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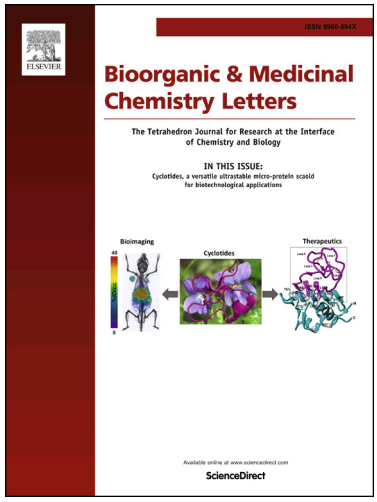

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PII: S0960-894X(17)31116-2
DOI: https://doi.org/10.1016/j.bmcl.2017.11.029
Reference: BMCL 25435

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 26 July 2017
Revised Date: 10 November 2017
Accepted Date: 14 November 2017

Please cite this article as: Zhurilo, N.I., Chudinov, M.V., Matveev, A.V., Smirnova, O.S., Konstantinova, I.D., Miroshnikov, A.I., Prutkov, A.N., Grebenkina, L.E., Pulkova, N.V., Shvets, V.I., Isosteric ribavirin analogues: synthesis and antiviral activities., *Bioorganic & Medicinal Chemistry Letters* (2017), doi: https://doi.org/10.1016/j.bmcl.2017.11.029

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Isosteric ribavirin analogues: synthesis and antiviral activities.

Nikolay I. Zhurilo[a], Mikhail V. Chudinov [a†], Andrey V. Matveev [a], Olga S. Smirnova[b], Irina D. Konstantinova[b], Anatoly I. Miroshnikov[b], Alexander N. Prutkov[c], Lyubov E. Grebenkina[c], Natalya V. Pulkova[c], Vitaly I. Shvets[a]

[a] *Lomonosov Institute of Fine Chemical Tehnologies, Moscow Technological University, Vernadskogo Pr. 78, 119454, Moscow, Russia.*

[b] *Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Miklukho-Maklaya St. 16/10, 117997 GSP, Moscow B-437, Russia.*

[c] *Moscow Politechnical University, Bolshaya Semenovskaya Str., 38, 107023, Moscow, Russia.*

† Corresponding author: mikle@irims.ru

Abstract

The novel isosteric ribavirin analogues were synthesized by two different ways. Some of them showed significant antiviral action against hepatitis C virus (HCV), herpes simplex (HCV-1) and influenza A virus comparable to that of ribavirin itself. The data obtained confirm the proposed theory of the ribavirin possible antiviral activity mechanism related with bioisosterism.

Ribavirin (Virazole, 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is a nucleoside analogue with a broad antiviral activity spectrum. It is active against influenzia virus, HCV, RSV, HSV etc. [1-6].

Ribavirin action mechanism is still not clarified, however, an existing hypothesis assume the heterocyclic base of ribavirin, 1,2,4-triazole-3-carboxamide (TKA), as a mimic of guanosine purine cycle. This steric similarity allows to ribavirin molecule to incorporate into the viral genome and cause irreversible mutations[7].

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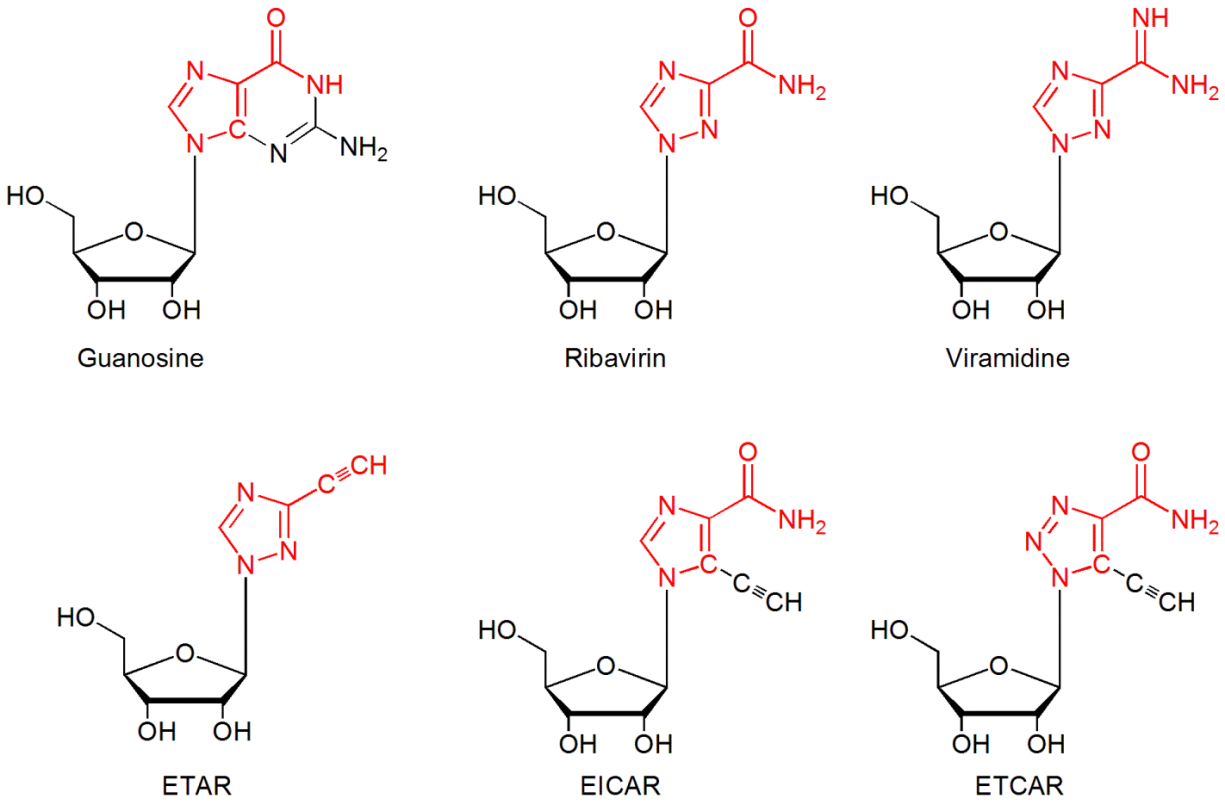

Figure 1. Structure of guanosine and some isosteric antivirals[8].

A wide variety of antiviral nucleoside analogues with corresponding structure mojeties are known for the moment (see Fig.1).

1,2,4-Triazole-3-carboxamide and other 1,2,4-triazole derivatives are substrates for purine nucleoside phosphorylases (NPs) in a manner similar to natural purine nucleosides. We showed earlier that various substituted amides of 1,2,4-triazole-3-carboxylic acid are also NP substrates[9] and demonstrate antiviral activity.

1,2,4-Oxadiazole ring is a common isosteric replacement of the amide group in drug design[10]. However, ribavirin analogues with carboxamide fragment substituted by isosteric 1,2,4-oxadiazole ring were not described before. Here we present a synthetic pathway to this type of ribavirin analogues as well as their antiviral activity.

There are three possible approaches to the synthesis of these compounds: i) ribavirin direct modification; ii) chemical or iii) enzymatic ribozylation of the corresponding heterocyclic bases. The first method allows obtaining only beta-ribosides, while the second one enables to get a set of analogues bearing various sugar mojeties. Enzymatic ribosylation is limited by the NP substrate specificity, while the chemical one is limited by the heterocyclic base stability in the ribosylation reaction conditions.

The first approach is presented on the Scheme 1. Starting from ribavirin a protected amidoxime **3**, a starting material for the ribavirin analogues **5a**-**d** synthesis, was obtained by several step pathway. Thus, compound **4**a was obtained by heating of **3** in triethylformate, while derivatives **4b**-**d** were synthesized by the amidoxime **3** reaction with the corresponding acyl chlorides.

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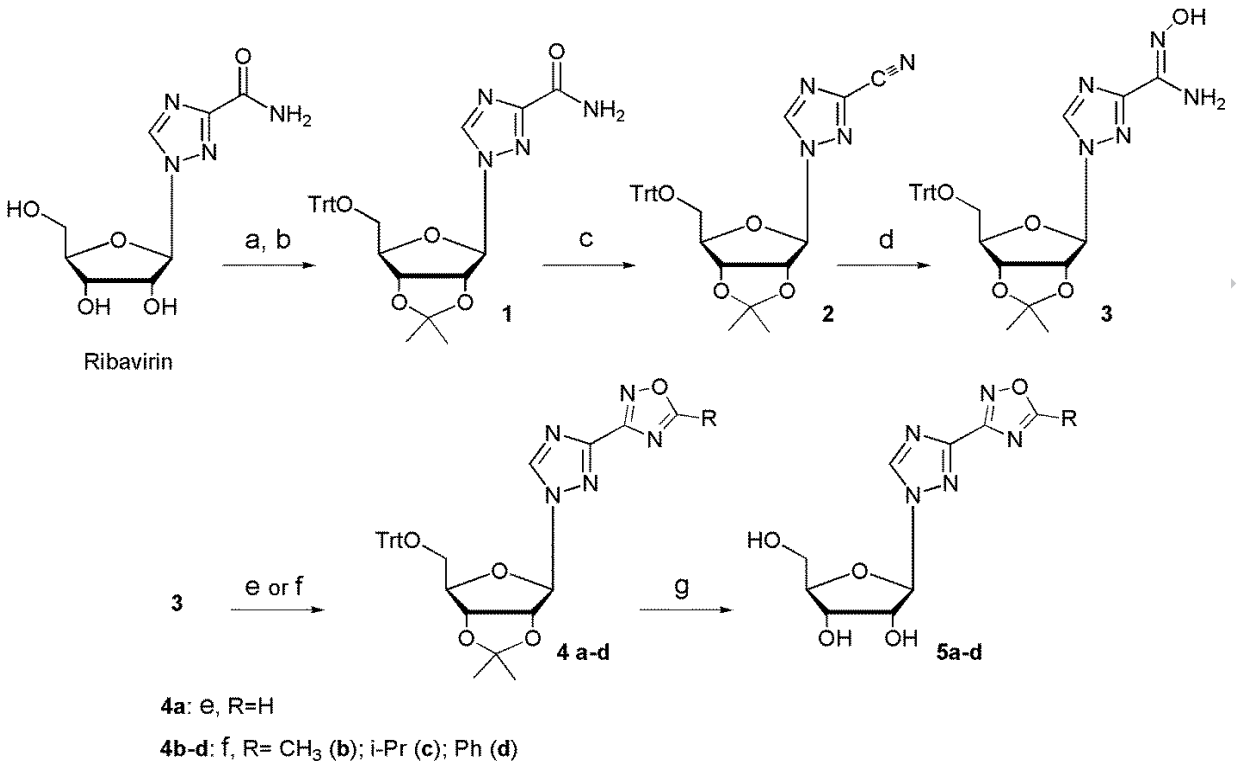
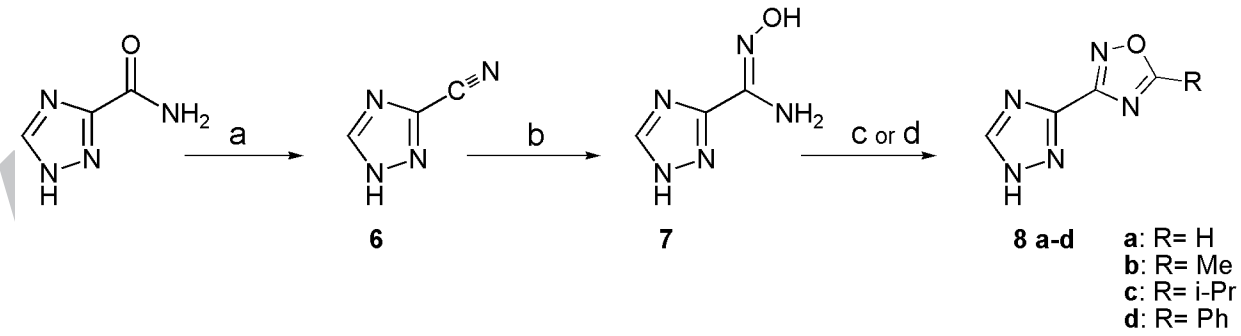


Schema 1. Synthesis of isosteric ribavirin analogues **5a-d** from ribavirin. Reagents and condition: (a) 2,2-dimethoxypropane, p-toluenesulfonic acid (2% mol), DMF, 2 hr, 100 ^{o}C; (b) trityl chloride, pyridine, 1 hr, 5 ^{o}C, 57% yield; (c) trifluoroacetic anhydride, pyridine, CH_2Cl_2, 12 hr, 0-5 ^{o}C, 70% yield; (d) NH_2OH (aq), MeOH, 12 hr, rt, 96% yield; (e) triethyl ortoformate, p-toluenesulfonic acid (10% mol), 3 hr, reflux, 96% yield; (f) acyl chloride, triethyl amine, CH_2Cl_2, 1.5 hr, rt, 73-89% yields; (g) trifluoroacetic acid-water (9:1), 40 min, rt, 48-73% yields.

Also heterocyclic bases **8a-d** were synthesized for the experiments with glycosylation and enzymatic transglycosilation reactions aiming to obtain isosteric ribavirin analogues (Scheme 2).



Schema 2. Synthesis of isosteric TKA bases. Reagents and condition: (a) trifluoroacetic anhydride, pyridine, dioxane, 10 min, -5 ^{o}C, 72 % yield; (b) hydroxylamine (50% in water), ethanol, 12hr, rt, 98% yield ; (c) triethyl ortoformate or triethyl ortoacetate, p-toluenesulfonic acid (2.5% mol), 3 hr, reflux, 68-71 % yields ; (d) acyl chloride, triethyl amine, CH_2Cl_2, 1.5 hr, 0^{o}C ->rt, then tetrabutyl ammonium fluoride (TBAF), toluene, 4 hr, reflux, 68-71 %.

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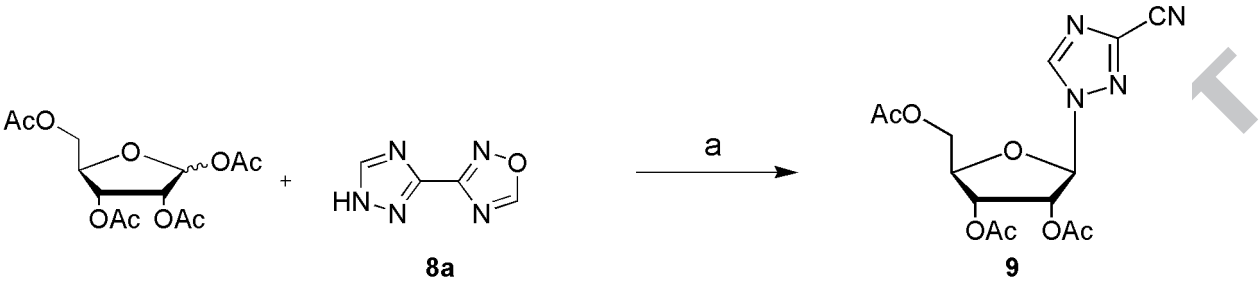
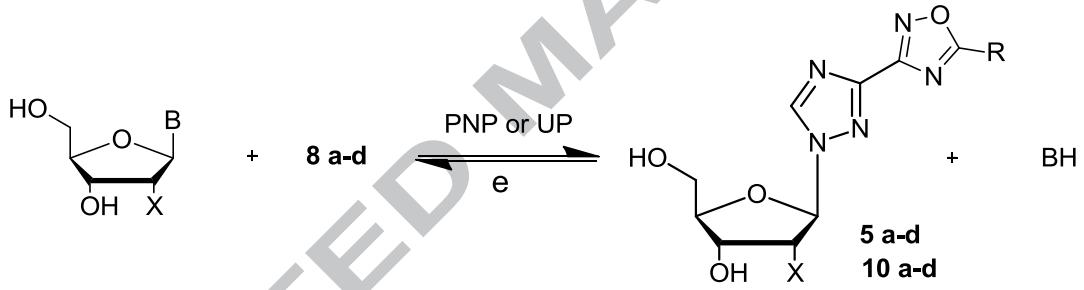
It was shown that the reaction of the base **8a** with ribose tetraacetate in standard conditions[11] yields mainly the derivative **9** (Scheme 3), consequently, this approach is inapplicable for the preparative synthesis of isosteric ribavirin analogues.



Schema 3. Reaction of heterocyclic base **8a** with ribose tetraacetate. Reagents and condition: (a) bis-p-nitrophenyl phosphate (3% mol), 160°C, 15 min.

The opposite was observed for the enzymatic transglycosylation of the nucleosides: all bases **8** regardless of the substituent size in the oxadiazole ring appeared to be good substrates for NP. This quite unexpectable behaviour was in accordance to a theory based on previous experiments in our laboratory[9,12]: the substituent size in the 3d position of 1,2,4-triazole ring has mininimal effect on the substrate properties.



Schema 4. Synthesis of isosteric ribavirin analogues via transglycosylation reaction.

Moreover, glycosylation of compound **8d** bearing bulky phenyl substituent proceeds notably faster than for compounds **8a**-**c** (Fig. 2).

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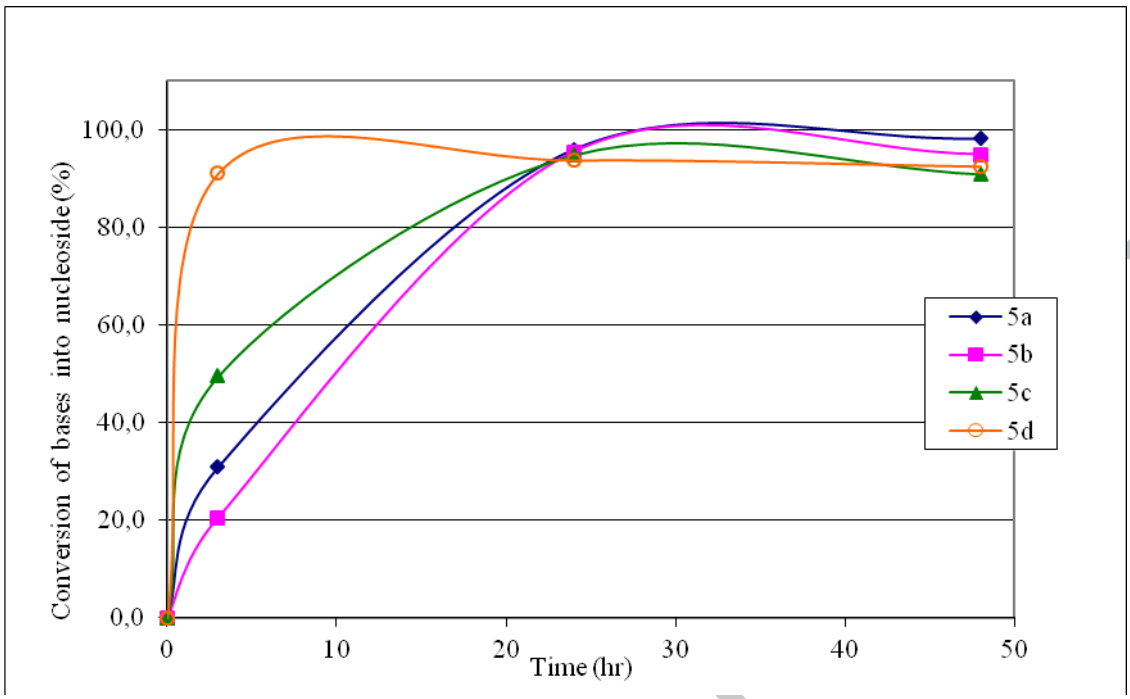

Figure 2. Dependence of bases **8a**-**d** conversion into riboside **5a**-**d**. Reagents and condition: base (1 mM) and Urd (2 mM) in 1.0 ml of the buffer (pH 7.0; 4 mM KH_2PO_4), the recombinant *E. coli* enzymes (70 e. u. of PNP and 90 e. u. of UP), 50 °C, 48 hr.

Synthesis of 2-deoxyribosides **10a**-**d** proceeded faster than that of ribosides, with a 95-98% conversion in 1 h, while riboside synthesis requires from 2 to 24 hours (Fig. 3).

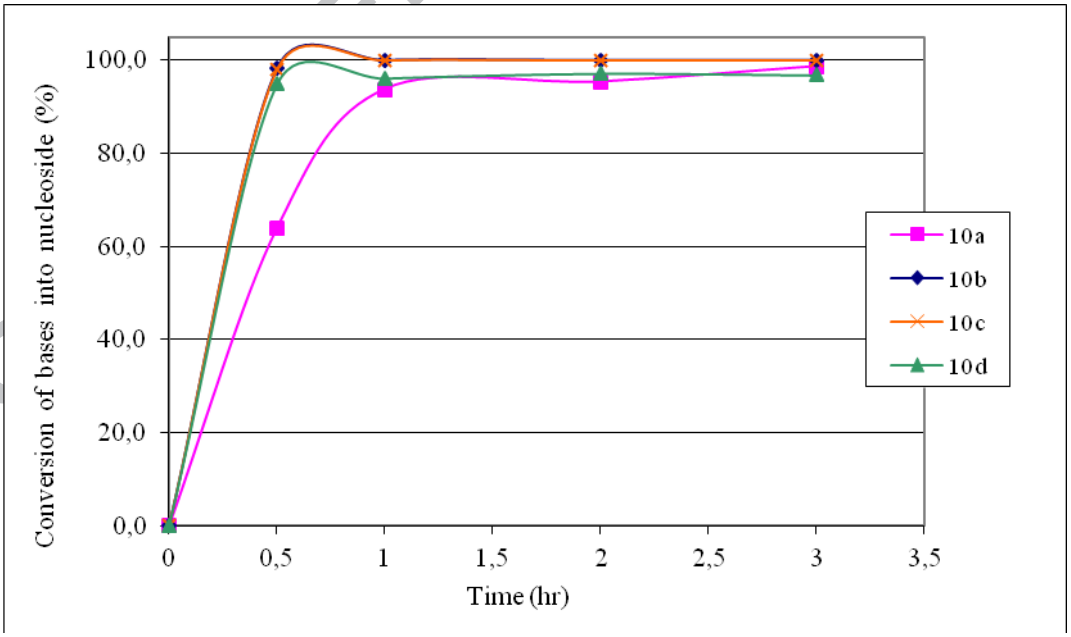

Figure 3. Dependence of bases **8a**-**d** conversion into 2'-deoxyriboside **10a**-**d**. Reagents and condition: base (1 mM) and dUrd (2 mM) in 1.0 ml of the buffer (pH 7.0; 4 mM KH_2PO_4), the recombinant E. coli enzymes (70 e. u. of PNP and 90 e. u. of UP), 50 °C, 3 hr.

The nucleosides **5a**-**d** and **10a**-**d** formation was confirmed by HPLC and LC-MS data. Nucleosides **5b** and **10b** were obtained in a sufficient amount for antiviral activity studies.

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The antiviral activity and cytotoxicity was estimated in vitro on influenza A virus H5N1 (A/duck/Novosibirsk/56/05) and HCV in *SPEV* cells as well as in *Vero E6* cells infected with herpes simplex virus (type 1, L2 strain), and the prevention of the development of the virus-induced cytopathic effect (CPE)[13] was evaluated. Cytotoxicity was estimated by monolayer condition using optical methods. The ribavirin analogues **5a**-**c** and **10b** showed no cytotoxicity against *Vero E6* cells up to a concentration of 1000 μg/ml, although compound **5d** showed moderate cytotoxicity (CC_{50}=380 μg/ml). All compounds showed no cytotoxicity against *SPEV* cells up to a concentration of 150 μg/ml (CC_{50}= 75 μg/ml for ribavirin).

Riboside **5d** exhibits a significant selective activity against HCV (IC_{50}=8.8 μg/ml, 12.5 μg/ml for ribavirin). Compound **5a** showed activity against HSV-1 comparable to the ribavirin activity at a multiplicity of infection (MOI) of 0.01 PFU/cell (IC_{50}=250 μg/ml, 125 μg/ml for ribavirin). Riboside **5b** exhibits a moderate activity against influenza A virus (IC_{50}=50 μg/ml, 12.5 μg/ml for ribavirin). Compounds **5c** and **10b** exhibit no antiviral activity.

Thus, we proposed two ways to efficient synthesis of the new ribavirine analogues: chemical and chemo-enzymatic. The preliminary antiviral activity studies for some of them have shown significant antiviral action comparable to that of ribavirin itself. The data obtained confirm the proposed theory of the ribavirin possible antiviral activity mechanism related with bioisosterism.

Acknowledgements

The work was supported by the grant no.20.9895.2017 of the Ministry of Education and Science of the Russian Federation. Also this work was supported by the Moscow Technological University grant for the support of young scientists and a grant of the president of Russian Federation for the leading scientific school state support (7946.2016.11). We thank V.L. Andronova, G.A. Galegov and P.G. Deryabin from Ivanovsky Research Institute of Virology (Moscow, Russia) for their assistance in the antiviral evaluation. We thank R.S. Esipov and T.I. Muravyova (IBCH RAS) for kindly providing for experiments enzymes nucleoside phosphorylases.

Supplementary data

Supplementary data (experimental procedures and spectroscopic characterizations data of the compounds (**1**-**9**)) associated with this article can be found in the Electronic Supplementary Information (ESI).

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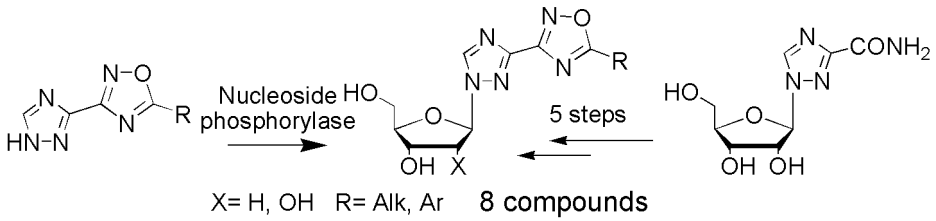

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