, the percentage of which decreases with time, while the percentage of N(4)-isomer **83** increases and the latter becomes the major product within 24 h. Besides, it was found[66] that compounds **82** and **83** can undergo reversible interconversion in a mixture of acetonitrile and TMSOTf.

This method was used to perform ribosylation of compounds **73** (Scheme 28).[60] The direction of insertion of the sugar moiety giving either the N(3)- or N(4)-isomer (compounds **84** or **85**, respectively) depends on the nature of the substituent R in the starting 2-R-1,2,4-triazolo[1,5-*a*]-pyrimidin-7-one **73**. In the case of R = H, the reaction produced only the N(3)-isomer; if R = SMe, only the N(4)-isomer. In the case of R = Me, a mixture of N(3)- and N(4)-isomers was obtained (42 and 58%, respectively). The removal of the acyl protecting group occurs in an ammonia solution in methanol with 40—60% yields.

2.3. 7-Aminoazolo[1,5-a]pyrimidines

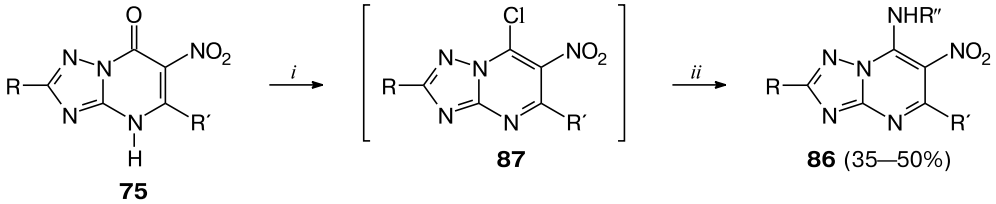
A versatile method for the synthesis of 7-amino-6-nitro-1,2,4-triazolo[1,5-*a*]pyrimidines **86** is based on the chlorodeoxygenation of readily available 6-nitro-1,2,4-triazolo[1,5-*a*]pyrimidin-7-ones **75** followed by the treatment with amines. The reaction of these compounds with phosphoryl trichloride in dry acetonitrile without the isolation of unstable chloro derivative **87** proved to be the optimal process (Scheme 29).[58,67,68]

The reduction of nitro compounds **86** with sodium dithionite affords 7-alkylamino-6-amino-1,2,4-triazolo-pyrimidines **88**, which are of interest by themselves and also as intermediates in the synthesis of 1,2,4-triazolo-[5,1-*b*]purines. Thus, the heating of diamines **88** under reflux in concentrated formic acid produces 2-R-5-R′-8-alkyl-1,2,4-triazolo[5,1-*b*]purines **89** (Scheme 30).[59,67]

Aza analogues of compounds **89** — 2*H*-azolo[1,5-*a*]-1,2,3-triazolo[4,5-*e*]pyrimidines **90** — were prepared by the cycloaddition of sodium azide to nitropyrimidines **91** acting as dipolarophiles (Scheme 31).[68,69]

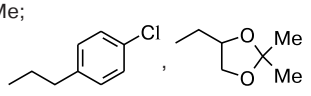
These compounds can be modified using the S_NH strategy, as illustrated by an example of 1,2,4-triazolo-[1,5-*a*]-1,2,3-triazolo[4,5-*e*]pyrimidine **90**.[70,71] Triazolo-pyrimidine **90** was found to react with a number of aromatic C-nucleophiles to form relatively stable adducts **92**, which can be subjected to oxidative aromatization giving 4-substituted 1,2,4-triazolo[1,5-*a*]-1,2,3-triazolo-[4,5-*e*]pyrimidines **93** (Scheme 32).[69]

Scheme 29



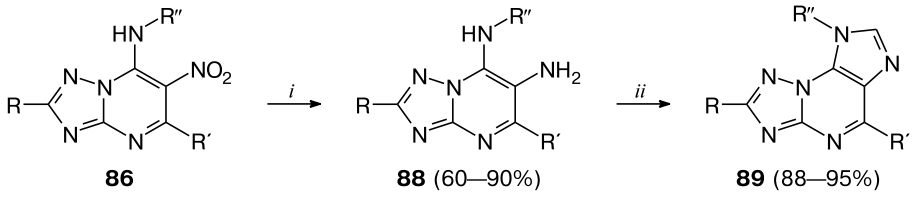
R = H, Me, SMe, CF_3, CO_2Et; R′ = H, Me;

R″ = Pr, Pr^i, Bu, Bu^i, Bu^t, CH_2CH_2OAc,

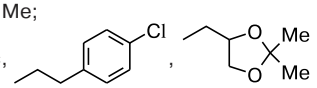


Reagents and conditions: *i*. $POCl_3$, MeCN, Py, reflux, 3 h; *ii*. R″NH_2, Et_3N, ~20 °C, 2 h.

Scheme 30

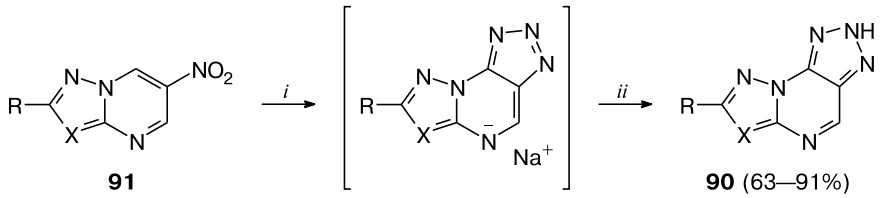


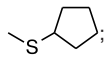
R = H, Me, SMe, CF_3, CO_2Et; R′ = H, Me;

R″ =Pr, Pr^i, Bu, Bu^i, Bu^t, CH_2CH_2OAc, 

Reagents and conditions: *i*. $Na_2S_2O_4$, EtOH, H_2O, reflux, 3 h; *ii*. HCO_2H, reflux, 2—3 h.

Scheme 31

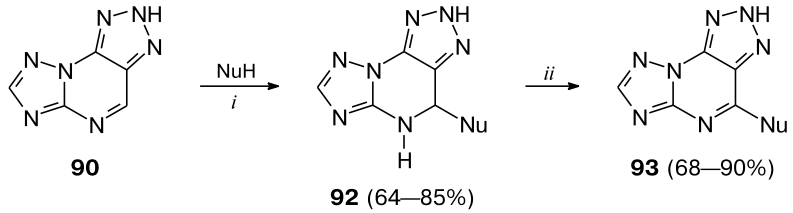


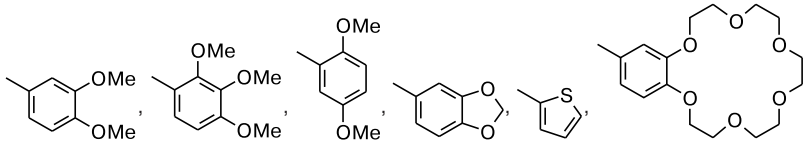
R = H, SMe, SPr^i, SCH_2CN, 

; X = N, CO_2Et

Reagents and conditions: *i*. NaN_3, DMF, 70—80 °C, 30—40 min; *ii*. HCl, H_2O, ~20 °C.

Scheme 32



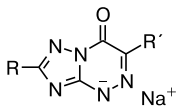
Nu = 

Reagents and conditions: *i*. CF_3CO_2H, ~20 °C, 12 h; *ii*. $PhI(OAc)_2$, AcOH, 80—90 °C, 10—15 min.

3. Antiviral activity

The design of agents effective against influenza and other viral infections remains a challenging task in medicinal chemistry because of high variability of the viruses. Interdisciplinary research performed at the Ural Federal University and the Postovsky Institute of Organic Synthesis of the Ural Branch of the Russian Academy of Sciences in collaboration with the Research Institute of Influenza of the Ministry of Health of the Russian Federation (St. Petersburg), the Virological Center of the Ministry of Defense of the Russian Federation (Sergiev Posad), the Institute of Military Medicine (St. Petersburg), and the National Research Institute for Veterinary Virology and Microbiology (Pokrov) resulted in the development of a new family of antiviral compounds, which includes pyrazolo- and 1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones and 1,2,4-triazolo[1,5-*a*]pyrimidin-7-ones.[7,9—13,18,24,47,64,65,72—86] The data on toxicity, metabolism, and mechanisms of action of these compounds

Table 3. Cytotoxicity and virus inhibitory activity of sodium salts of 2-R-6-R′-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones



R	R′	MTC[a]	C^b	Antiviral activity[c] ($logID_{50}$)	
		μg mL^{-1}		A(H1N1) A/Puerto Rico/8/34	A(H3N2) A/Victoria/35/72
SMe	NO_2	>400	100	2.0	2.25
SEt	NO_2	>200	100	1.0	1.0
SPr	NO_2	>200	100	1.0	1.0
SBu	NO_2	>200	—	—	0
H	F	100	50	0	—
Me	F	100	50	0.5	—
SMe	F	100	50	0	—
SMe	Cl	>100	100	1.0	1.0
SMe	Br	>100	100	1.5	1.0
SMe	Ph	100	50	1.0	—
SMe	CO_2Et	100	50	0.5	—
SMe	CN	100	50	1.0	—
—	—	—	—	5.5[d]	5.0[d]

[a] Here and in Table 4, MTC is the minimum toxic concentration.

[b] Here and in Tables 4 and 5, *C* is the working concentration.

[c] Here and in Tables 4 and 5, this value is the difference between the titers of the virus in the control and the experiment.

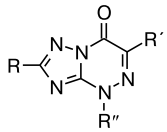
[d] Control (here and in Tables 4 and 5, the starting titer of the virus or the infectious activity of the virus in the absence of agents).

were obtained. Compounds effective against diseases caused by influenza, tick-borne encephalitis, and viral hemorrhagic fever viruses were found.

3.1. Anti-influenza activity

Salts of 2-R-6-R′-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones (Table 3) and N(4)-substituted 2-R-6-R′-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones (Table 4) exhibit antiviral activity against the influenza type A viruses A(H1N1) A/Puerto Rico/8/34 and A(H3N2) A/Victoria /35/72 in *in vitro* experiments. The efficacy of the agent ($logID_{50}$) is considered to be weak if the difference between the titers of the influenza virus in the control and experiment is less than 1.0; moderate, in the range of 1.0—3.0; high, at >3.0.

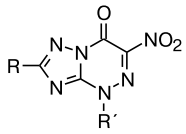
Table 4. Cytotoxicity and virus inhibitory activity of 2-R-4-R″-6-R′-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones



R	R′	R″	MTC	*C*	Antiviral activity ($logID_{50}$)	
			μg mL^{-1}		A(H1N1) A/Puerto Rico/8/34	A(H3N2) A/Victoria/35/72
SPr	H	NO_2	>100	100	—	1.5
PrSO	H	NO_2	>200	100	1.0	1.0
Pr^{i}SO	H	NO_2	>200	100	1.0	1.0
$EtSO_2$	H	NO_2	>200	100	0.5	0.5
Pr^iSO_2	H	NO_2	>200	100	1.0	1.0
SMe	Me	NO_2	>100	100	1.0	0.0
SMe	Et	NO_2	>200	100	1.0	0.0
SMe	Bu^t	NO_2	100	50	2.0	1.5
SMe	$CH_2OC(O)Bu^t$	NO_2	100	50	1.0	1.0
Pr^iSO_2	$CH_2OC(O)Bu^t$	NO_2	>100	100	—	1.0
SMe	$CH_2OC(O)Bu^t$	$SCH_2C(NH_2)CO_2H$	200	62.5	3.0	3.5
Me	Me	$NH(CH_2)_3Me$	>100	100	—	1.0
—	—	—	—	—	5.5*	5.0*

* Control.

Table 5. Virus inhibitory activity of 4-allyloxymethyl- and 4-propargyloxymethyl-6-nitro-1,2,4-triazolo[3,2-*c*]-1,2,4-triazin-7-ones



R	R′	*C* /μg mL^{-1}	Antiviral activity (logID_{50})					
			B B/Samara /253/99	A/H3N2 A/Hong Kong 1/68	A/H3N2 A/St. Petersburg /22/99	A/H5N1 A/Duck/Singapore R/F119-3/97	AH0N1 A/Mongolia/ 56/87	AH0N1 A/PR/ 8/34
H	CH_2=$CHCH_2OCH_2$	20	2.5	0.5	—	1.5	2.5	2.5
H	CH_2=$CHCH_2OCH_2$	40	2.5	2.0	—	3.0	3.0	4.0
Me	CH_2=$CHCH_2OCH_2$	20	1.0	0.5	—	1.5	2.5	2.5
Me	CH_2=$CHCH_2OCH_2$	40	2.5	2.0	—	3.0	3.0	4.0
SMe	CH_2=$CHCH_2OCH_2$	20	2.5	2.0	—	2.5	4.0	2.5
SMe	CH_2=$CHCH_2OCH_2$	40	3.0	2.5	—	3.0	4.0	4.0
H	CH≡CCH_2OCH_2	20	—	—	3.0	—	—	—
H	CH≡CCH_2OCH_2	40	—	—	4.0	—	—	—
Me	CH≡CCH_2OCH_2	20	—	—	2.9	—	—	—
Me	CH≡C CH_2OCH_2	40	—	—	4.0	—	—	—
SMe	CH≡CCH_2OCH_2	20	—	—	2.6	—	—	—
SMe	CH≡CCH_2OCH_2	40	—	—	4.0	—	—	—
Rimantadine		20	0	2.5	2.9	3.0	0	1.0
		40	0.5	2.5	3.3	3.0	1.5	1.5
—	—	—	4.5*	3.5*	5.1*	4.0*	5.0*	5.5*

* Control.

The assessment of antiviral activity against the influenza viruses A(H3N2), A(H5N1), and A(H0N1) (rimantadine-resistant strains) and influenza type B virus in *in vitro* experiments demonstrated that 4-allyloxymethyl(propargyloxymethyl)-6-R′-1,2,4-triazolo[3,2-*c*]-1,2,4-triazin-7-ones **46** and **48** exhibit activity against influenza A virus comparable with that of rimantadine and are more effective against influenza B virus than the latter drug. In further studies, modified nucleosides **43** proved to be active against the rimantadine-resistant strains A(H0N1) and were found to significantly inhibit the replication of influenza B virus, against which rimantadine in inactive (Table 5).[10,43]

The evaluation of antiviral activity of the synthesized compounds against respiratory syncytial virus demonstrated that 4-(allyloxymethyl)-6-carbethoxy- and 4-(propargyloxymethyl)-6-nitro-1,2,4-triazolo[3,2-*c*]-1,2,4-triazin-7-ones inhibit the replication of this virus. A decrease in infectious activity of the virus (logID_{50}) on treatment with the agents under study was 1.0—1.85 (22—67%).[43]

The characteristic and quite unusual property of the 6-nitroazolo[5,1-*c*]-1,2,4-triazin-7-one family is that these compounds show higher antiviral activity in experimental animal models than in *in vitro* experiments. Sodium salts of 2-methyl- and 2-ethylthio-6-nitro-1,2,4-triazolo-[5,1-*c*]-1,2,4-triazin-7-ones exhibited antiviral activity against the influenza virus (A strain/hen/Kurgan/2/05 (H5N1)) in experimental animal models.[9,50,72—74] Sodium salt of 6-cyano-2-methylthio-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-one showed a protection index comparable with that of the drug tamiflu (55.6%) in *in vivo* experiments using a model of lethal influenza infection in mice infected with the virus A/California/05/09 pdm2009.[86]

In a series of azolopyrimidines, sodium and arginine salts of 5-methyl-6-nitro-1,2,4-triazolo[1,5-*a*]pyrimidin-7-one showed a high protection index (70—80%) in experiments using a model of lethal influenza infection in mice infected with the influenza virus strains A/Aichi/2/68 (H3N2), A/California/07/09 (H1N1) pdm2009, B/Lee/40 and with respiratory syncytial virus;[64,65] the protection index of 2-methylthio-5-methyl-6-nitro-1,2,4-triazolo-[1,5-*a*]pyrimidin-7-one was 20%.[77]

Triazavirin (sodium salt of 2-methylthio-6-nitro-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-one, dihydrate) is the first agent that was developed bases on this class of compounds. This agent has passed all phases of clinical trials as an anti-influenza agent and was included on 28.08.2014 in the Drug Register of the Russian Federation (No. LP-002604). The Pharmaceutical plant Medsintez and the Ural Center for Biopharma Technologies manufactured this drug on an industrial scale.[9,14,29,50,72,74—76,83,85,87—89] Since 2015, triazavirin has been sold through the retail pharmacy network.

Triazavirin possesses antiviral activity against a broad range of influenza viruses (H1N1, H5N1, H5N2, H7N3, H9N2), respiratory syncytial infection, parainfluenza viruses, adenoviruses, and so on. The efficacy index against

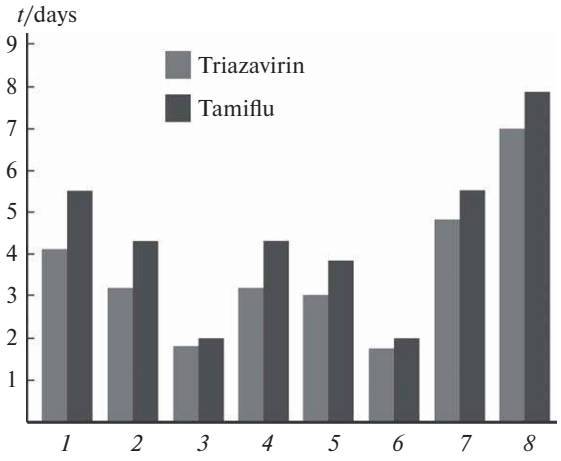


Fig. 5. Duration of the main symptoms of the disease (*t*) on treatment with triazavirin and tamiflu: *1*, period to recovery; *2*, temperature; *3*, fever; *4*, headache; *5*, myalgia; *6*, pain and burning in the eyes; *7*, sore throat; *8*, cough (epidemic season 2012—2013, 127 patients with influenza aged from 18 to 65).

influenza type A and B viruses is 65—80%. It is important that triazavirin is effective in all phases of development of the viral infection both in prophylactic and therapeutic strategies. It is also essential that triazavirin is a low-toxicity class IV drug.[50,75,76,85]

Clinical studies (phase I—III trials) demonstrated that the use of triazavirin in etiotropic therapy of influenza reduces the duration of the main symptoms of the disease (intoxication, fever, and catarrhal symptoms), quickly normalizes body temperature in therapeutic groups, and decreases the influenza virus reisolation rate.[85,87,88] A comparative evaluation of the efficacy demonstrated that triazavirin is superior to tamiflu in most parameters (Fig. 5).

In parallel with clinical studies, the mechanism of action of triazavirin was investigated. It was established that this agent targets the viral protein hemagglutinin, which was experimentally confirmed by surface plasmon resonance. The computer simulation of the interaction of triazavirin with hemagglutinin of the pandemic influenza virus A/California/04/2009 (H1N1) was performed. The following short sequences responsible for noncovalent interactions of the influenza virus hemagglutinin with triazavirin were identified: CKLRGV (Cys—Lys—Leu—Arg—Gly—Val), LGK (Leu—Gly—Lys), and FYKNLIW (Phe—Tyr—Lys—Asn—Leu—Iso—Trp) (Fig. 6).[14]

An important role of interactions between triazavirin and SH groups of amino acids was experimentally demonstrated, and the presence of disulfide bonds between the cysteines 59, 292, 296, and 320 in the tertiary structure of hemagglutinin was confirmed. These data provide evidence for the participation of both triazavirin and the protein

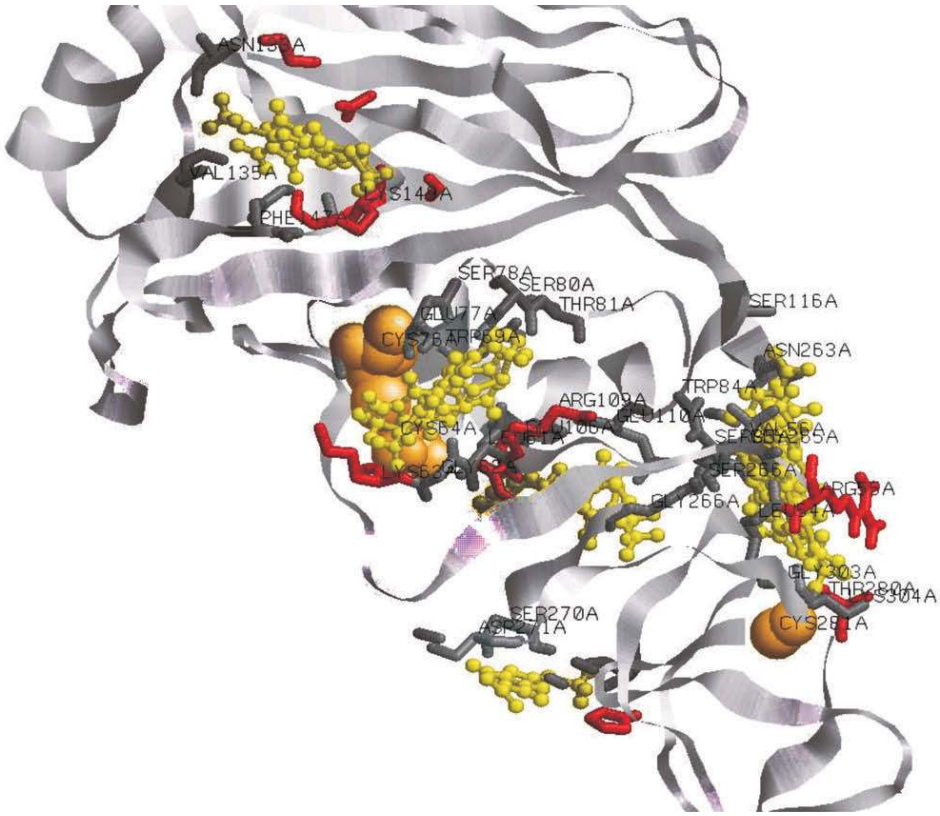


Fig. 6. Results of multiple docking of triazavirin to influenza virus H1 hemagglutinin. The best mutual arrangements of the molecules in terms of interaction energy are shown.
Note. Fig. 6 is available in full color on the web page of the journal (http://www.link.springer.com).

```
  1 mkailvvlly tfatanadtl cigyhannst dtvdtvlekn vtvthsvnll edkhngkLCK
 61 LRGVAPLHLG KCNiagwilg npeceslsta sswsyivetp ssdngtcypg dfidyeelre
121 qlssvssfer feifpktssw pnhdsnkgvt aacphagaks fyknliwlvk kgnsypklsk
181 syindkgkev lvlwgihhps tsadqqslyq nadtyvfvgs sryskkfkpe iairpkvrdq
241 egrmnyywtl vepgdkitfe atgnlvvpry afamernags giiisdtpvh DCNTTCQtpk
                                                    YG NCNTKCQ
301 gaintslpfq nihpitigkc pkyvkstklr latglrnips iqsrglfgai agfieggwtg
361 mvdgwygyhh qneqgsgyaa dlkstqnaid eitnkvnsvi ekmntqftav gkefnhlekr
421 ienlnkkvdd gfldiwtyna ellvllener tldyhdsnvk nlyekvrsql knnakeignq
481 cfefyhkcdn tcmesvkngt ydypkyseea klnreeidgv klestriyqi laiystvass
541 lvlvvslgai sfwmcsngsl qcrici
```

Fig. 7. Amino acid sequence of hemagglutinin of influenza A virus A/California/04/2009 (H1N1). Model peptides are highlighted.

disulfide isomerase (PDI) that catalyzes the formation and isomerization of disulfide bonds. To prove this assumpion, the following model peptides were used: HA-I, DCNTTCQ; HA-II, YGNCNTKCQ; HA-III, LCKLGGIAPLHLGKCN-amid. These peptides contain two cysteine residues each. In the amino acid sequence, these peptides are shown in bold capital letters (Fig. 7). After the incubation of the model peptides with triazavirin under physiological conditions, the interaction products were analyzed by mass spectrometry (Fig. 8). Triazavirin was shown to facilitate the S—S bond formation. Apart from intramolecular S—S bonds, intermolecular disulfide bonds are formed, the latter giving rise to dimeric structures. Both the monomeric and dimeric S—S forms are clearly manifested in the mass spectra, which is indicative of the oxidative function of triazavirin.[14]

These data suggest an important role of interactions of triazavirin with SH groups of amino acids in the formation and isomerization of disulfide bonds, which disrupt the three-dimensional structure of hemagglutinin and the life cycle of the virus.[14,83]

These results are in good agreement with the pharmacokinetic data,[50] according to which triazavirin, acting as an oxidant for thiol groups, is transformed from the nitro derivative into the corresponding amino compound — 6-amino-2-methylthio-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-one.

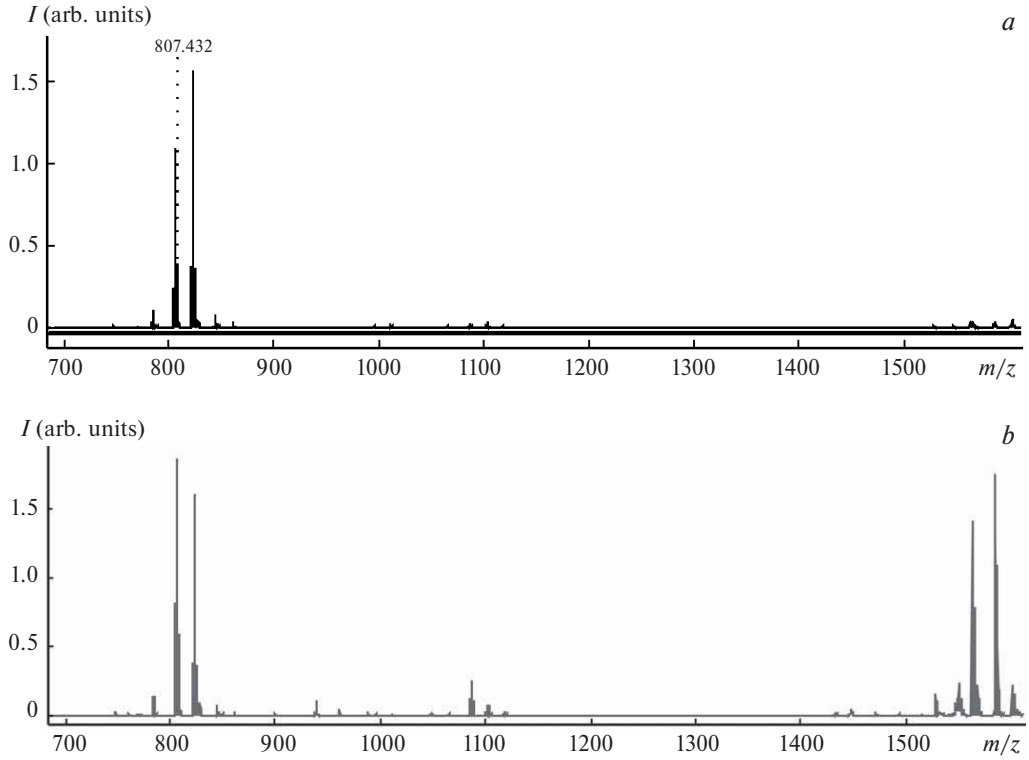


Fig. 8. Fragments of MALDI mass spectra: the model peptide DCNTTCQ (HA-I) (*a*) and HA-I upon treatment with triazavirin (*b*).

3.2. Antiherpetic activity

Herpes viruses are widely distributed in nature and cause dangerous infectious diseases that are a significant public health concern. More than half the world′s population is infected with the herpes virus and up to 20% of people have certain signs of the disease. Besides, herpes infections often accompany AIDS caused by human immunodeficiency virus, which complicates the treatment of the disease and often leads to lethal outcome. Nucleoside analogues modified both at the base and ribose moieties have played a great role in the construction of antiherpetic agents. Several acyclic guanosine analogues, such as acyclovir, penciclovir, and ganciclovir, are commonly used as pharmaceuticals.[1,2]

In the series of azolo[5,1-*c*]-1,2,4-triazines, 6-phenyl-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones **36**, **38**, **40**, and **44**, which bear substituents at the 4 position of the heterocycle that mimic, to a certain extent, the glycosidic moiety of a nucleoside, exhibit antiviral activity against herpes simplex virus 1 (HSV-1).[10,42,47,78] These compounds can be considered as nucleosides modified at the base and ribose moieties. The antiviral activity and cytotoxicity of these compounds were tested in the Vero cell culture infected with herpes simplex virus 1 at different multiplicities of infection. The results of assays are given in Table 6.

Compounds containing the 2-hydroxyethoxymethyl group suppressed the viral replication by 50% at a concentration higher than 250 μmol L^{-1}, while compounds containing the hydroxybutene moiety inhibited the viral replication by 50% at concentrations of 15—30 μmol L^{-1} and showed the highest selectivity index compared to the other compounds at two different plaque-forming unit (PFU) per cell.

It should be noted that all compounds are low toxic for the non-infected cell culture. Their cytotoxicity is comparable with that of the antiherpetic agent acyclovir and is lower than that of ganciclovir.

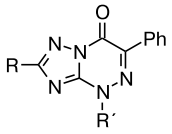
One of mechanisms of antiviral activity of nucleoside analogues is the inhibition of viral DNA synthesis catalyzed by viral DNA polymerase. 4-(4-Hydroxybutyl)- and 4-(2-hydroxyethyl)-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-one triphosphates inhibit the incorporation of [α-^{32}P]ATP at the 3′-end of the primer matrix complex (half-maximal inhibitory concentration is 100—160 μmol L^{-1}). The efficacy of inhibition proved to be 3—3.5 times lower than that of acyclovir triphosphate.[41]

4-Propargyloxymethyl-2-R-6-nitro-1,2,4-triazolo-[5,1-*c*]-1,2,4-triazin-7-ones **48** (R = H, Me, SMe) showed low efficacy against herpes simplex virus (EC strain).[10] Sodium salts of 2-propyl- and 2-butyl-6-nitro-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones **5** (X = N, R = SPr, SBu) demonstrated high activity. These compounds completely suppress the viral cytopathic effect at a concentration of 500 μg mL^{-1}.[13]

3.3. Antiviral activity against the causative agent of West Nile fever

An increase in morbidity caused by the West Nile fever (WNF) viruses stimu lates the search for new antiviral agents against WNF and evaluation of the efficacy of the available antiviral agents. Outbreaks of this infection are

Table 6. Cytotoxicity and anti-HSV-1 activity of 6-phenyl-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones **36**, **38**, **40**, and **44**



R	R′	CD_{50} /mmol L^{-1}	Antiviral activity against multiple infection			
			0.01 PFU per cell		0.001 PFU per cell	
			ID_{50}/mmol L^{-1}	IS	ID_{50}/mmol L^{-1}	IS
H	$(CH_2)_4OH$	>0.8	0.06	>13	0.05	>16
Me	$(CH_2)_4OH$	>0.93	0.12	>7.7	0.06	>15.4
H	$CH_2CH{=}CHCH_2OH$	>0.5	0.25	>2	—	—
Me	$CH_2CH{=}CHCH_2OH$	0.95	0.03	32	0.015	63
SMe	$CH_2CH{=}CHCH_2OH$	0.53	0.015	35	≥0.007	≤76
H	$(CH_2)_2OH$	>0.5	0.25	>2	—	—
Me	$(CH_2)_2OH$	>1.0	0.12	>8.3	—	—
SMe	$(CH_2)_2OH$	>0.5	0.12	>4	—	—
H	$CH_2O(CH_2)_2OH$	0.5	0.25	2	—	—
Me	$CH_2O(CH_2)_2OH$	>0.5	0.5	>1	—	—
SMe	$CH_2O(CH_2)_2OH$	>0.5	0.25	>2	—	—

Note. CD_{50} is the cytotoxic concentration causing 50% non-infected cell death; ID_{50} is the concentration of the drug causing a 50% reduction in viral cytopathic effect; IS is the selectivity index (CD_{50}/ID_{50}); PFU is a plaque-forming unit. The values averaged over three independent experiments are given.

reported annually, one-half of West Nile fever cases being accompanied by neurological manifestations, such as encephalitis and meningoencephalitis. Before the outbreak of WNF in Russia in 1999, etiotropic and nonspecific agents were absent, and the therapy was based on pathogenic agents. In a series of chemotherapeutic drugs, amixine showed moderate efficacy against the WNF virus and ribavirin exhibited high efficacy.[90]

The evaluation of antiviral efficacy of compounds of the azoloazine series against WNF viruses in the GMK-AH-(1D) cell culture demonstrated that sodium salt of 2-ethylthio-6-nitro-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-one and arginine salt of 2-methylthio-6-nitro-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-one suppress the replication of WNF virus (Eg101 strain) and protect 30—50% of albino mice against experimental WNF.[12,81] In *in vivo* experiments, 2-methylthio-5-methyl-6-nitro-1,2,4-triazolo[1,5-*a*]pyrimidin-7-one[77] and anomalous nucleoside, which was prepared based on the compound of this class, *viz.*, 2-methylthio-4-[(2-hydroxyethoxy)methyl]-5-methyl-1,2,4-triazolo-[1,5-*a*]pyrimidin-7-one,[82] were shown to be effective against WNF.

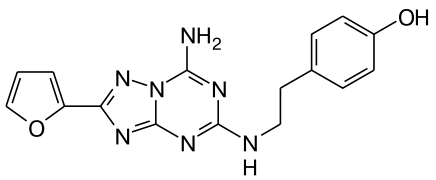
3.4. Antiviral activity against tick-borne encephalitis virus

In most regions of Russia, tick-borne encephalitis (TBE) occupies an important place among zoonotic viral infections with regards to epidemiological significance, clinical disease severity, and lethality.[91] The nosoarea of tick-borne encephalitis in Siberia, the Urals, the Volga-Vyatka district and also in northern Europe is currently being expanded.

In a series of azoloazines, sodium salt of 2-methylthio-6-nitro-1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-one exhibits antiviral activity. In the transplantable porcine kidney cell culture at a maximum tolerable dose (128 μg mL^{-1}), this compound efficiently suppresses the TBE virus replication. A decrease in the level of virus accumulation is 2.3 lg.[89] In *in vivo* experiments, this compound protects 45—55% of albino mice when used in prophylactic or therapeutic strategies, as well as in the immediate treatment strategy. [83—85]

4. Effect on adenosine receptors

A series of selective agonists and antagonists of A_{2A} adenosine receptors ($A_{2A}AR$) are already in clinical trials as agents for the treatment of Parkinson´s disease, inflammations, cancer, ischemia, diabetic nephropathy, and central nervous system disorders.[92,93] Some fused azoloazines, which are structural analogues of purines, are used as medications for the prevention and treatment of sepsis *via* regulation of adenosine receptors.[94—96] Azoloazines containing the bridgehead nitrogen atom in the molecular skeleton occupy an important place among these effectors of A_{2A} receptors. These compounds include, *e.g.*, 2-furyl-5-(4-hydroxyphenyl)ethylamino-7-amino-1,2,4-triazolo[5,1-*a*]-1,3,5-triazine (**94**).[97—99]



94

We proposed[58] 6-nitro-1,2,4-triazolo[1,5-*a*]pyrimidin-7-ones **75** (see Scheme 25) and 7-alkylamino-6-nitro-1,2,4-triazolo[1,5-*a*]pyrimidines **86** (see Scheme 29) as new antiseptics.

The values of $pK_i = -\log[K_i]$, where K_i is the equilibrium constant for the formation of the complex (L•R) of the ligand (L) with the receptor (R) ($K_i = [R•L]/[R]•[L]$) were determined (Table 7)[59] by means of computer simulation using the flexible docking algorithm that is utilized in the Surflex-Dock program implemented in the Sybyl-X 1.1 suite.[100] Many of the compounds that were examined form complexes with the receptor *via* hydrogen bonding, thereby demonstrating moderate interaction. Exceptions are compound **75** with R = 2-furyl and R′ = H, characterized by $pK_i > 7$, and 7-*N*-[2-(4-chlorophenyl)ethylamino]-6-nitro-1,2,4-triazolo[1,5-*a*]pyrimidine **86**, which exhibited the most effecting binding in the series of triazolopyrimidines.[59]

The results of *in vivo* testing of the above-mentioned compounds are in general agreement with the calculated data. The screening of compounds for biological activity was performed using a septic shock model induced by lipopolysaccharide from *Salmonella typhosa* (Sigma—Aldrich) in mice. Compound **75** with R = 2-furyl and R′ = H was shown to have the highest activity (80% survival rate of mice with artificially induced septic shock). Compound **86** with R = R′ = H and R″ = 2-(4-chlorophenyl)ethyl, which exhibited the highest affinity according to calculations, except for compound **94**, also showed significant activity in animals, preventing death from septic shock in 60% of test group animals (see Table 7).[59]

Based on survival rates of animals with septic shock, promising compounds were revealed by screening in order to evaluate their effect on the pathological manifestation of acute respiratory distress syndrome in animals infected with *Klebsiella pneumoniae*. This inflammatory lung injury is a severe life-threatening syndrome, which is developed in the body, including the process associated with septic inflammation.

All the selected compounds were used in combination with meropenem exhibiting antibacterial activity. Thus, the administration of heterocycles **86** (R = R″ = H,

Table 7. Results of docking tests for 1,2,4-triazolo[1,5-*a*]pyrimidine derivatives **75** and **86** and compound **94** to A_{2A} adenosine receptors and the effect of compounds **75** and **86** on the survival rate of albino mice with septic sh ock

Compound	R	R′	R′′	pK_i	Effect (dead/total)	Survival rate (%)
75	2-Furyl	H	—	7.14	1/5[a]	80
75	H	H	—	5.23	4/5	20
75	3-Pyridyl	Me	—	5.99	5/5	0
75	H	Me	—	5.27	5/5	0
75	Me	Me	—	5.11	5/5	0
86	H	OH	H	5.34	2/5[a]	60
86	H	H	Bu	6.53	4/5	20
86	H	H	4-$ClC_6H_4(CH_2)_2$	7.54	2/5[a]	60
86	H	Me	$(CH_2)_2OAc$	6.48	2/5[a]	60
94	—	—	—	10.00	—	—
Hydrocortisone[b]	—	—	—	—	2/5[a]	60
Placebo	—	—	—	—	5/5	0
—	—	—	—	—	0/5[c]	100[c]

[a] $p < 0.05$.
[b] 15 mg kg^{-1}.
[c] Intact animals.

R′ = OH) and **75** (R is 2-furyl, R′ = H) was shown to reduce extravascular lung water (to 53 and 62%, respectively, *versus* 100% when treated with placebo). This correlates with the lower-grade systemic inflammation associated with the migration of a considerable amount of leukocytes toward infection loci.

5. Antidiabetic activity

Type 2 diabetes mellitus (T2DM) is one of the major public health problems. It was estimated that there were 415 million people with diabetes in 2015 and that this number will rise to 642 millions by 2040 (~90% patients with T2DM). Diabetes mellitus is a cause of 5 million deaths annually. Despite a large number of drugs approved for clinical use having various mechanisms of action, the target level of glycated hemoglobin HbA1c lower than 7% was not reached in 63% of patients subjected to therapy.[101] Consequently, the development of new antidiabetic drugs, which are both targeted to certain pathogenic events in T2DM and aimed at preventing its complications, remains a challenging problem.

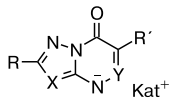
Besides, azolo-annulated 1,2,4-triazines and pyrimidines under consideration may be attractive compounds for the search for new antidiabetic agents. Structurally similar 6-aminomethyl-7-(2,4-dichlorophenyl)-2,5-dimethyl-1,2,4-triazolo[1,5-*a*]pyrimidine,[100] 6-aminomethyl-7-(2,4-dichlorophenyl)-2,5-dimethylpyrazolo[1,5-*a*]pyrimidine,[102,103] and *N*-[2-{2-[(2*S*)-2-cyanopyrrolidin-1-yl]-2-oxoethyl}amino-2-methylpropyl]-2-methylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide hydrochloride (anagliptin)[103] were characterized as selective dipeptidyl peptidase 4 (DPP-4) inhibitors active in the nanomolar range.

Pyrazolo- and 1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones synthesized by our research team were tested for antidiabetic properties, including antiglycation activity, cross-link breaking activity, and ability to inhibit enzymes, which play an important role in glycemic control (DPP-4, glycogen phosphorylases, and α-glucosidases).[15,104]

5.1. Antiglycation activity

Antiglycation activity was assessed by measuring the effect of the compounds on nonenzymatic glycation of bovine serum albumin (BSA) by glucose. Most of the tested pyrazolo- and 1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-one derivatives (**4**, **7**, **11**), 1,2,4-triazolo[1,5-*a*]pyrimidin-7-ones (**70**, **76**), and 7-amino-6-nitro-1,2,4-triazolo[1,5-*a*]pyrimidines (**86**) exhibited pronounced antiglycation properties, *i.e.*, prevent nonenzymatic glycation of the protein, which is a key mechanism of development of micro- and macroangiopathic complications.[15,104,105] Investigations of the structure—activity relationship demonstrated that azolo[5,1-*c*]-1,2,4-triazin-7-one derivatives containing the pyrazole moiety are more active than the corresponding 1,2,4-triazolo[5,1-*c*]-1,2,4-triazin-7-ones. Compounds **4**, **7**, **11** and 1,2,4-triazolo[1,5-*a*]pyrimidin-7-ones **70**, **76** are significantly superior to aminoguanidine as the reference compound[15,105] (Table 8). 7-Amino-6-nitro-1,2,4-triazolo[1,5-*a*]pyrimidines are less active[105] (Table 9).

Table 8. Antiglycation activity (IC_{50}) of azolo[5,1-*c*]-1,2,4-triazin-7-ones **4**, **7**, **11** and 1,2,4-triazolo[1,5-*a*]pyrimidin-7-ones **70**, **76**



R	X	Y	R′	Kat^+	IC_{50}/μmol L^{-1}
H	CCO_2Et	N	CO_2Et	Na^+	53.96
H	CCO_2Na	N	CO_2Et	Na^+	96.14
H	CCO_2Et	N	NO_2	Na^+	338.55
H	CCO_2H	N	NO_2	Na^+	85.81
H	CCO_2Et	N	CN	Na^+	116.77
H	CCO_2Na	N	CN	Na^+	122.12
H	CCO_2Et	N	$C=N(OH)NH_2$	Na^+	70.58
H	CCO_2Na	N	$C=N(OH)NH_2$	Na^+	158.81
H	CCO_2Et	N	$C(=O)NHBu^t$	Na^+	48.13
H	CCO_2Na	N	$C(=O)NHBu^t$	Na^+	130.80
H	N	CMe	NO_2	H^+	136.72
SMe	N	CH	NO_2	H^+	111.99
Me	N	CMe	NO_2	H^+	202.53
H	N	CMe	NO_2	Na^+	161.28
CF_3	N	CMe	NO_2	H^+	690.75
3-Pyridyl	N	CMe	NO_2	H^+	202.44
2-Furyl	N	CH	NO_2	H^+	50.35
2-Furyl	N	CH	NO_2	Arg^+	120.04
2-Furyl	N	CH	NO_2	$H_2N^+(Me)CH_2(CH(OH))_4CH_2OH$	120.04
—	—	—	—	—	765.00*

* For aminoguanidine.

Recent data suggest that the mechanism of action of known inhibitors of glycation end product formation is associated primarily with the chelation of heavy metal cations involved in catalysis of oxidation reactions.[106] Our data confirm this hypothesis. Thus, the most pronounced antiglycation properties are observed for compounds containing nitro, ester, *N*-(*tert*-butyl)carboxamide, or *N*-hydroxycarboxamide groups with strong chelating properties.

A study of reglycation activity of azolo[5,1-*c*]-1,2,4-triazin-7-one derivatives in a model system *in vitro* demonstrated that 2-isopropylthio-6-carbethoxy-1,2,4-triazolo-[5,1-*c*]-1,2,4-triazin-7-one and 3-carboxy-6-carbethoxypyrazolo[5,1-*c*]-1,2,4-triazin-7-one exhibit activity comparable with that of the reference drug — alagebrium. The other compounds showed lower activity or proved to be inactive.[15] The absence of a correlation between two contiguous activities — antiglycation and reglycation — calls for a more detailed research on the mechanism of action of these compounds.

Table 9. Antiglycation activity of 7-amino-6-nitro-1,2,4-triazolo[1,5-*a*]pyrimidines **86**

R	R′	R″	IC_{50}/μmol L^{-1}
H	Me	$(CH_2)_2OAc$	179.56
H	Me	Pr^i	580.66
CF_3	H	Bu	—
CF_3	Me	Bu	—
CF_3	Me	Pr^i	690.75
2-Furyl	H	Bu	—
H	—	—	136.72
MeS	—	—	111.99

5.2. Inhibition of dipeptidyl peptidase 4

The inhibition of dipeptidyl peptidase 4 by azolo-[5,1-*c*]-1,2,4-triazin-7-one derivatives was evaluated by measuring their effect on human blood serum DPP-4 *in vitro*.[15] In the first screening step, the compounds were studied at the final concentration of 0.1 μmol L^{-1}. Most of the compounds under consideration (**4**, **7**, **11**) did not exhibit activity or showed weak inhibitory properties. 3-Carbethoxy- and 3-cyano-6-nitropyrazolo[5,1-*c*]-1,2,4-triazin-7-ones and also 6-amino-3-carboxypyrazolo-[5,1-*c*]-1,2,4-triazin-7-one displayed significant DPP-4 inhibitory activity, although this activity is lower than that of the reference agent vildagliptin.

In a series of 7-amino-6-nitro-1,2,4-triazolo[1,5-*a*] pyrimidines **86**, 7-(2-acetyloxyethyl)amino-5-methyl-6-nitro-1,2,4-triazolo[1,5-*a*]pyrimidine and 7-butylamino-5-methyl-6-nitro-1,2,4-triazolo[1,5-*a*]pyrimidine were found to have statistically significant inhibitory activity against DPP-4.[105]

In general, most of azolo[5,1-*c*]-1,2,4-triazin-7-ones exhibit pronounced antiglycation activity. Several promis-

ing DPP-4 inhibitors were found. Besides, these compounds show weaker reglycation activity.

Conclusions

The data summarized in this review show that azolo[1,5-*a*]pyrimidine and azolo[5,1-*c*]-1,2,4-triazine derivatives, particularly those containing the nitro group, have attracted great interest due to a wide range of biological activities. Along with the improvement of available methods of synthesis, efforts are aimed at developing new reactions for the preparation of novel compounds and optimization of their structures in order to enhance biological activity. The most favorable methods for the synthesis of these compounds are based on aminoazoles fused to the 1,2,4-triazine or pyrimidine ring. The advantage of this approach is that it can be used to prepare a wide diversity of azoloazines containing a bridgehead nitrogen atom and different substituents both in the azole and azine moieties.

Besides, the azoloazines under consideration can be employed to construct more complex heterocyclic systems, *e.g.*, azolo[5,1-*b*]purines and azapurines.

The fact that azolo[1,5-*a*]pyrimidines and azolo[5,1-*c*]-1,2,4-triazines are structurally similar to purines suggests that these compounds can be involved in the viral nucleic acid synthesis. Therefore, the synthesis of not only new compounds of this class but also natural nucleoside analogues based on these compounds is relevant.

The available methods of synthesis and the data on biological activity of purine-isosteric azolo[5,1-*c*]-1,2,4-triazines and azolo[1,5-*a*]pyrimidines provide a reliable basis for the design of effective drugs.

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References

1. E. De Clercq, *J. Clinic. Virology*, 2001, **22**, 73.
2. E. De Clercq, *Med. Res. Rev.*, 2008, **28**, 929.
3. C. E. Muller, T. Seior, *Pharm. Acta Helv.*, 1993, **68**, 77.
4. G. Gallos, T. D. Ruyle, C. W. Emala, H. T. Lee, *Am. J. Physiology. Renal Physiology*, 2005, **289**, 369.
5. B. V. Chi, B. Peng, K. Gnanasambandam, *J. Med. Chem.*, 2004, **47**, 4291.
6. K. V. Sivak, A. V. Vasin, V. V. Egorov, V. B. Tsvetkov, N. N. Kuzmich, V. A. Savina, O. I. Kiselev, *Mol. Biol.*, 2016, **50**, 200.
7. V. L. Rusinov, E. N. Ulomsky, O. N. Chupakhin, V. N. Charushin, *Russ. Chem. Bull.*, 2008, **57**, 985.
8. V. L. Rusinov, A. Yu. Petrov, I. Ya. Postovsky, *Chem. Heterocycl. Compd.*, 1980, **18**, 974.
9. Pat. RF 2294936; *Byul. Izobret.* [*Inventor Bull.*], 2007, 7 (in Russian).
10. Pat. RF 2340614; *Byul. Izobret.* [*Inventor Bull.*], 2008, 34 (in Russian).
11. Pat. RF 2343154; *Byul. Izobret.* [*Inventor Bull.*], 2009, 1 (in Russian).
12. Pat. RF 2404182; *Byul. Izobret.* [*Inventor Bull.*], 2010, 32 (in Russian).
13. Pat. RF 2402552; *Byul. Izobret.* [*Inventor Bull.*], 2010, 30 (in Russian).
14. O. N. Chupakhin, V. N. Charushin, V. L. Rusinov, *Herald Russ. Acad. Sci.*, 2016, **86**, 206.
15. V. L. Rusinov, I. M. Sapozhnikova, A. M. Bliznik, O. N. Chupakhin, V. N. Charushin, A. A. Spasov, P. M. Vassiliev, V. A. Kuznetsova, A. I. Reshchenko, D. A. Babkov, *Arch. Pharm. (Weinheim, Ger.)*, 2017, **350**, e1600361.
16. L. G. Egorova, A. Yu. Petrov, V. L. Rusinov, O. N. Chupakhin, *Chem. Heterocycl. Compd.*, 1984, **21**, 591.
17. I. V. Blazhennikova, A. F. Kurpyakova, V. N. Bykov, D. S. Geibo, A. S. Nikiforov, A. V. Stepanov, V. N. Charushin, O. N. Chupakhin, S. K. Kotovskaya, V. L. Rusinov, *Eksp. Klinicheskaya Farmakologiya* [*Exper. Clinical Pharmacology*], 2015, **78**, 34 (in Russian).
18. Pat. RF 2547835; *Byul. Izobret.* [*Inventor Bull.*], 2015, 10 (in Russian).
19. G. Tennant, R. J. S. Vevers, *J. Chem. Soc., Perkin Trans 1*, 1976, 421.
20. M. H. Elnagdi, M. R. H. El-Moghayar, F. D. Hleita, E. A. A. Hafez, S. M. Fahmy, *J. Org. Chem.*, 1976, **41**, 3781.
21. T. M. Kandeel, V. B. Baghos, I. S. Mohareb, M. H. Elnagdi, *Arch. Pharm. (Weinheim, Ger.)*, 1983, **316**, 713.
22. E. N. Ulomsky, S. L. Deev, V. L. Rusinov, O. N. Chupakhin, *Russ. J. Org. Chem.*, 1999, **35**, 1355.
23. E. N. Ulomsky, N. R. Medvedeva, A. V. Shepochkin, O. S. El´tsov, V. L. Rusinov, O. N. Chupakhin, E. G. Deeva, O. I. Kiselev, *Chem. Heterocycl. Compd.*, 2011, **46**, 1164.
24. Pat. RF 2493158; *Byul. Izobret.* [*Inventor Bull.*], 2013, 26 (in Russian).
25. V. L. Rusinov, T. L. Pilicheva, O. N. Chupakhin, N. A. Klyuev, D. T. Allakhverdieva, *Chem. Heterocycl. Compd.*, 1985, **22**, 543.
26. O. N. Chupakhin, E. N. Ulomsky, S. L. Deev, V. L. Rusinov, *Synth. Commun.*, 2001, **31**, 2351.
27. T. S. Shestakova, S. L. Deev, E. N. Ulomskii, V. L. Rusinov, M. I. Kodess, O. N. Chupakhin, *Arkivoc*, 2008, 69.
28. S. L. Deev, Z. O. Shenkarev, T. S. Shestakova, O. N. Chupakhin, V. L. Rusinov, A. S. Arseniev, *J. Org. Chem.*, 2010, **24**, 8487.
29. T. S. Shestakova, I. A. Khalymbadzha, S. L. Deev, O. S. El´tsov, V. L. Rusinov, Z. O. Shenkarev, A. S. Arseniev, O. N. Chupakhin, *Russ. Chem. Bull.*, 2011, **60**, 729.
30. T. S. Shestakova, Z. O. Shenkarev, S. L. Deev, O. N. Chupakhin, I. A. Khalymbadzha, V. L. Rusinov, A. S. Arseniev, *J. Org. Chem.*, 2013, **78**, 6975.
31. V. L. Rusinov, E. N. Ulomsky, O. N. Chupakhin, G. L. Rusinov, A. I. Chernishev, G. G. Aleksandrov, *Chem. Heterocycl. Compd.*, 1987, **23**, 1236.
32. V. L. Rusinov, E. N. Ulomsky, O. N. Chupakhin, M. M. Zubairov, A. B. Kapustin, N. I. Mitin, M. I. Zhirovetsky, I. A. Vinograd, *Pharm. Chem. J.*, 1990, **24**, 646.
33. E. N. Ulomsky, S. L. Deev, T. S. Shestakova, V. L. Rusinov, O. N. Chupakhin, *Russ. Chem. Bull.*, 2002, **51**, 1737.
34. O. N. Chupakhin, V. L. Rusinov, E. N. Ulomsky, N. R. Medvedeva, I. M. Sapozhnikova, *Butlerovskie Soobshcheniya* [*Butlerov Commun.*], 2012, **31**, 43 (in Russian).
35. J. Farras, E. Fos, R. Ramos, J. Vilarrasa, *J. Org. Chem.*, 1988, **53**, 887.

36. E. N. Ulomsky, S. L. Deev, V. L. Rusinov, O. N. Chupakhin, *Dokl. Chem.*, 2001, **379**, 196.
37. E. N. Ulomsky, S. L. Deev, A. V. Tkachev, I. K. Moiseev, V. L. Rusinov, *Russ. J. Org. Chem.*, 2002, **38**, 272.
38. H. Vorbrüggen, *Acc. Chem. Res.*, 1995, **28**, 509.
39. H. Vorbrüggen, B. Bennua, *Chem. Ber.*, 1981, **114**, 1279.
40. S. L. Deev, T. S. Shestakova, V. L. Rusinov, O. N. Chupakhin, A. S. Shashkov, *Arkivoc*, 2009, 196.
41. T. Goslinski, B. Golankewiecz, E. De Clercq, J. Balzarini, *J. Med. Chem.*, 2002, **45**, 5052.
42. S. L. Deev, M. V. Yasko, I. L. Karpenko, A. L. Andronova, G. A. Galegov, T. S. Shestakova, E. N. Ulomsky, V. L. Rusinov, O. N. Chupakhin, *Bioorg. Chem.*, 2010, **38**, 265.
43. V. L. Rusinov, O. N. Chupakhin, S. L. Deev, T. S. Shestakova, E. N. Ulomskii, L. I. Rusinova, O. I. Kiselev, E. G. Deeva, *Russ. Chem. Bull.*, 2010, **59**, 136.
44. S. Phadtare, D. Kessel, T. H. Corbett, H. E. Renis, B. F. Court, J. Zemlicka, *J. Med. Chem.*, 1991, **34**, 421.
45. T. S. Shestakova, L. S. Luk´yanova, T. A. Tseitler, S. L. Deev, E. N. Ulomsky, V. L. Rusinov, M. I. Kodess, O. N. Chupakhin, *Russ. Chem. Bull.*, 2008, **57**, 2423.
46. A. A. Arzumanov, N. B. Dyatkina, *Nucleosides Nucleotides*, 1994, **13**, 1031.
47. Pat. RF 2345080; *Byul. Izobret.* [*Inventor Bull.*], 2009, 3 (in Russian).
48. V. L. Rusinov, I. M. Sapozhnikova, N. R. Medvedeva, V. V. Egorov, O. I. Kiselev, E. G. Deeva, A. V. Vasin, O. N. Chupakhin, *Chem. Heterocycl. Compd.*, 2015, **51**, 275.
49. E. N. Ulomsky, E. V. Tsoi, V. L. Rusinov, O. N. Chupakhin, G. L. Kalb, I. M. Sosonkin, *Chem. Heterocycl. Compd.*, 1992, **28**, 570.
50. I. Karpenko, S. Deev, O. Kiselev, V. Charushin, V. Rusinov, E. Ulomsky, E. Deeva, D. Yanvarev, A. Ivanov, O. Smirnova, S. Kochetkov, O. Chupakhin, M. Kukhanova, *Antimicrob. Agents Chemotherapy*, 2010, **54**, 2017.
51. A. Yu. Petrov, V. L. Rusinov, O. N. Chupakhin, *Chem. Heterocycl. Compd.*, 1982, **18**, 992.
52. V. L. Rusinov, E. N. Ulomsky, O. N. Chupakhin, A. Yu. Petrov, E. A. Sharonov, *Chem. Heterocycl. Compd.*, 1989, **25**, 209.
53. T. W. Green, P. G. M. Wuts, *Protective Groups in Organic Synthesis*, Wiley-Intersci., New York, 1999.
54. V. L. Rusinov, A. Yu. Petrov, O. N. Chupakhin, N. A. Klyuev, G. G. Aleksandrov, *Chem. Heterocycl. Compd.*, 1985, **21**, 576.
55. E. K. Voinkov, E. N. Ulomskiy, V. L. Rusinov, O. N. Chupakhin, K. V. Savateev, V. V. Fedotov, E. B. Gorbunov, M. L. Isenov, O. S. Eltsov, *Mendeleev Commun.*, 2016, **26**, 172.
56. E. K. Voinkov, E. N. Ulomsky, V. L. Rusinov, O. N. Chupakhin, E. B. Gorbunov, R. A. Drokin, V. V. Fedotov, *Chem. Heterocycl. Compd.*, 2015, **51**, 1057.
57. K. V. Savateev, E. N. Ulomsky, V. L. Rusinov, M. L. Isenov, O. N. Chupakhin, *Russ. Chem. Bull.*, 2015, **64**, 1378.
58. V. L. Rusinov, A. Yu. Petrov, T. L. Pilicheva, O. N. Chupakhin, G. V. Kovalev, E. R. Komina, *Pharm. Chem. J.*, 1986, **20**, 113.
59. K. V. Savateev, E. N. Ulomsky, V. V. Fedotov, V. L. Rusinov, K. V. Sivak, M. M. Lyubshin, N. N. Kuzmich, A. G. Aleksandrov, *Russ. J. Bioorg. Chem.*, 2017, **43**, 402.
60. O. N. Chupakhin, S. L. Deev, T. S. Shestakova, O. L. Eltsov, V. L. Rusinov, *Heterocycles*, 2010, **80**, 1149.
61. V. P. Kislyi, A. L. Laikhter, B. L. Ugrak, V. V. Semenov, *Russ. Chem. Bull.*, 1994, **43**, 70.
62. V. L. Rusinov, M. N. Kushnir, E. N. Ulomsky, N. A. Klyuev, S. V. Shorshnev, G. G. Aleksandrov, O. N. Chupakhin, *Russ. J. Org. Chem.*, 1993, **29**, 525.
63. T. P. Kofman, T. A. Uvarova, G. Yu. Kartseva, T. L. Uspenskaya, *Russ. J. Org. Chem.*, 1997, **33**, 1784.
64. Pat. RF 2330036; *Byul. Izobret.* [*Inventor Bull.*], 2008, 21 (in Russian).
65. Pat. RF 2529487; *Byul. Izobret.* [*Inventor Bull.*], 2014, 27 (in Russian).
66. I. A. Khalymbadzha, T. S. Shestakova, J. O. Subbotina, O. S. El´tsov, V. L. Rusinov, O. N. Chupakhin, I. L. Karpenko, M. V. Jasko, M. K. Kukhanova, S. L. Deev, *Tetrahedron*, 2014, **70**, 1298.
67. K. V. Savateev, E. N. Ulomsky, S. S. Borisov, E. K. Voinkov, V. V. Fedotov, V. L. Rusinov, *Chem. Heterocycl. Compd.*, 2014, **50**, 880.
68. E. B. Gorbunov, G. L. Rusinov, E. N. Ulomsky, M. L. Isenov, V. N. Charushin, *Chem. Heterocycl. Compd.*, 2015, **51**, 491.
69. E. B. Gorbunov, G. L. Rusinov, E. N. Ulomskii, O. S. El´tsov, V. L. Rusinov, V. N. Charushin, O. N. Chupakhin, *Tetrahedron Lett.*, 2016, **57**, 2303.
70. *Metal-Free C—H Functionalization of Aromatics: Nucleophilic Displacement of Hydrogen*, Eds V. N. Charushin, O. N. Chupakhin, Springer, Heidelberg—New York—Dordrecht—London, 2014.
71. O. N. Chupakhin, V. N. Charushin, *Tetrahedron Lett.*, 2016, **57**, 2665.
72. S. Ya. Loginova, S. V. Borisevich, V. A. Maksimov, V. P. Bondarev, S. K. Kotovskaya, V. L. Rusinov, V. N. Charushin, *Antibiotiki Khimioterapiya* [*Antibiotics Chemotherapy*], 2007, **52**, 18 (in Russian).
73. S. Ya. Loginova, S. V. Borisevich, V. A. Maksimov, V. P. Bondarev, S. K. Kotovskaya, V. L. Rusinov, V. N. Charushin, O. N. Chupakhin, *Antibiotiki Khimioterapiya* [*Antibiotics Chemotherapy*], 2010, **55**, 25 (in Russian).
74. S. Ya. Loginova, S. V. Borisevich, V. A. Maksimov, V. P. Bondarev, S. K. Kotovskaya, V. L. Rusinov, V. N. Charushin, O. N. Chupakhin, *Antibiotiki Khimioterapiya* [*Antibiotics Chemotherapy*], 2011, **56**, 10 (in Russian).
75. S. Ya. Loginova, S. V. Borisevich, V. L. Rusinov, E. N. Ulomsky, V. N. Charushin, O. N. Chupakhin, *Antibiotiki Khimioterapiya* [*Antibiotics Chemotherapy*], 2012, **57**, 8 (in Russian).
76. E. G. Deeva, V. L. Rusinov, V. N. Charushin, O. N. Chupakhin, O. I. Kiselev, *Razrabotka i registratsiya lekarstvennykh sredstv* [*Drug Development and Registration*], 2014, 144 (in Russian).
77. Pat. RF 2365591; *Byul. Izobret.* [*Inventor Bull.*], 2009, 24 (in Russian).
78. Pat. RF 2376307; *Byul. Izobret.* [*Inventor Bull.*], 2009, 35 (in Russian).
79. Pat. RF 2455304; *Byul. Izobret.* [*Inventor Bull.*], 2012, 19 (in Russian).
80. Pat. RF 2516936; *Byul. Izobret.* [*Inventor Bull.*], 2014, 14 (in Russian).
81. Pat. RF 2536874; *Byul. Izobret.* [*Inventor Bull.*], 2014, 27 (in Russian).
82. Pat. RF 2537295; *Byul. Izobret.* [*Inventor Bull.*], 2014, 34 (in Russian).
83. S. Ya. Loginova, S. V. Borisevich, V. L. Rusinov, E. N. Ulomsky, V. N. Charushin, O. N. Chupakhin, P. V. Sorokin,

Antibiotiki Khimioterapiya [*Antibiotics Chemotherapy*], 2015, **60**, 8 (in Russian).
84. S. Ya. Loginova, S. V. Borisevich, V. L. Rusinov, E. N. Ulomsky, V. N. Charushin, O. N. Chupakhin, P. V. Sorokin, *Antibiotiki Khimioterapiya* [*Antibiotics Chemotherapy*], 2015, **60**, 11 (in Russian).
85. *Triazavirin* [*Triazavirin*], Eds O. N. Chupakhin, O. I. Kiselev, Ekaterinburg, 2016, 254 pp. (in Russian).
86. Pat. RF 2607628; *Byul. Izobret.* [*Inventor Bull.*], 2017, 1 (in Russian).
87. O. I. Kiselev, E. G. Deeva, T. I. Mel´nikova, K. N. Kozeletskaya, K. N. Kiselev, V. L. Rusinov, V. N. Charushin, O. N. Chupakhin, *Voprosy virusologii* [*Problems of Virology*], 2012, **57**, 9 (in Russian).
88. E. G. Deeva, O. I. Kiselev, T. I. Mel´nikova, K. A. Zagorodnikova, V. N. Charushin, V. L. Rusinov, E. N. Ulomsky, O. N. Chupakhin, *Epidemiologiya i Infektsionnye Bolezni* [*Epidemiology and Infectious Diseases*], 2013, 20 (in Russian).
89. S. Ya. Loginova, S. V. Borisevich, V. L. Rusinov, E. N. Ulomsky, V. N. Charushin, O. N. Chupakhin, *Antibiotiki Khimioterapiya* [*Antibiotics Chemotherapy*], 2014, **59**, 3 (in Russian).
90. S. Ya. Loginova, A. V. Koval´chuk, S. V. Borisevich, *Voprosy virusologii* [*Problems of Virology*], 2004, 147 (in Russian).
91. E. I. Bolotin, *Parazitologiya* [*Parasitology*], 1999, **33**, 369 (in Russian).
92. J. A. McCullers, *Clin. Microbiol. Rev.*, 2006, **19**, 571.
93. F. Xu, H. Wu, V. Katritch, G. W. Han, K. A. Jacobson, Z.-G. Gao, V. Cherezov, R. C. Stevens, *Science*, 2011, **332**, 322.
94. A. Ohta, M. Sitkovsky, *Nature*, 2001, **414**, 916.
95. A. Y. Wen, K. M. Sakamoto, L. S. Miller, *J. Immunol.*, 2010, **185**, 6413.
96. K. Reinhart, M. Bauer, N. C. Riedemann, C. S. Hartog, *Clin. Microbiol. Rev.*, 2012, **25**, 609.
97. P. G. Baraldi, M. A. Tabrizi, S. Gessi, P. A. Borea, *Chem. Rev.*, 2008, **108**, 238.
98. S. Sheth, R. Brinto, D. Mukherjea, L. P. Rybak, V. Ramkumar, *Int. J. Mol. Sci.*, 2014, **15**, 2024.
99. G. Lebon, T. Warne, P. C. Edwads, K. Bennett, C. J. Langmead, A. G. W. Leslie, C. G. Tate, *Nature*, 2011, **47**1.
100. A. N. Jain, *J. Med. Chem.*, 2003, **46**, 499.
101. *Mishen´-orientirovannyi poisk antidiabeticheskikh sredstv* [*Target-Oriented Search for Antidiabetic Agents*], Eds A. A. Spasov, V. I. Petrov, Volgograd, 2016, 231 pp. (in Russian).
102. R. P. Brigance, W. Meng, A. Fura, T. Harrity, A. Wang, R. Zahler, M. S. Rirby, L. G. Hamann, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 4395.
103. N. Kato, M. Oka, T. Murase, M. Yoshida, M. Sakairi, S. Yamashita, Y. Yasuda, A. Yoshikawa, Y. Hayashi, M. Makino, M. Takeda, Y. Mirensha, T. Kakigami, *Bioorg. Med. Chem.*, 2011, **19**, 7221.
104. Pat. RF 2612300; *Byul. Izobret.* [*Inventor Bull.*], 2017, 7 (in Russian).
105. A. A. Spasov, D. A. Babkov, V. A. Sysoeva, R. A. Litvinov, D. D. Shamshina, O. N. Chupakhin, V. L. Rusinov, E. N. Ulomsky, K. V. Savateev, V. V. Fedotov, P. A. Slepukhin, *Arch. Pharm. (Weinheim, Ger.)*, 2017, **350**, e1700226.
106. D. Kim, L. Wang, M. Beconi, G. J. Eiermann, M. H. Fisher, H. He, G. J. Hickey, J. E. Kowalchick, B. Leiting, K. Lyons, F. Marsilio, M. E. McCann, R. A. Patel, A. Petrov, G. Scapin, S. B. Patel, R. S. Roy, J. K. Wu, M. J. Wyvratt, B. B. Zhang, L. Zhu, N. A. Thornberry, A. E. Weber, *J. Med. Chem.*, 2005, **48**, 141.

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