Fine Chemical Technologies, 2019, Vol. 14, No. 4, pp. 7–23.
Original Russian Text © Mikhail V. Chudinov, published in Tonkie Khimicheskie Tekhnologii, 2019, Vol. 14, No. 4, pp. 7–23

REVIEW ARTICLES

ОБЗОРНЫЕ СТАТЬИ

https://doi.org/10.32362/2410-6593-2019-14-4-7-23
UDC 615.281.8, 615.277.3, 544.165

Ribavirin and its analogs: Can you teach an old dog new tricks?

Mikhail V. Chudinov

MIREA – Russian Technological University (M.V. Lomonosov Institute of Fine Chemical Technologies), Moscow 119571, Russia
@Corresponding author, e-mail: chudinov@mirea.ru

The review article focuses on the current state of synthetic and biological studies of ribavirin analogs. Ribavirin is a broad-spectrum nucleoside antiviral drug with a 50-year long history of research and application, but its mechanism of action still remains unclear. This article examines contemporary views on the antiviral and antitumor effects of ribavirin and its analogs and describes the contradictions and gaps that exist in our knowledge. In recent years, new nucleoside analogs of ribavirin have been synthesized. These ribavirin derivatives modified at the heterocyclic base, have the potential to become the antiviral and antitumor agents of the new generation. Thus, this paper presents a systematic review of antiviral activities, antitumor activities and structure–activity relationship (SAR) correlations of 39 ribavirin analogs created in the past 15 years. Biological targets and possible mechanisms of action of these new compounds are also discussed, as well as the prospects and possible directions for further research.

Keywords: *ribavirin, ribavirin analogs, biological mechanism, bioisosterism, antiviral drugs, antitumor drugs.*

Рибавирин и его аналоги: можно ли старую собаку научить новым фокусам

М.В. Чудинов

МИРЭА – Российский технологический университет (Институт тонких химических технологий имени М.В. Ломоносова), Москва 119571, Россия
@Автор для переписки, e-mail: chudinov@mirea.ru

Обзор посвящен современному состоянию синтетических и биологических исследований аналогов рибавирина. Рибавирин – нуклеозидный противовирусный препарат широкого спектра действия с 50-ти-летней историей исследований и применения, но механизмы его действия до сих пор остаются неясными. В обзоре кратко изложены современные взгляды на биологические механизмы противовирусного и противоопухолевого действия рибавирина и его аналогов, существующие в этих взглядах противоречия и пробелы. В течение последних лет получены новые нуклеозидные аналоги – производные рибавирина по гетероциклическому основанию, потенциально представляющие собой противовирусные и противоопухолевые средства нового поколения. В статье дан систематический обзор исследований противовирусной и противоопухолевой активности и корреляций «структура – активность», в общей сложности, для 39 аналогов рибавирина, представленных за последние 15 лет, обсуждаются биологические мишени и возможные механизмы действия этих новых соединений, а также перспективы и направление дальнейших исследований.

Ключевые слова: *рибавирин, аналоги рибавирина, механизмы биологического действия, биоизостеризм, противовирусные препараты, противоопухолевые препараты.*

Introduction

Diseases caused by viral infections are the reality we face. It is no secret that the majority of the available "powerful medicines against colds and the flu" are just costly placebos, whereas the treatment of serious, life-threatening illnesses such as difficult cases of influenza, viral hepatitis and hemorrhagic fever, requires a different approach. Among the drugs that doctors turn to when everything else has been tried, ribavirin occupies a special place. This molecule, 1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide, is also called virazole (**1**) (Fig. 1).

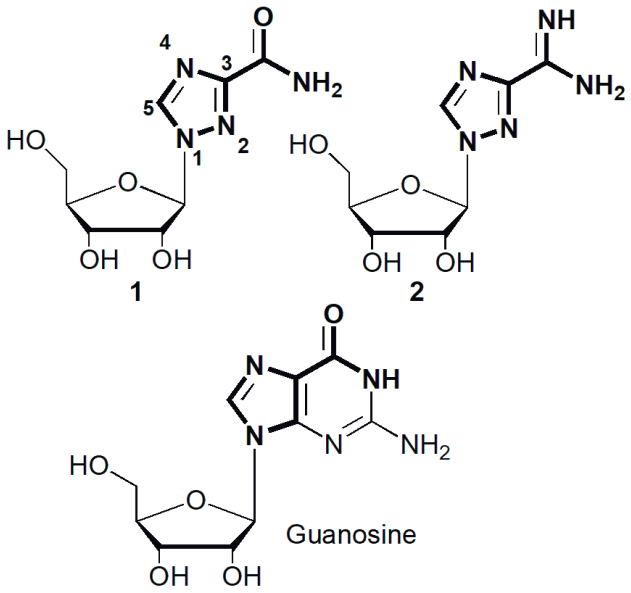

Fig. 1. Ribavirin (**1**) and related compounds, viramidine (**2**) and guanosine (isosteric fragments in heterocycles are highlighted in the bold line).

Having been introduced into clinics almost 50 years ago, ribavirin is still in use, despite several generations of new medications which came after it. All of the drug's drawbacks, such as systemic toxicity, low efficiency of monotherapy for various infections, the significant cost of the course of treatment, are compensated by ribavirin's wide range of antiviral activity. The molecule is active *in vitro* against many RNA- and DNA-containing viruses [1–10], and its *in vivo* activity is just slightly lower. Until recently, the drug was used (in combination with pegylated interferon alfa, INF-α) as the sole effective medication against hepatitis C and in the treatment of severe viral infections, for example, Crimean–Congo hemorrhagic fever, yellow fever, life-threatening cases of influenza. Nonetheless, the introduction of protease inhibitors, such as Sofosbuvir and Simeprevir, seems to have limited the use of ribavirin in hepatitis C treatment – and, fortunately, the other rest of the abovementioned infections are not very common. Does it mean that ribavirin, like many old drugs, is losing its relevance?

According to the Web of Science database, 10 014 articles containing the word "ribavirin" in the title have been published between 1975 and 2019, and 23 480 articles mentioned the drug amongst the keywords. Most of the research was purely medical, and only about 8500 articles were related to chemistry or the molecular biology of the cell. The number of such publications increased every year, from 45 in 1995 to 651 in 2015. After 2015 there was a significant loss of interest to the drug, potentially due to the introduction of protease inhibitors for hepatitis C treatment. Even so, there were 451 publications about it in 2018.

The uniqueness of ribavirin is in its diverse mechanisms of action which are not fully understood even today. So far, multiple attempts to modify the molecule and generate a substance as effective as ribavirin but without its drawbacks, have yielded just

one relatively successful outcome—viramidine (**2**) (1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamidine, taribavirin). This substance (which is less toxic) is a prodrug of ribavirin and is being tested in a Phase IV clinical trial. The research carried out within the last decade opens up new prospects for ribavirin and its derivatives and analogs.

1. Metabolism and mechanism of action of ribavirin and its structural analogs: contemporary concepts

Ribavirin's metabolism and mechanisms of antiviral action are in the focus of multiple review articles [11–21], so less detail of these processes will be presented here. In brief, ribavirin enters the cell through the cytoplasmic membrane with the help of nucleoside transporters CNT3 and ENT1. Then it is phosphorylated in the cytosol by the adenosine kinase, hADK (EC:2.7.1.20) (Fig. 2). Phosphorylation may also occur with the help the cytosolic purine 5'-nucleotidase (EC:3.1.3.5) [22]. Ribavirin 5'-*O*-monophosphate (RMP) (**3**) is processed further by kinases [23] into 5'-di- and triphosphates (**4** and **5**, respectively), which serve as substrates for many enzymes of the host cell and viruses.

Viramidine **2** is also a substrate for hADK, but it is phosphorylated 10–300 times slower than ribavirin, and is 10^4–10^5 times slower than adenosine [22]. Ribavirin's systemic toxicity is explained by the accumulation of its phosphates in erythrocytes, leading to hemolytic anemia in 10% of the patients undergoing a prolonged treatment with it [24]. Other cell types (for example, hepatocytes) remove the metabolites rather quickly ($T_{1/2}$ < 2 h) when the extracellular drug concentration decreases. The metabolites are dephosphorylated back into ribavirin by inosine triphosphate pyrophosphatase (EC:3.6.1.9) [25, 26], then ribavirin undergoes reversible phosphorolysis by purine nucleoside phosphorylase (PNP) (EC:3.6.1.9) [27] and/or hydrolysis of the carboxamide group by adenosine deaminase (ADA) (EC:3.5.4.4) [28, 29]. Thus, the main products of ribavirin's catabolism are 1,2,4-triazole-3-carboxamide (**7**) and 1,2,4-triazole-3-carboxylic acid (**8**), which are released from the body.

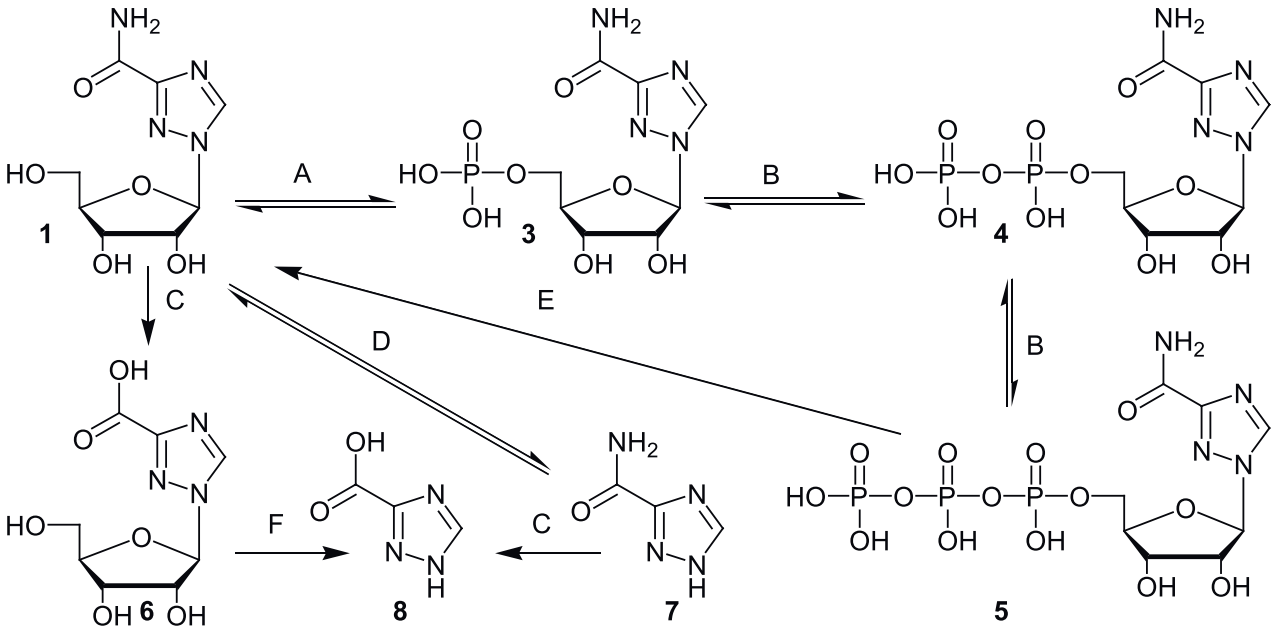

Fig. 2. Metabolism of ribavirin. A – hADK; B – various kinases; C – ADA; D – PNP; E – inosine triphosphate pyrophosphatase; F – various nucleosidases.

Ribavirin is not a substrate or inhibitor for cytochrome P450 (CYP450) and thus is not involved in oxidative catabolism. So, the molecule's metabolic cascade engages the same enzymatic systems as purine nucleosides do. The fact that most of the previously synthesized ribavirin analogs are inactive is often explained by the high substrate specificity of these enzymatic systems. It is usually thought that ribavirin is active against viral

infections only in the 5'-phosphate form [15, 30]. Whereas ribavirin itself, an isostere of guanine and adenosine, is recognized by hADK quite well (phosphorylation rate is only 1200 times lower than for adenosine), other molecules, for example, 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxylic acid (**6**) and 1-β-D-ribofuranosyl-1,2,4-triazole, are not phosphorylated by kinases at all [31]. It is probably due to this that they exhibit no antiviral activity. According to contemporary concepts, there are six suggested mechanisms of ribavirin's action. Fig. 3, based on review [21], shows the schematic representation of these mechanisms.

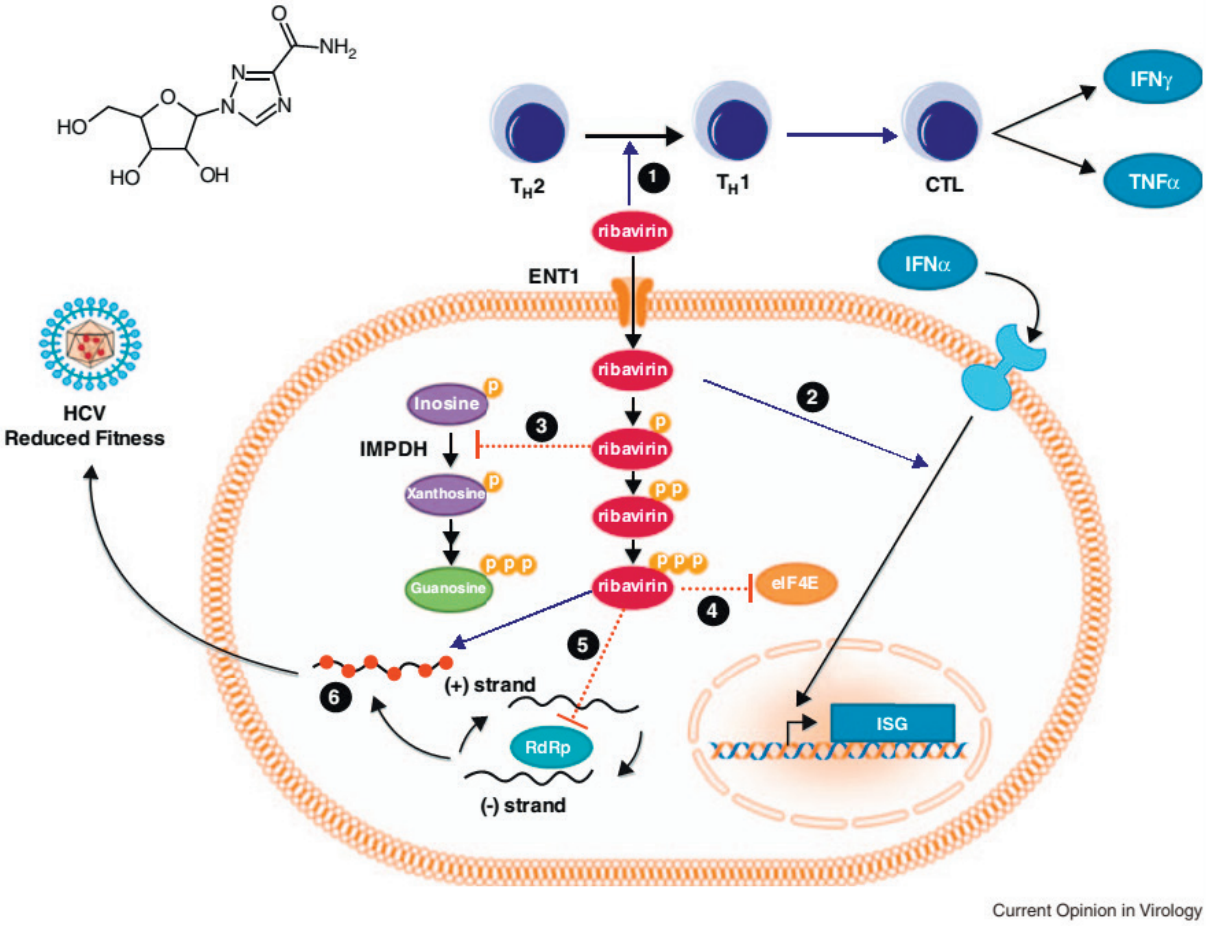

Fig. 3. Mechanisms of ribavirin's action against HCV [21].
1) Modulation of the cell's immune response by lymphocytes Th1 and Th2;
2) modulation of ISG expression; 3) inhibition of inosine monophosphate dehydrogenase (IMPDH);
4) inhibition of eukaryotic translation initiation factor eIF4E;
5) direct inhibition of viral RNA-dependent RNA polymerases (RdRp);
6) viral mutagenesis.

Mechanisms 3–6 are directly related to the phosphorylated metabolites of ribavirin, and the structural bases of the immunomodulating activity in mechanisms 1 and 2 are still unknown. Mechanism 1, related to T lymphocytes, is systemic, and this kind of activity cannot be tested using cell models.

The mechanism which has been studied most (and most proven) is the inhibition of inosine monophosphate dehydrogenase (IMPDH) (EC 1.1.1.205) by RMP (3). IMPDH is a key enzyme in *de novo* synthesis of purine nucleotides. RMP blocks guanosine triphosphate (GTP) synthesis by binding to the enzyme's active site in a reversible manner. This slows down the synthesis of nucleic acids, as well as all other processes in the cell which require GTP as a substrate, including the process of viral genome replication [32]. The change in GTP concentration affects the functioning of the host cell, being the reason behind ribavirin's toxicity.

IMPDH inhibition leads to a decrease in GTP levels to ~60% of the normal level. Afterwards, however, GTP concentration is stabilized and does not decrease further, while ribavirin's concentration increases. At the same time, the dose-dependent manner of the drug's antiviral activity persists [33–36], indicating the presence of other antiviral mechanisms. The change in concentration balance of nucleotide substrates for the viral polymerase may cause the substitution of GTP with other substrates and accumulation of lethal mutations in the viral genome (mechanism 6 in Fig. 3).

Another possible mechanism of action is the direct inhibition of viral polymerases by the "wrong" substrate, ribavirin 5'-*O*-triphosphate (RTP) (5), similar to how azidothymidine terminates elongation of HIV DNA by reverse transcriptase (mechanism 5 in Fig. 3). This mechanism was proved by *in vitro* experiments for hepatitis C virus [37] and influenza virus [38, 39]. There is proof that RTP may be the substrate/inhibitor for other viral fragments [40, 41]. RTP is the substrate for capping enzymes (for example, the D1 of the vaccinia virus) [42], and is able to form a "wrong" RNA cap, containing RMP residues, not 7-*N*-methylguanosine. This cap inhibits the eukaryotic translation initiation factor eIF4E, and the viral RNA cannot be translated (mechanism 4 in Fig. 3).

It is known that ribavirin monotherapy is effective against the Lassa virus [4, 5, 43], human orthopneumovirus (respiratory syncytial virus) [44], but not against chronic hepatitis C. However, when combined with INF-α, a protein which modulates the immune response, ribavirin is an effective medication against hepatitis C [45]. As it turns out, ribavirin switches the phenotype of T lymphocytes produced by the immune system, from Th2 to Th1, thus changing the type of the cell's immune response (mechanism 1 in Fig. 3) [46, 47]. Moreover, the interferon produced by the immune system when binding to receptors on the surface of a cell attacked by a virus, launches a signaling cascade which leads to the expression of interferon stimulated genes (ISG), putting the cell in a "defensive" position (mechanism 2 in Fig. 3). ISG function is a "hot topic" in current research [48–55], but little is known so far. For example, the ISG_{15} protein in human cells is an important part of the innate immune system and is responsible for antiviral activity [56]. Ribavirin has an effect on the signaling cascade of interferon by modulating ISG expression. It was confirmed by *in vitro* [57–60] and *in vivo* [61–63] experiments, but the structural basis of this effect is unknown. There are suggestions [64] that the decrease in GTP levels due to IMDPH inhibition is related to the disruption of the enzymatic cascade, which regulates nitric oxide (NO) levels in the cell (NO is cytotoxic for T lymphocytes, and Th1 cells are less resistant to it).

The immunomodulating activity of ribavirin was discovered in the mid-2000s, when major research on synthesis of the analogs and structure optimization seemed quite complete. The biological properties of most ribavirin analogs had been tested on cheap available cell lines. This is why it is possible to discover immunomodulating activity for the substances which had already been studied and rejected. The diversity of viral infections for which new analogs had been tested also needs to be taken into account; results are difficult to compare since the targets are so different. A substance active against one virus may often be useless against another.

Ribavirin was created as an antiviral drug and is still used as such today, although the idea of utilizing it as a cytostatic agent is quite old [65]. Today, there is information about 28 trials (10 are complete and 6 are in Phase IV) on clinicaltrials.gov [66], where ribavirin is being tested in treatments of cancers of varying kinds. Ribavirin's mechanisms of antitumor activity are quite similar to the antiviral mechanisms described above: the inhibition of enzymatic cascades related to nucleic acids metabolism [67–69], the violation of translation mechanisms [70, 71], immune response modulation [72].

Of all 1,2,4-triazole nucleosides, including those with antiviral activity, almost none have been studied in order to uncover their mechanisms of action, apart from ribavirin itself and a couple of prospective drugs, e.g. viramidine. None of the suggested mechanisms can fully explain ribavirin's biological activity, or the structure–activity relationship for the drug's analogs and derivatives. All theories collided with facts that did not fit sooner or later; there is a number of unresolved issues with each suggested mechanism. IMPDH inhibition, as mentioned before, only leads to lower GTP levels in the cell, which could explain the antiviral effect by the disruption of viral genome replication. However, the comparison of the spectrum and intensity of antiviral activity of ribavirin and other known IMPDH inhibitors shows that, despite the fact that equal inhibition is achieved, the antiviral effects are rather different [32]. Actual interaction with viral enzymes is usually observed in *in vitro* experiments, where ribavirin concentrations are 10–1000 times higher than clinically relevant [16, 73]. Immunomodulating activity is also characteristic for levovirine, or 1-(β-L-ribofuranosyl)-1,2,4-triazole-3-carboxamide, which is the L-enantiomer of ribavirin [74]. This substance cannot be a substrate for hADK because of the configuration of the glycoside fragment, and is, perhaps, not phosphorylated in the body.

There have been quite a few attempts to understand the relationship between the structures of ribavirin analogs and their metabolism and activity [12, 31, 75–77]. However, obvious correlations have not been found. For example, there was an attempt to explain why there is almost no activity in analogs with pentose glycoside residues, other than D-ribose. It could be due to the fact that such compounds do not interact with the human nucleoside diphosphate kinase (hNDKA) (EC:2.7.4.6), an enzyme which catalyzes the reversible transition of nucleoside diphosphates into triphosphates [23]. In this scenario, the dose-dependent manner of the antiviral activity of ribavirin's acyclic analogs, demonstrated using the *in vitro* adenovirus model [78], could not be explained. The attempt to explain the activity of such compounds by their non-specific hydrolysis, followed by the transition from 1,2,4-triazole-3-carboxamide (**7**) into ribavirin with the help of PNP, does not seem very convincing. This is because hydrolysis in cell models is not very likely, and the rate of glycoside bond rupture in a specialized model system correlates weakly with the observed activity [79]. We can conclude that almost 50 years of research have produced more questions than answers.

The molecule of ribavirin has, roughly speaking, three places where modification is possible: glycoside fragment, carboxamide group and the 5-position of the heterocyclic base. Another possible modification route is the change from the 1,2,4-triazole heterocyclic system to another azole, for example, imidazole or 1,2,3-triazole. All these possibilities were reported in the early days of research on synthesis [80, 81], but they did not produce a lot of interesting results. Ribavirin itself has remained the best one amongst its structural analogs, in terms of activity and spectrum of action. Even small changes in the molecule would lead to either complete loss of activity or significant decrease in efficacy and increase in selectivity. For example, 2'-deoxyribavirin is inactive [79]; 5-methylribavirin is inactive as well, according to some data [82]. However, other research suggests that it inhibits reproduction of certain viruses *in vitro*, i.e. the Tahyna virus, the Dhori virus and the vaccinia virus [83], but the effect is much lower than that of ribavirin. The substitution of oxygen with sulfur in the carboxamide group results in the narrower spectrum of action; 1-(β-D-ribofuranosyl)-1,2,4-triazole-3-thiocarboxamide inhibits herpes simplex virus *in vitro*, with 80% efficacy compared to ribavirin, but it is inactive to other viruses that were tested: adenovirus, parainfluenza virus and rhinovirus [84]. Many compounds related to ribavirin but with a different heterocyclic system, such as 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (AICAR, acadesine) and 5-hydroxy-1-β-D-ribofuranosylimidazole-4-carboxamide (mizoribine), also have pharmacological prospects, but their biological activity is different [85]. So is there any need to search further when there seems to be no higher peak to reach?

2. New analogs of ribavirin: synthesis and biological properties

2.1. Isosteric analogs for carboxamide group

The only "old" compound which has prospects for use is viramidine **2**, where the carboxamide is replaced with the isosteric amidine group. This molecule's metabolism is different from that of ribavirin [86–88]; it is phosphorylated much slower, and active metabolites do not accumulate in erythrocytes. Viramidine is transformed into ribavirin by ADA in liver cells, where its antiviral activity is supposed to manifest. Moreover, viramidine inhibits PNP [89], blocking one of the pathways of ribavirin's catabolism [90]. Various substituents, such as alkyl groups and amino acid residues (Fig. 4), have been introduced into the amidine group of viramidine [91]. Some of these substances exhibit significant antiviral activity *in vitro* (Table 1).

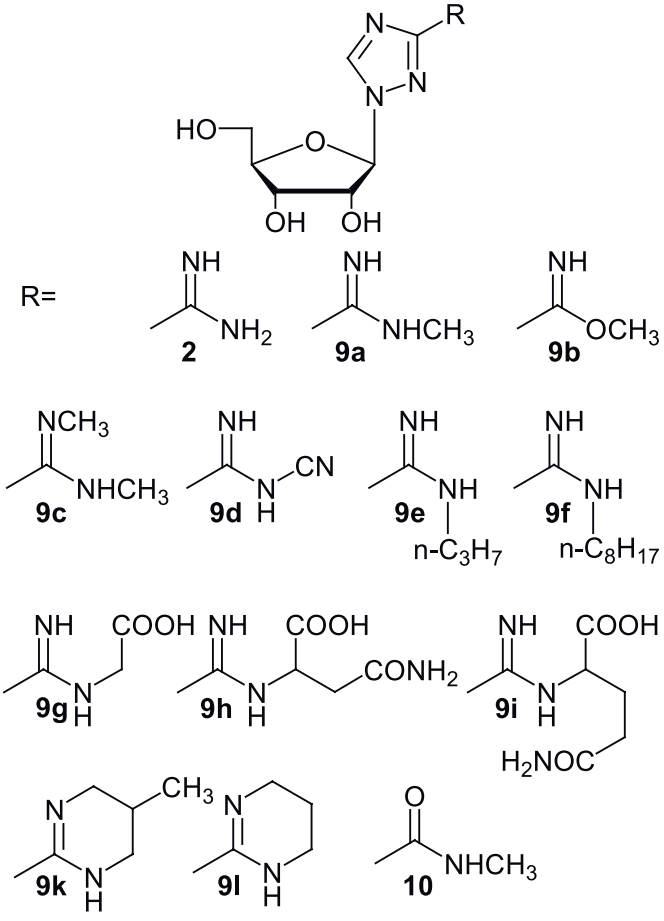

Fig. 4. Viramidine analogs with different substituents [91].

Substituents alter the antiviral activity quite a lot. At the same time, it is hard to explain it by metabolic transition into ribavirin – by deamination or hydrolysis of the amidine group. Compound **9a** is very active, but its hydrolysis product **10** is not. We can see that activity and toxicity decrease with the growing size of the substituent, although this rule has exceptions. Molecule **9e** (homolog of **9a**) is more active and toxic than **9a**, while having a longer alkyl chain, but the spectrum of activity is smaller.

Another isosteric analog of ribavirin is ETAR, 1-(β-D-ribofuranosyl)-3-ethynyl-1,2,4-triazole (**11**) (Fig. 5) [92], in which the carboxamide is replaced with the ethynyl group. The molecule exhibits strong inhibitory properties, better than ribavirin, towards a number of flaviviruses, for example Dengue virus, in cell models [93]. The research suggests that its mechanism of action involves IMPDH inhibition. However, the prospects of this drug are low due to its high toxicity.

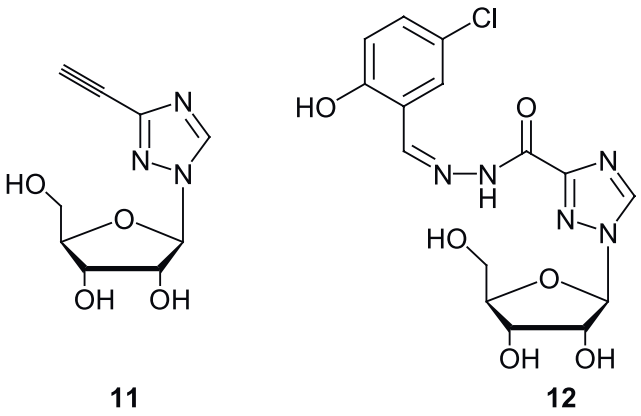

Fig. 5. Active isosteric analogs of ribavirin.

Table 1. Antiviral activity and cytotoxicity of viramidine and its analogs with substituents, measured *in vitro* using Vero cell line

IC_{50}, μg/ml	Compound												
	2	**9a**	**9b**	**9c**	**9d**	**9e**	**9f**	**9g**	**9h**	**9i**	**9k**	**9l**	**10**
SFSV	36	104	98	94	*	73	339	566	484	547	*	*	*
PTV	83	250	201	181	*	41	*	**	1600	2690	*	*	*
DGV4	100	n	76	162	250	n	n	n	n	n	n	n	n
RSV	16	24	n	n	n	n	n	n	n	n	n	n	n
VV	59	198	n	184	n	n	n	n	n	n	n	n	n
IFAV	48	n	63	n	n	n	n	n	n	n	n	n	n
IFBV	48	n	n	n	n	n	n	n	n	n	n	n	n
PIFV3	n	125	n	n	n	n	n	n	n	n	n	n	n
CC_{50}, μg/ml	250–1000	>1000	250–840	250–1000	250–1000	660–1000	>1000	1000–3200	>3200	>3200	>1000	>3200	>320

SFSV – Sandfly fever Sicilian virus; PTV – Punta Toro virus; DGV4 – Dengue 4 virus; RSV – human orthopneumovirus (respiratory syncytial virus); VV – vaccinia virus; IFAV – influenza A virus; IFBV – influenza B virus; PIFV3 – parainfluenza virus type 3.

* inactive; ** inhibition does not reach 50%; n – not tested.

Viramidine inhibits PNP, an enzyme which is targeted in chemotherapy of diseases like cancer [94]. The work of Liu et al. [95] investigates cytotoxic properties of ribavirin analogs, where the carboxamide group is replaced with a hydrazone group. One of the suggested compounds, (*Z*)-*N*'-(1-(5-chloro-2-hydroxyphenyl)ethylidene)-1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carbohydrazide (**12**), inhibits lung cancer (line A549) growth at the concentration of 20 μM.

The research focusing on hADK specificity for ribavirin analogs, where the carboxamide is replaced with an isostere, and potentially related antiviral activity, has not shown a clear correlation between the kinase's ability to phosphorylate a molecule and the activity of the latter [77]. For example, hADK activity towards compounds **9a** and **9b** is 5% and less than 2%, respectively, compared to the enzyme's activity towards ribavirin. At the same time, the inactive methyl amide **10** is phosphorylated only 10 times slower than ribavirin. The patent application [96], where some ribavirin isosteres for the carboxamide group are described as "inhibitors of viral polymerases", has not been approved, and no data on the activity of those compounds were shown in this patent application or elsewhere.

There are alternative phosphorylation pathways which are not dependent on hADK, as well as other types of pharmacological activity. In the studies on

the substrate specificity of the genetically engineered PNP from *E. coli* [97, 98], a number of ribavirin analogs were obtained, with the *N*-alkylated amide group. As it turned out, even bulky substituents in the carboxamide fragment did not affect transglycosylation, which is catalyzed by PNP (Fig. 6).

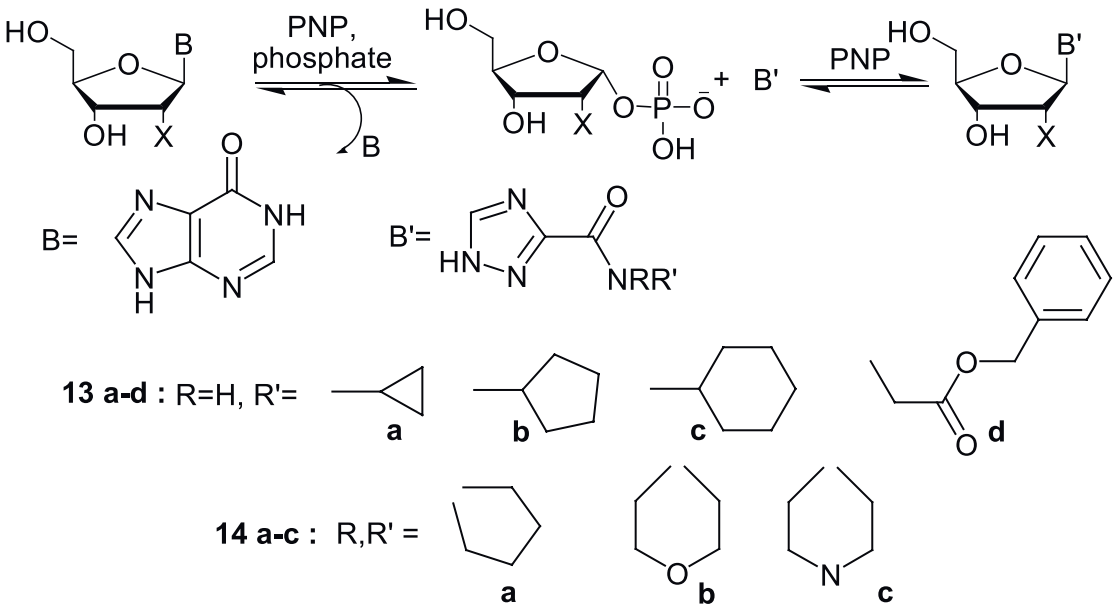

Fig. 6. Synthesis of *N*-alkylamide analogs of ribavirin by chemo-enzymatic transglycosylation.

Alkylamide analogs of ribavirin, **13b** and **13c**, have shown a relatively high *in vitro* activity against influenza A virus and herpes simplex virus (an RNA and a DNA virus, respectively) in different cell lines. Interestingly, according to the existing concepts [99], metabolism of such compounds should not lead to ribavirin, but rather to the inactive 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxylic acid. It is impossible to explain the observed activity by a metabolic transformation into an active compound, as in the case of molecules **9a** and **10**. It's important to note that, when ribavirin in all its roles (maybe apart from that of the immunomodulating molecule) imitates natural purine nucleosides and participates in their enzymatic cascades, the analogs **13b–c** can hardly partake in the majority of these processes, because of steric factors. This indicates the existence of yet unknown mechanisms of antiviral activity for compounds of this type.

The research paper [100] suggests using another approach to modify the carboxamide group (Fig. 7). The 3-position in the designed molecules contains 1,2,4-oxadiazole instead of the carboxamide, the former being the heterocyclic bioisostere of the latter.

The 3-(1,2,4-triazolyl)-1,2,4-oxadiazoles, as it was discovered, are perfect substrates for PNP; that is why nucleosides **14a–d** can be synthesized by ribavirin

[1]Galegov G.A., Deryabin P.G., Andronova V.L. (in press).

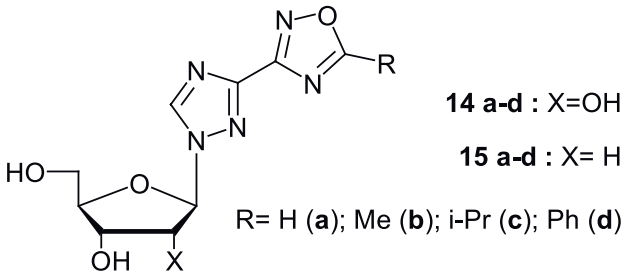

Fig. 7. 3-Oxadiazole analogs of ribavirin.

modification, as well as by chemo-enzymatic methods from natural nucleoside substrates, that allows to produce 2'-deoxy analogs **15a–d**. The most active compound (against hepatitis C virus *in vitro*), amongst the isosteres **14** and **15**, is molecule **14d** (IC_{50} = 8.8 μg/ml; 12.5 μg/ml for ribavirin). At the same time, its toxicity for the tested cell line is much lower than ribavirin's. Substances **14a** and **14b** also exhibit some activity against herpes simplex virus and influenza A virus, respectively. Compounds of this type cannot be metabolized into ribavirin, and the structure of the most active molecule **14d** contains a bulky aromatic substituent, that sterically prevents it from participating in processes such as replication.

2.2. Ribavirin analogs with substitutions at the 5-position of the heterocycle

Of the publications within the past 10 years dedicated to structural analogs of ribavirin, the majority focus on derivatives of 1,2,4-triazole-3-carboxylic acids, with substituents at the 5-position. Most of these works, especially aspects of synthesis, are carefully summarized in the review by Xia et al. [101]. A significant number of the described compounds contain D-ribose or an acyclic methoxyethanol residue as the glycoside fragment, and the substituent in the 3- or 5-position of the triazole cycle (depending on the glycoside position) contains an aryl fragment, attached to the triazole by spacers of various nature (Fig. 8). The structural similarity, to a great extent, is related to the methodology of these research papers based on a serial modification of the sole nucleoside precursor by one or two chemical reactions (most commonly, Pd-catalyzed cross-coupling).

Compounds containing a substituted 1,2,3-triazole in the 5-position, **16–24** (Fig. 9), have been found effective against the tobacco mosaic virus (TMV) [102–104]. The experiments involved the "half-leaf juice rubbing" model, wherein 50% of the surface of a tobacco plant leaf were treated with the tested substance, and then the plant was infected with TMV. The comparison of the affected areas

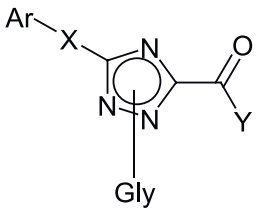

Fig. 8. The general structure of ribavirin analogs substituted at the 5-position. X – spacer group (ethynyl, vinyl, 1,2,3-triazolyl, NH, S); Y – NH_2, OEt; Gly – glycoside fragment (ribose, methoxyethanol, H).

on treated and untreated surfaces showed the level of antiviral activity. The antiviral effect is exhibited not only by nucleosides or acyclic nucleoside analogs, but also by their protected derivatives and by heterocyclic bases themselves (which have the highest activity). In this case, it is hard to suggest a mechanism that would include synthesis of a nucleoside from the base, with the help of PNP, because such a bulky substituent in the 5-position limits the substrate specificity of the enzyme [97, 105]. We can speculate that antiviral activity is determined by the heterocyclic base and the glycoside fragment has a transport function.

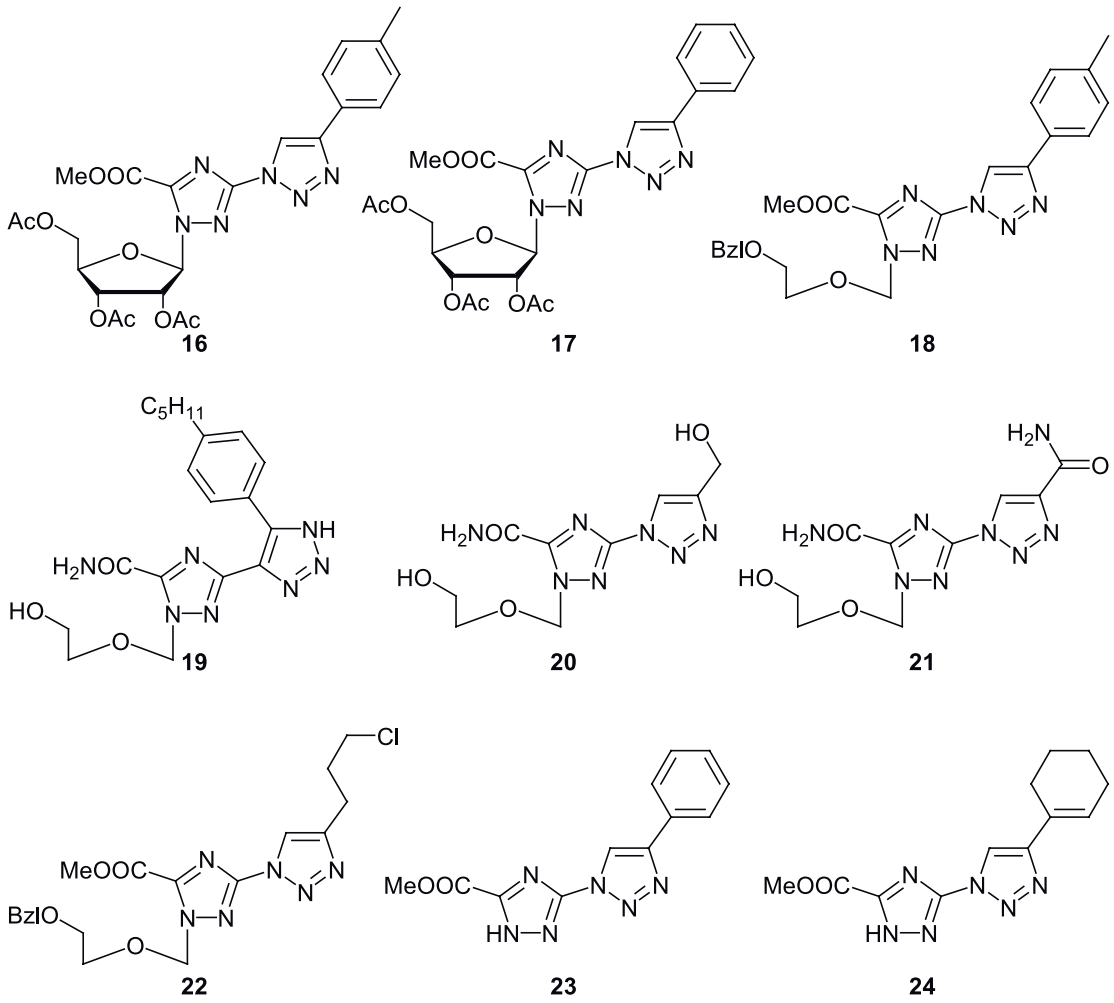

Fig. 9. Ribavirin analogs active against TMV.

Compounds **25–28** (Fig. 10) with an ethynyl spacer are quite active against hepatitis C virus (HCV) (Table 2) in cell models which contain the viral replicon [106–108].

In this case, there is a similar and consistent pattern: the carbohydrate fragment seems not to be too critical for activity, although it has influence on the toxicity of the compound. The authors of the study have analyzed the structure–activity relationship (SAR) by synthesizing a number of compounds and demonstrating that the important part is the rigid spacer group in the 5-position of the 1,2,4-triazole ring, as well as the position and type of the substituent in the arylethynyl fragment. When the triple bond in compound **28** is replaced with a single bond, activity is lost. This indicates the importance of the molecule's geometry and/or the presence of π-conjugation between aromatic fragments in the base, for the substance to be active against HCV. Derivatives with a substituent in the 4-position of the aryl ring are active, and the antiviral activity increases with the growth of lipophilicity and the substituent size. However, it is difficult to suggest a mechanism in this case. One of the most active compounds is not a nucleoside, but a protected derivative **25**, which is unable to participate in metabolic cascades. Also, it is the only active derivative without an aryl ring.

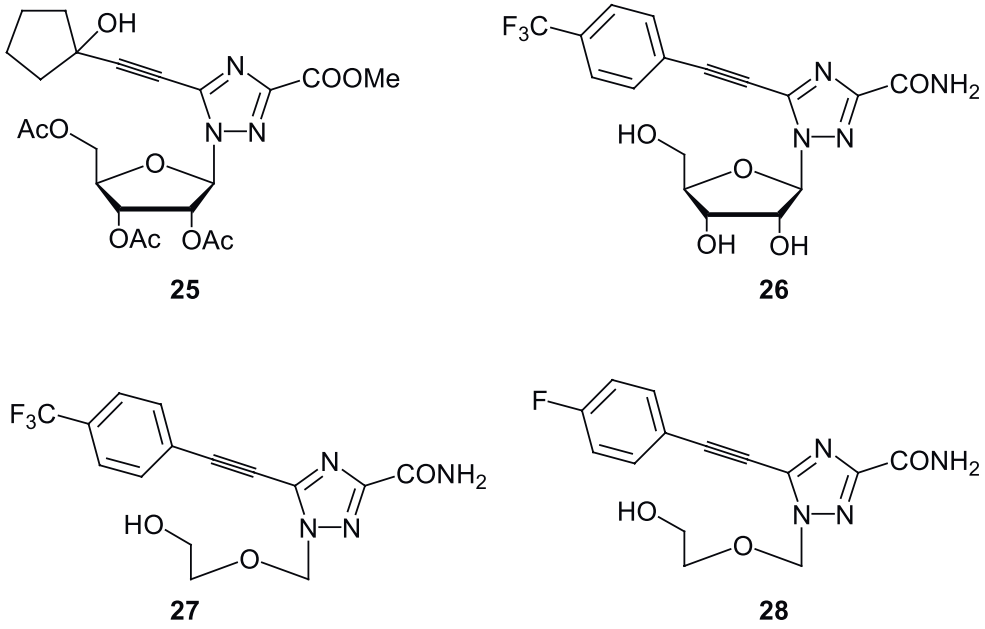

Fig. 10. Ribavirin analogs active against HCV.

Table 2. Activity of arylethynyl analogs of ribavirin against HCV virus *in vitro*

Cell line / Compound	Huh-5-2		Huh-9-13		Huh-6	
	EC_{50}	CC_{50}	EC_{50}	CC_{50}	EC_{50}	CC_{50}
25	52.3±3.1	>105	54.3±18	>105	25.1±4.8	>105
26	17.7±1.9	82.5±9.7	19.4±7.0	>120	43.7±20	>120
27	14.1±3.7	56.5±14	36.7±23	79.0±19	50.8±21	87.5±14
28	72.3±9.9	>160	125±6.6	>160	95.3±3.3	132±18
Ribavirin	28.7±8.2	86.0±45	84.0±15	229	33.0	>100

EC_{50} (μM) – concentration at which inhibition occurs with 50% efficiency; inhibition of replication of subgenome replicon of HCV in a respective cell line;
CC_{50} (μM) – concentration at which 50% cells die.

Nucleoside analogs with simple alkyl substituents in the 5-position of the triazole cycle are almost inactive against hepatitis C virus in cell models *in vitro* [105]. Analogs of compound **26** (Fig. 11) that have a double bond are active in the *trans* configuration (EC_{50} = 9 μM, CC_{50} > 30 μM) and inactive in the *cis* configuration [109].

For ribavirin analogs substituted at the 5-position, antitumor activity is observed for compounds **30–37**, which are structurally similar to the anti-HCV and anti-TMV substances described earlier; although there are compounds of other types in the series (Fig. 12) [108, 110–115].

Fig. 11. Active (**29a**) and inactive (**29b**) vinyl analogs of compound **26**.

Xia et al. [111, 113, 116] have shown that the anticancer activity of 5-arylethynyl analogs is due to the induced caspase-dependent apoptosis. The antitumor effect was demonstrated using a cell line of pancreatic cancer, MiaPaCa-2, which is drug resistant. Apoptosis is launched since the expression of the heat shock protein Hsp27 is suppressed because of these nucleoside compounds. Other mechanisms have been suggested as well: the inhibition of androgen receptors [116] and immunomodulating activity of derivatives with a 1,2,3-triazole spacer [114].

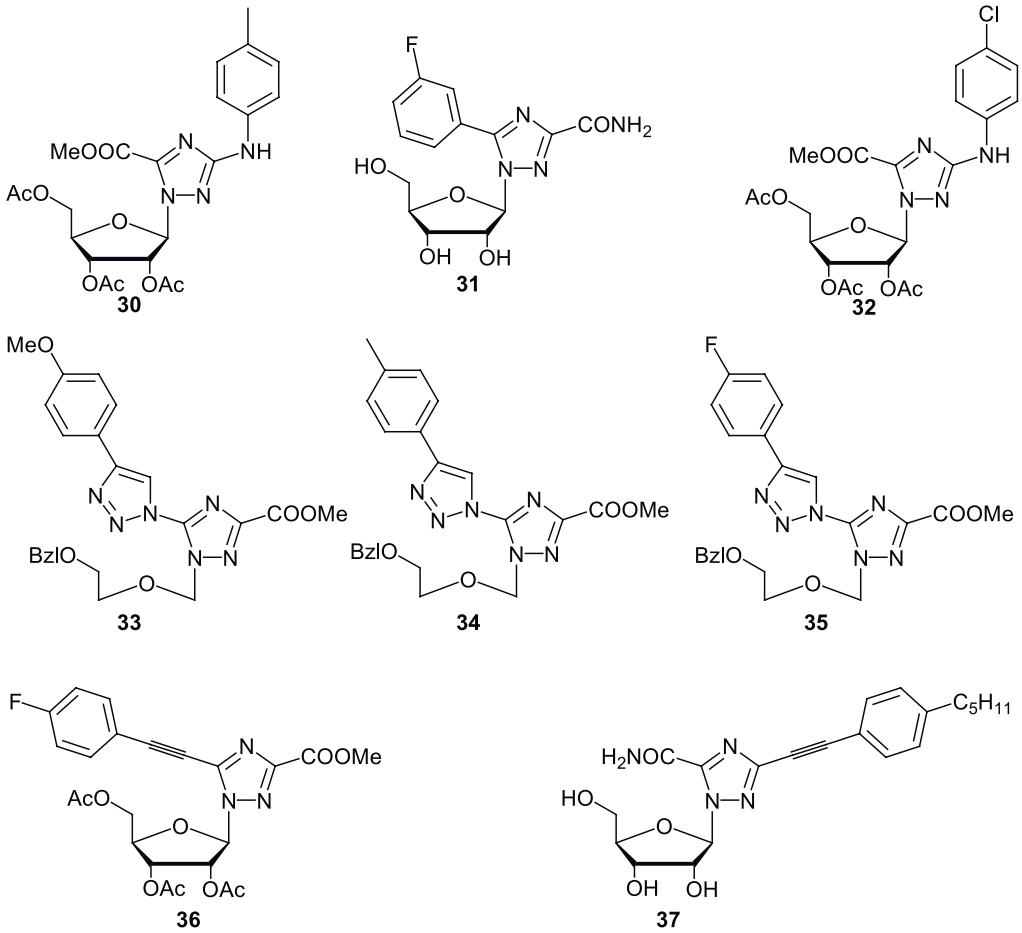

Fig. 12. Ribavirin analogs with a substituent at the 5-position that have antitumor activity.

Interestingly, the SAR parameters obtained for some analogs of compound **37** show almost the same consistent pattern as the anti-HCV derivatives do. A rigid spacer group and a lipophilic substituent in the 4-position of the aromatic ring are needed. At the same time, the roles of the glycoside fragment and the carboxamide group in the 3-position are unclear. Computer modeling of the structures of the active compounds and X-ray crystallography demonstrate the coplanarity of the connected aromatic fragments in the base. Researchers are of the opinion that this structural motif imitates planar conjugated aromatic systems in natural purine bases and is required for the activity of the compound [115].

Conclusions

The majority of the publications summarized here focus on biological properties of large libraries of target compounds. Some conclusions can be made regarding the structure–activity relationship (SAR) for ribavirin

analogs of the last generation, compounds **16–37**, which contain bulky substituents in the 5- (or 3-) position of the heterocyclic base. The first and most important observation is that these substances, while having structures quite different from the parent molecule, cannot have the same mechanisms of biological activity as ribavirin itself. This is because they do not imitate the structure of a purine base and cannot be substrates for the enzymes of the purine metabolic cascade. Inhibition of IMPDH or viral polymerases by these compounds is also not very likely, due to the high substrate specificity of these enzymes; at least, the possible mechanism of such inhibition would be very different from ribavirin's. The isosteres of ribavirin **13**–**15**, on the contrary, may be supposed to imitate the purine structure, and their possible mechanisms of action may be the same as for ribavirin. Our second conclusion is that compounds **16–37**, which exhibit various types of activity, are very similar in structure, and the presence of the glycoside fragment (that makes the molecule a nucleoside analog) is not required. It is possible that the structural basis for mechanisms of activity is not connected to the nucleoside structure of the molecule, but only to the heterocyclic base, 1,2,4-triazole, which is attached to an aromatic lipophilic fragment by a rigid spacer group. This idea is supported by multiple facts: the abovementioned, high anti-TMV activity of bitriazole bases **23** and **24**; the varying activity of the analogs, with substituents at the N1 and N2 atoms of the triazole and acyclic derivatives; the high activity of many protected precursors. However, there is no direct proof of this hypothesis, because antiviral and anticancer activity of aglycones has not been studied.

In line with these conclusions, a significant antiviral activity of compound AMP-006, 5-(tetrahydrofuran-2-yl)-1,2,4-triazole-3-carboxamide (**38**) (Fig. 13) has been reported [118]. This molecule, while being a close structural analog of ribavirin, is not at all a nucleoside. It has no hydroxy groups and it is not hydrolyzed by enzymes, but the molecule has very low cytotoxicity, and is active *in vitro* against the influenza A virus and the herpes virus, being just a little bit less effective than ribavirin. Nucleoside **39**, which is obtained from it synthetically, does not have antiviral activity[2].

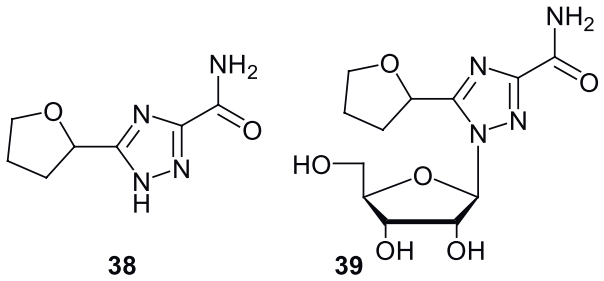

Fig. 13. AMP-006 (**38**) and its inactive nucleoside derivative **39**.

Given the fact that mechanisms of ribavirin's activity are not known in detail, the role of this drug (and its new structural analogs) in the fight against viral diseases and tumors is still very promising. It seems that in near future these molecules will still be playing an important part.

The author declares no conflict of interest.

References:

1. Sidwell R.W., Huffman J.H., Khare L.G P., Allen B., Witkowski R.J.T., Robins K. Broad-spectrum antiviral activity of virazole: 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide. *Science.* 1972;177(4050):705-706. https://doi.org/10.1126/science.177.4050.705

2. Oxford J.S. Inhibition of the replication of influenza A and B viruses by a nucleoside analogue (ribavirin). *J. Gen. Virol.* 1975;28(3):409-14. https://doi.org/10.1099/0022-1317-28-3-409

3. Hruska J.F., Bernstein J.M., Douglas R.G. Jr., Hall C.B. Effects of ribavirin on respiratory syncytial virus *in vitro*. *Antimicrob. Agents Chemother.* 1980;17(5):770-775. https://doi.org/10.1128/AAC.17.5.770

4. McCormick J.B., King I.J., Webb P.A., Scribner C.L., Craven R.B., Johnson K.M., Elliott L.H., Belmont-Williams R. Lassa fever. Effective therapy with ribavirin. *N. Engl. J. Med.* 1986;314(1):20-26. https://doi.org/10.1056/NEJM198601023140104

5. Huggins J.W. Prospects for treatment of viral hemorrhagic fevers with ribavirin, a broad-spectrum antiviral drug. *Rev. Infect. Dis.* 1989;11(Suppl_4):S750-S761. https://doi.org/10.1093/clinids/11.Supplement_4.S750

6. Shigeta S., Mori S., Baba M., Ito M., Honzumi K., Nakamura K., Oshitani H., Numazaki Y., Matsuda A., Obara T. Antiviral activities of ribavirin, 5-ethynyl-1-β-D-ribofuranosyl-imidazole-4-carboxamide, and 6'-(R)-6'-C-methylneplanocin A against several ortho- and paramyxoviruses. *Antimicrob. Agents Chemother.* 1992;36(2):435-439. https://doi.org/10.1128/AAC.36.2.435

7. Jordan I., Briese T., Fischer N., Lau J.Y., Lipkin W.I. Ribavirin inhibits West Nile virus replication and cytopathic effect in neural cells. *J. Infect. Dis.* 2000;182(4):1214-1217. https://doi.org/10.1086/315847

8. Kim Y., Lee C. Ribavirin efficiently suppresses porcine nidovirus replication. *Virus Res.* 2013;171(1):44-53. https://doi.org/10.1016/j.virusres.2012.10.018

9. Kihira S., Uematsu J., Kawano M., Itoh A., Ookohchi A., Satoh S., Maeda Y., Sakai K., Yamamoto H., Tsurudome M., O'Brien M., Komada H. Ribavirin inhibits human parainfluenza virus type 2 replication *in vitro*. *Microbiology and Immunology.* 2014;58(11):628-635. https://doi.org/10.1111/1348-0421.12192

10. Ramirez-Olivencia G., Estebanez M., Membrillo F.J.,

[2]Prutkov A.N., Chudinov M.V., Galegov G.A., Deryabin P.G., Andronova V.L. (in press).

Ybarra M.D.C. Use of ribavirin in viruses other than hepatitis C. A review of the evidence. *Enferm. Infect. Microbiol. Clin.* 2018. (in press). https://doi.org/10.1016/j.eimc.2018.05.008

11. Streeter D.G., Witkowski J.T., Khare G.P., Sidwell R.W., Bauer R.J., Robins R.K., Simon L.N. Mechanism of action of 1-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (Virazole), a new broad-spectrum antiviral agent. *Proc. Natl. Acad. Sci. USA.* 1973;70(4):1174-1178. https://doi.org/10.1073/pnas.70.4.1174

12. Miller J.P., Kigwana L.J., Streeter D.G., Robins R.K., Simon L.N., Roboz J. The relationship between the metabolism of ribavirin and its proposed mechanism of action. *Ann. N. Y. Acad. Sci.* 1977;284(1):211-229. https://doi.org/10.1111/j.1749-6632.1977.tb21953.x

13. Crotty S., Cameron C., Andino R. Ribavirin's antiviral mechanism of action: Lethal mutagenesis? *J. Mol. Med.* (Berl.). 2002;80(2):86-95. https://doi.org/10.1007/s00109-001-0308-0

14. Hong Z., Cameron C.E. Pleiotropic mechanisms of ribavirin antiviral activities. *Progr. Drug Res.* Basel: Birkhäuser Basel. 2002;59:41-69. https://doi.org/10.1007/978-3-0348-8171-5_2

15. Parker W.B. Metabolism and antiviral activity of ribavirin. *Virus Res.* 2005;107(2):165-171. https://doi.org/10.1016/j.virusres.2004.11.006

16. Dixit N.M., Perelson A.S. The metabolism, pharmacokinetics and mechanisms of antiviral activity of ribavirin against hepatitis C virus. *Cell. Mol. Life Sci.* 2006;63(7-8):832-842. https://doi.org/10.1007/s00018-005-5455-y

17. Graci J.D., Cameron C.E. Mechanisms of action of ribavirin against distinct viruses. *Rev. Med. Virol.* 2006;16(1):37-48. https://doi.org/10.1002/rmv.483

18. Te H.S., Randall G., Jensen D.M. Mechanism of action of ribavirin in the treatment of chronic hepatitis C. *Gastroenterol. Hepatol.* (N. Y.). 2007;3(3):218-225. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3099343

19. Chung R.T., Gale M.Jr., Polyak S.J., Lemon S.M., Liang T.J., Hoofnagle J.H. Mechanisms of action of interferon and ribavirin in chronic hepatitis C: Summary of a workshop. *Hepatology* (Baltimore, Md.). 2008;47(1):306-320. https://doi.org/10.1002/hep.22070

20. Shiffman M.L. What future for ribavirin? *Liver Int.* 2009;29(Suppl 1):68-73. https://doi.org/10.1111/j.1478-3231.2008.01936.x

21. Paeshuyse J., Dallmeier K., Neyts J. Ribavirin for the treatment of chronic hepatitis C virus infection: A review of the proposed mechanisms of action. *Curr. Opin. Virol.* 2011;1(6):590-598. https://doi.org/10.1016/j.coviro.2011.10.030

22. Wu J.Z., Larson G., Walker H., Shim J. H., Hong Z. Phosphorylation of ribavirin and viramidine by adenosine kinase and cytosolic 5'-nucleotidase II: Implications for ribavirin metabolism in erythrocytes. *Antimicrob. Agents Chemother.* 2005;49(6):2164-2171. https://doi.org/10.1128/AAC.49.6.2164-2171.2005

23. Gallois-Montbrun S., Chen Y., Dutartre H., Sophys M., Morera S., Guerreiro C., Schneider B., Mulard L., Janin J., Veron M., Deville-Bonne D., Canard B. Structural analysis of the activation of ribavirin analogs by NDP kinase: Comparison with other ribavirin targets. *Mol. Pharmacol.* 2003;63(3):538-546. https://doi.org/10.1124/mol.63.3.538

24. Russmann S., Grattagliano I., Portincasa P., Palmieri V., Palasciano G. Ribavirin-induced anemia: Mechanisms, risk factors and related targets for future research. *Cur. Med. Chem.* 2006;13(27):3351-3357. https://doi.org/10.2174/092986706778773059

25. Nystrom K., Pettersson G., Wanrooij P.H., Brunet S., Said J., Ortolani G., Waldenstrom J., Adamek L., Tang K.W., Norberg P., Chabes A., Hellstrand K., Norder H., Lagging M. Inosine triphosphate pyrophosphatase enhances the effect of ribavirin on hepatitis C virus cell culture infection. *J. Hepatol.* 2017;66(1):321. http://dx.doi.org/10.1016/S0168-8278(17)30965-0

26. Nystrom K., Wanrooij P. H., Waldenstrom J., Adamek L., Brunet S., Said J., Nilsson S., Wind-Rotolo M., Hellstrand K., Norder H., Tang K.W., Lagging M. Inosine triphosphate pyrophosphatase dephosphorylates ribavirin triphosphate and reduced enzymatic activity potentiates mutagenesis in hepatitis C virus. *J. Virol.* 2018; 92(19):e01087-18. https://doi.org/10.1128/JVI.01087-18

27. Furihata T., Kishida S., Sugiura H., Kamiichi A., Iikura M., Chiba K. Functional analysis of purine nucleoside phosphorylase as a key enzyme in ribavirin metabolism. *Drug Metabolism and Pharmacokinetics.* 2014;29(2):211-214. https://doi.org/10.2133/dmpk.DMPK-13-NT-065

28. Page T., Connor J.D. The metabolism of ribavirin in erythrocytes and nucleated cells. *Int. J. Biochem.* 1990;22(4):379-383. https://doi.org/10.1016/0020-711X(90)90140-X

29. Wu J.Z., Walker H., Lau J.Y.N., Hong Z. Activation and deactivation of a broad-spectrum antiviral drug by a single enzyme: Adenosine deaminase catalyzes two consecutive deamination reactions. *Antimicrob. Agents Chemother.* 2003;47(1):426-431. https://doi.org/10.1128/AAC.47.1.426-431.2003

30. Martin P., Jensen D.M. Ribavirin in the treatment of chronic hepatitis C. *J. Gastroenterol. Hepatol.* 2008;23(6):844-855. https://doi.org/10.1111/j.1440-1746.2008.05398.x

31. Drabikowska A.K., Dudycz L., Shugar D. Studies on the mechanism of antiviral action of 1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (ribavirin). *J. Med. Chem.* 1979;22(6):653-657. https://doi.org/10.1021/jm00192a009

32. Nair V., Shu Q. Inosine monophosphate dehydrogenase as a probe in antiviral drug discovery. *Antivir. Chem. & Chemother.* 2007;18(5):245-258. https://doi.org/10.1177%2F095632020701800501

33. Wray S.K., Gilbert B.E., Noall M.W., Knight V. Mode of action of ribavirin: Effect of nucleotide pool alterations on influenza virus ribonucleoprotein synthesis. *Antiviral Res.* 1985; 5(1):29-37. https://doi.org/10.1016/0166-3542(85)90012-9

34. Crotty S., Maag D., Arnold J.J., Zhong W., Lau J.Y., Hong Z., Andino R., Cameron C.E. The broad-spectrum antiviral ribonucleoside ribavirin is an RNA virus mutagen. *Nat. Med.* 2000; 6(12):1375-1379. https://doi.org/10.1038/82191

35. Lanford R.E., Chavez D., Guerra B., Lau J.Y., Hong Z., Brasky K.M., Beames B. Ribavirin induces error-prone replication of GB virus B in primary tamarin hepatocytes. *J. Virol.* 2001;75(17):8074-8081. https://doi.org/10.1128/jvi.75.17.8074-8081.2001

36. Olschlager S., Neyts J., Gunther S. Depletion of GTP pool is not the predominant mechanism by which ribavirin exerts its antiviral effect on Lassa virus. *Antiviral Res.* 2011;91(2):89-93. https://doi.org/10.1016/j.antiviral.2011.05.006

37. Vo N.V., Young K.C., Lai M.M. Mutagenic and inhibitory effects of ribavirin on hepatitis C virus RNA polymerase. *Biochemistry.* 2003;42(35):10462-10471. https://doi.org/10.1021/bi0344681

38. Wray S.K., Gilbert B.E., Knight V. Effect of ribavirin triphosphate on primer generation and elongation during

influenza virus transcription *in vitro*. *Antiviral Res.* 1985;5(1):39-48. https://doi.org/10.1016/0166-3542(85)90013-0

39. Eriksson B., Helgstrand E., Johansson N.G., Larsson A., Misiorny A., Noren J.O., Philipson L., Stenberg K., Stening G., Stridh S., Oberg B. Inhibition of influenza virus ribonucleic acid polymerase by ribavirin triphosphate. *Antimicrob. Agents Chemother.* 1977;11(6):946-951. https://doi.org/10.1128/aac.11.6.946

40. Heck J.A., Lam A.M.I., Narayanan N., Frick D.N. Effects of mutagenic and chain-terminating nucleotide analogs on enzymes isolated from hepatitis C virus strains of various genotypes. *Antimicrob. Agents Chemother.* 2008;52(6):1901-1911. https://dx.doi.org/10.1128%2FAAC.01496-07

41. Benarroch D., Egloff M.P., Mulard L., Guerreiro C., Romette J.L., Canard B. A structural basis for the inhibition of the NS5 Dengue virus mRNA 2'-O-methyltransferase domain by ribavirin 5'-triphosphate. *J. Biol. Chem.* 2004;279(34):35638-35643. https://doi.org/10.1074/jbc.M400460200

42. Goswami B.B., Borek E., Sharma O.K., Fujitaki J., Smith R.A. The broad spectrum antiviral agent ribavirin inhibits capping of mRNA. *Biochem. Biophys. Res. Commun.* 1979;89(3):830-836. https://doi.org/10.1016/0006-291X(79)91853-9

43. Carrillo-Bustamante P., Nguyen T.H.T., Oestereich L., Günther S., Guedj J., Graw F. Determining Ribavirin's mechanism of action against Lassa virus infection. *Scientific Reports.* 2017;7(1):11693. https://doi.org/10.1038/s41598-017-10198-0

44. Hall C., Walsh E.E., Hruska J.F., Betts R.F., Hall W.J. Ribavirin treatment of experimental respiratory syncytial viral infection: A controlled double-blind study in young adults. *JAMA.* 1983;249(19):2666-2670. https://doi.org/10.1001/jama.1983.03330430042027

45. Reichard O., Schvarcz R., Weiland O. Therapy of hepatitis C: Alpha interferon and ribavirin. *Hepatology.* 2003;26(S3):108S-111S. https://doi.org/10.1002/hep.510260719

46. Hultgren C., Milich D.R., Weiland O., Sallberg M. The antiviral compound ribavirin modulates the T helper (Th) 1/Th2 subset balance in hepatitis B and C virus-specific immune responses. *J. Gen. Virol.* 1998;79(10):2381-2391. https://doi.org/10.1099/0022-1317-79-10-2381

47. Tam R.C., Pai B., Bard J., Lim C., Averett D.R., Phan U.T., Milovanovic T. Ribavirin polarizes human T cell responses towards a Type 1 cytokine profile. *J. Hepatol.* 1999;30(3):376-382. https://doi.org/10.1016/S0168-8278(99)80093-2

48. Metz P., Reuter A., Bender S., Bartenschlager R. Interferon-stimulated genes and their role in controlling hepatitis C virus. *J. Hepatol.* 2013;59(6):1331-1341. https://doi.org/10.1016/j.jhep.2013.07.033

49. Schneider W.M., Chevillotte M.D., Rice C.M. Interferon-stimulated genes: A complex web of host defenses. *Ann. Rev. Immunol.* 2014;32:513-545. https://doi.org/10.1146/annurev-immunol-032713-120231

50. Schoggins J.W. Interferon-stimulated genes: Roles in viral pathogenesis. *Curr. Opin. Virol.* 2014;6:40-46. https://doi.org/10.1016/j.coviro.2014.03.006

51. Sun J., Rajsbaum R., Yi M. Immune and non-immune responses to hepatitis C virus infection. *World J. Gastroenterol.* 2015;21(38):10739-10748. https://doi.org/10.3748/wjg.v21.i38.10739

52. Wong M.T., Chen S.S. Emerging roles of interferon-stimulated genes in the innate immune response to hepatitis C virus infection. *Cell Mol. Immunol.* 2016;13(1):11-35. https://doi.org/10.1038/cmi.2014.127

53. Hayes C.N., Chayama K. Interferon stimulated genes and innate immune activation following infection with hepatitis B and C viruses. *J. Med. Virol.* 2017;89(3):388-396. https://doi.org/10.1002/jmv.24659

54. Niedzwiedzka-Rystwej P., Ratajczak W., Tokarz-Deptula B., Deptula W. Mechanisms of type I interferon action and its role in infections and diseases transmission in mammals. *Acta Biochim. Pol.* 2017;64(2):199-205. https://doi.org/10.18388/abp.2016_1403

55. Wang W., Xu L., Su J., Peppelenbosch M.P., Pan Q. Transcriptional regulation of antiviral interferon-stimulated genes. *Trends. Microbiol.* 2017;25(7):573-584. https://doi.org/10.1016/j.tim.2017.01.001

56. Morales D.J., Lenschow D.J. The antiviral activities of ISG15. *J. Mol. Biol.* 2013;425(24):4995-5008. https://doi.org/10.1016/j.jmb.2013.09.041

57. Thomas E., Feld J.J., Li Q., Hu Z., Fried M.W., Liang T.J. Ribavirin potentiates interferon action by augmenting interferon-stimulated gene induction in hepatitis C virus cell culture models. *Hepatology.* 2011;53(1):32-41. https://doi.org/10.1002/hep.23985

58. Meier V., Burger E., Mihm S., Saile B., Ramadori G. Ribavirin inhibits DNA, RNA, and protein synthesis in PHA-stimulated human peripheral blood mononuclear cells: Possible explanation for therapeutic efficacy in patients with chronic HCV infection. *J. Med. Virol.* 2003;69(1):50-8. https://doi.org/10.1002/jmv.10264

59. Taylor M.W., Grosse W.M., Schaley J.E., Sanda C., Wu X., Chien S.C., Smith F., Wu T.G., Stephens M., Ferris M.W., McClintick J.N., Jerome R.E., Edenberg H.J. Global effect of PEG-IFN-alpha and ribavirin on gene expression in PBMC *in vitro*. *J. Interferon Cytokine Res.* 2004;24(2):107-18. https://doi.org/10.1089/107999004322813354

60. Stevenson N.J., Murphy A.G., Bourke N.M., Keogh C.A., Hegarty J.E., O'Farrelly C. Ribavirin enhances IFN-alpha signalling and MxA expression: A novel immune modulation mechanism during treatment of HCV. *PLoS One.* 2011;6(11):e27866. https://doi.org/10.1371/journal.pone.0027866

61. Feld J.J., Lutchman G.A., Heller T., Hara K., Pfeiffer J.K., Leff R.D., Meek C., Rivera M., Ko M., Koh C., Rotman Y., Ghany M.G., Haynes-Williams V., Neumann A.U., Liang T.J., Hoofnagle J.H. Ribavirin improves early responses to peginterferon through improved interferon signaling. *Gastroenterology.* 2010;139(1):154-162.e4. https://doi.org/10.1053/j.gastro.2010.03.037

62. Conte E., Modica A., Cacopardo B., Messina L., Nigro L., Messina A. Ribavirin up-regulates IL-12 p40 gene expression and restores IL-12 levels in Leishmania-treated PBMCs. *Parasite Immunol.* 2005;27(12):447-51. https://doi.org/10.1111/j.1365-3024.2005.00796.x

63. Tokumoto Y., Hiasa Y., Uesugi K., Watanabe T., Mashiba T., Abe M., Kumagi T., Ikeda Y., Matsuura B., Onji M. Ribavirin regulates hepatitis C virus replication through enhancing interferon-stimulated genes and interleukin 8. *J. Infect. Dis.* 2012;205(7):1121-1130. https://doi.org/10.1093/infdis/jis025

64. Kast R.E. Ribavirin in cancer immunotherapies - Controlling nitric oxide helps generate cytotoxic lymphocyte. *Cancer Biology & Therapy.* 2002;1(6):626-630. https://doi.org/10.4161/cbt.310

65. Müller W.E.G., Maidhof A., Taschner H., Zahn R.K. Virazole (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide); A cytostatic agent. *Biochem. Pharmacol.* 1977;26(11):1071-1075. https://doi.org/10.1016/0006-2952(77)90246-5

66. https://clinicaltrials.gov/ct2/results?term=ribavirin&cond=cancer

67. Petrelli R., Torquati I., Felczak K., Wilson D.J., Cappellacci L. Novel inhibitors of inosine monophosphate dehydrogenase as potential anti-cancer drugs: A patent review (2002-2014). In: Topics in Anti-Cancer Research. V. 3. *Bentham Publ.*, 2014:37-102. https://doi.org/10.2174/978160805090891140301

68. Ochiai Y., Sano E., Okamoto Y., Yoshimura S., Makita K., Yamamuro S., Ohta T., Ogino A., Tadakuma H., Ueda T., Nakayama T., Hara H., Yoshino A., Katayama Y. Efficacy of ribavirin against malignant glioma cell lines: Follow-up study. *Oncol. Rep.* 2018;39(2):537-544. https://doi.org/10.3892/or.2017.6149

69. Pankiewicz K.W., Felczak K. From ribavirin to NAD analogues and back to ribavirin in search for anticancer agents. *Heterocyclic Commun.* 2015;21(5):249-257.

70. Shi F., Len Y., Gong Y., Shi R., Yang X., Naren D., Yan T. Ribavirin inhibits the activity of mTOR/eIF4E, ERK/Mnk1/eIF4E signaling pathway and synergizes with tyrosine kinase inhibitor Imatinib to impair Bcr-Abl mediated proliferation and apoptosis in Ph+ leukemia. *PLoS One.* 2015;10(8):e0136746. https://doi.org/10.1371/journal.pone.0136746

71. De la Cruz-Hernandez E., Medina-Franco J.L., Trujillo J., Chavez-Blanco A., Dominguez-Gomez G., Perez-Cardenas E., Gonzalez-Fierro A., Taja-Chayeb L., Duenas-Gonzalez A. Ribavirin as a tri-targeted antitumor repositioned drug. *Oncol. Rep.* 2015;33(5):2384-2392. https://doi.org/10.3892/or.2015.3816

72. Shelton J., Lu X., Hollenbaugh J.A., Cho J.H., Amblard F., Schinazi R.F. Metabolism, biochemical actions, and chemical synthesis of anticancer nucleosides, nucleotides, and base analogs. *Chem. Rev.* 2016;116(23):14379-14455. https://doi.org/10.1021/acs.chemrev.6b00209

73. Naik G.S., Tyagi M.G. A pharmacological profile of ribavirin and monitoring of its plasma concentration in chronic hepatitis C infection. *J. Clin. Exp. Hepatol.* 2012;2(1):42-54. https://doi.org/10.1016/S0973-6883(12)60090-5

74. Ramasamy K.S., Tam R.C., Bard J., Averett D.R. Monocyclic l-nucleosides with type 1 cytokine-inducing activity. *J. Med. Chem*. 2000;43(5):1019-1028. https://doi.org/10.1021/jm9905514

75. Harris S., Robins R.K. Ribavirin: Structure and Antiviral Activity Relationships. Ribavirin – A Broad Spectrum Antiviral Agent. New York: Academic Press, 1980:1-21.

76. Streeter D.G., Miller J.P., Robins R.K., Simon L.N. The enzymic conversion of 1,2,4-triazole-3-carboxamide to ribavirin-5'-phosphate and its relationship to the proposed mechanism of action. *Ann. N. Y. Acad. Sci.* 1977;284(1):201-210. https://doi.org/10.1111/j.1749-6632.1977.tb21952.x

77. Kumarapperuma S.C., Sun Y., Jeselnik M., Chung K., Parker W.B., Jonsson C.B., Arterburn J.B. Structural effects on the phosphorylation of 3-substituted 1-β-D-ribofuranosyl-1,2,4-triazoles by human adenosine kinase. *Bioorg. Med. Chem. Lett.* 2007;17(11):3203-3207. https://doi.org/10.1016/j.bmcl.2007.03.018

78. Tsilevich T.L., Schaveleva I.L., Nosach N.L., Govtnovataya V.L., Smirnov I.P., Kochetkova S.V., Gottich B.P., Florent'ev V.L. Acyclic ribavirin analogues. Synthesis and antiviral activity. *Bioorganicheskaya Khimiya (Russian Journal of Bioorganic Chemistry).* 1988;14(5):689-693 (in Russ.).

79. Witkowski J.T., Robins R.K. N-Substituted 1,2,4-triazoles : Pat. US 3991078. Appl. 03/18/1974; publ. 11/09/1976. 5 p.

80. Witkowski J.T., Robins R.K. Synthesis and Chemistry of Certain Azole Nucleosides. In: Chemistry and Biology of Nucleosides and Nucleotides. Eds. R.E.Harmon, R.K. Robins, L.B. Townsend. New York: Academic Press, 1978:267-286. https://doi.org/10.1016/B978-0-12-326140-3.50023-1

81. Preobrazhenskaya M.N., Korbukh I.A. The Synthesis and Reactions of Pyrrole, Pyrazole, Triazole, Indole, Indazole, and Benzotriazole Nucleosides and Nucleotides. In: Chemistry of Nucleosides and Nucleotides. Ed. L.B. Townsend. V. 3. New York: Springer US, 1994:1-105. https://doi.org/10.1007/978-1-4757-9667-4_1

82. Naik S.R., Witkowski J.T., Robins R.K. Synthesis of nucleosides of 5-substituted 1,2,4-triazole-3-carboxamides. *J. Het. Chem.* 1974;11(1):57-61. https://doi.org/10.1002/jhet.5570110112

83. Konstantinova I.D., Fateev I.V., Muzyka I.S., Galkina I.V., Butenko A.M., Galegov G.A., Belov A.V., Larichev V.F., Deryabin P.G., Shvets V.I., L'vov D.K., Miroshnikov A.I. A biotechnological method for obtaining of ribavirin 5-methyl substitutes and study on their antiviral activity. *Biotechnology in Russia.* 2008;(4):98-112. Translated from Biotechnologiya, 2008;(4):69-79.

84. Witkowski J.T., Robins R.K., Khare G.P., Sidwell R.W. Synthesis and antiviral activity of 1,2,4-triazole-3-thiocarboxamide and 1,2,4-triazole-3-carboxamidine ribonucleosides. *J. Med. Chem.* 1973;16(8):935-937. https://doi.org/10.1021/jm00266a014

85. Zeidler J., Baraniak D., Ostrowski T. Bioactive nucleoside analogues possessing selected five-membered azaheterocyclic bases. Eur. *J. Med. Chem.* 2015;97:409-418. https://doi.org/10.1016/j.ejmech.2014.11.057

86. Lin C.-C., Lourenco D., Xu G., Yeh L.-T. Disposition and metabolic profiles of [^{14}C]viramidine and [^{14}C]ribavirin in rat and monkey red blood cells and liver. *Antimicrob. Agents Chemother.* 2004;48(5):1872-1875. https://doi.org/10.1128/aac.48.5.1872-1875.2004

87. Lin C.-C., Luu K., Lourenco D., Yeh L.-T. Pharmacokinetics and metabolism of [^{14}C]viramidine in rats and cynomolgus monkeys. *Antimicrob. Agents Chemother.* 2003;47(8):2458-2463. https://dx.doi.org/10.1128%2FAAC.47.8.2458-2463.2003

88. Lin C.C., Philips L., Xu C., Yeh L.T. Pharmacokinetics and safety of viramidine, a prodrug of ribavirin, in healthy volunteers. *J. Clin. Pharmacol.* 2004;44(3):265-275. https://doi.org/10.1177/0091270004262974

89. Sanghvi Y.S., Hanna N.B., Larson S.B., Fujitaki J.M., Willis R.C., Smith R.A., Robins R.K., Revankar G.R. Synthesis and evaluation of 5-amino-1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamidine and certain related nucleosides as inhibitors of purine nucleoside phosphorylase. *J. Med. Chem.* 1988;31(2):330-335. https://doi.org/10.1021/jm00397a010

90. Wu J.Z., Larson G., Hong Z. Dual-action mechanism of viramidine functioning as a prodrug and as a catabolic inhibitor for ribavirin. *Antimicrob. Agents Chemother.* 2004;48(10):4006-4008. https://dx.doi.org/10.1128%2FAAC.48.10.4006-4008.2004

91. Gabrielsen B., Phelan M.J., Barthel-Rosa L., See C., Huggins J.W., Kefauver D.F., Monath T.P., Ussery M.A., Chmurny G.N. Synthesis and antiviral evaluation of *N*-carboxamidine-substituted analogs of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamidine hydrochloride. *J. Med. Chem.* 1992;35(17):3231-3238. https://doi.org/10.1021/jm00095a020

92. Arterburn J., Kumarapperuma S., Jeselnik M., Chung D.-H., Sun Y., Parker W., Chu Y.K., Jonsson C. Design, synthesis and evaluation of 3-ethynyl-azole nucleosides with antiviral

activity against hantaviruses. *Antiviral Res.* 2008;78(2):A33. http://dx.doi.org/10.1016/j.antiviral.2008.01.057

93. McDowell M., Gonzales S.R., Kumarapperuma S. C., Jeselnik M., Arterburn J.B., Hanley K.A. A novel nucleoside analog, 1-β-D-ribofuranosyl-3-ethynyl-[1,2,4]-triazole (ETAR), exhibits efficacy against a broad range of flaviviruses *in vitro*. *Antiviral Res.* 2010;87(1):78-80. https://doi.org/10.1016/j.antiviral.2010.04.007

94. Bzowska A., Kulikowska E., Shugar D. Purine nucleoside phosphorylases: properties, functions, and clinical aspects. *Pharmacology & Therapeutics.* 2000;88(3):349-425. https://doi.org/10.1016/S0163-7258(00)00097-8

95. Liu W.Y., Li H.Y., Zhao B.X., Shin D.S., Lian S., Miao J.Y. Synthesis of novel ribavirin hydrazone derivatives and anti-proliferative activity against A549 lung cancer cells. *Carbohydr. Res.* 2009;344(11):1270-1275. https://doi.org/10.1016/j.carres.2009.05.017

96. Arterburn J.B., Jonsson C.B., Parker W.B. Azole nucleosides and use as inhibitors of RNA and DNA viral polymerases. Int. Pat. Appl. WO2008067002A2. Appl. 09/11/2006; publ. 06/05/2008. 64 p.

97. Konstantinova I.D., Chudinov M.V., Fateev I.V., Matveev A.V., Zhurilo N.I., Shvets V.I., Miroshnikov A.I. Chemoenzymatic method of 1,2,4-triazole nucleoside synthesis: Possibilities and limitations. *Russ. J. Bioorg. Chem.* 2013;39(1):53-71. https://doi.org/10.1134/S1068162013010056

98. Smirnova O.S., Konstantinova I.D., Fateev I.V., Zhurilo N.I., Chudinov M.V., Miroshnikov A.I. Biotechnological process for the preparation of an antiviral drug ribavirin analogues substituted on the amide group. *FEBS J.* 2013; 280 (Suppl):369. https://doi.org/10.1111/febs.12340

99. Goswami A., Van Lanen S.G. Enzymatic strategies and biocatalysts for amide bond formation: Tricks of the trade outside of the ribosome. *Mol. Biosyst.* 2015;11(2):338-353. https://doi.org/10.1039/c4mb00627e

100. 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. *Bioorg. Med. Chem. Lett.* 2018;28(1):11-14. https://doi.org/10.1016/j.bmcl.2017.11.029

101. Xia Y., Qu F., Peng L. Triazole nucleoside derivatives bearing aryl functionalities on the nucleobases show antiviral and anticancer activity. *Mini-Reviews in Med. Chem.* 2010;10(9):806-821. https://doi.org/10.2174/138955710791608316

102. Xia Y., Fan Z., Yao J., Liao Q., Li W., Qu F., Peng L. Discovery of bitriazolyl compounds as novel antiviral candidates for combating the tobacco mosaic virus. *Bioorg. Med. Chem. Lett.* 2006;16(10):2693-2698. https://doi.org/10.1016/j.bmcl.2006.02.023

103. Wang M., Zhu R., Fan Z., Fu Y., Feng L., Yao J., Maggiani A., Xia Y., Qu F., Peng L. Bitriazolyl acyclonucleosides synthesized via Huisgen reaction using internal alkynes show antiviral activity against tobacco mosaic virus. *Bioorg. Med. Chem. Let*t. 2011; 21(1): 354-357. https://doi.org/10.1016/j.bmcl.2010.10.141

104. Xia Y., Li W., Qu F., Fan Z., Liu X., Berro C., Rauzy E., Peng L. Synthesis of bitriazolyl nucleosides and unexpectedly different reactivity of azidotriazole nucleoside isomers in the Huisgen reaction. *Org. Biomol. Chem.* 2007;5(11):1695-1701. https://doi.org/10.1039/b703420b

105. Chudinov M.V., Matveev A.V., Prutkov A.N., Konstantinova I.D., Fateev I.V., Prasolov V.S., Smirnova O.A., Ivanov A.V., Galegov G.A., Deryabin P.G. Novel 5-alkyl(aryl)-substituted ribavirine analogues: synthesis and antiviral evaluation. *Mendeleev Commun.* 2016;26(3):214-216. https://doi.org/10.1016/j.mencom.2016.04.012

106. Zhu R., Wang M., Xia Y., Qu F., Neyts J., Peng L. Arylethynyltriazole acyclonucleosides inhibit hepatitis C virus replication. *Bioorg. Med. Chem. Lett.* 2008;18(11):3321-3327. https://doi.org/10.1016/j.bmcl.2008.04.026

107. Neyts J., Peng L., Que F., Zhu R. Novel viral replication inhibitors: Int. Pat. Appl. WO2009015446A2. Appl. 07/27/2007; publ. 02/05/2009. 50 p.

108. Wan J., Xia Y., Liu Y., Wang M., Rocchi P., Yao J., Qu F., Neyts J., Iovanna J. L., Peng L. Discovery of novel arylethynyltriazole ribonucleosides with selective and effective antiviral and antiproliferative activity. *J. Med. Chem.* 2009;52(4):1144-1155. https://doi.org/10.1021/jm800927r

109. Chudinov M.V., Prutkov A.N., Matveev A.V., Grebenkina L.E., Konstantinova I.D., Berezovskaya Y.V. An alternative route to the arylvinyltriazole nucleosides. *Bioorg. Med. Chem. Lett.* 2016;26(14):3223-3225. https://doi.org/10.1016/j.bmcl.2016.05.072

110. Peng L., Rocchi P., Iovanna J., Xia Y., Qu F., Wan J., Liu Y., Wang M. Novel triazole derivatives, their preparation and their application in therapeutics: US Pat. Appl. 2011136754A1; appl. 02/14/2011; publ. 06/09/2011. 26 p.

111. Xia Y., Liu Y., Wan J., Wang M., Rocchi P., Qu F., Iovanna J.L., Peng L. Novel triazole ribonucleoside down-regulates heat shock protein 27 and induces potent anticancer activity on drug-resistant pancreatic cancer. *J. Med. Chem.* 2009;52(19):6083-6096. https://doi.org/10.1021/jm900960v

112. Liu Y., Xia Y., Fan Y., Maggiani A., Rocchi P., Qu F., Iovanna J.L., Peng L. N-Aryltriazole ribonucleosides with potent antiproliferative activity against drug-resistant pancreatic cancer. *Bioorg. Med. Chem. Lett.* 2010;20(8):2503-2507. https://doi.org/10.1016/j.bmcl.2010.02.104

113. Xia Y., Liu Y., Rocchi P., Wang M., Fan Y., Qu F., Iovanna J. L., Peng L. Targeting heat shock factor 1 with a triazole nucleoside analog to elicit potent anticancer activity on drug-resistant pancreatic cancer. *Cancer Lett.* 2012;318(2):145-153. https://doi.org/10.1016/j.canlet.2011.09.043

114. Xia Y., Wang M., Demaria O., Tang J., Rocchi P., Qu F., Iovanna J. L., Alexopoulou L., Peng L. A novel bitriazolyl acyclonucleoside endowed with dual antiproliferative and immunomodulatory activity. *J. Med. Chem.* 2012;55(11):5642-5646. https://doi.org/10.1021/jm300534u

115. Chen M.M., Zhou Z.W., Suo Y.X., Li M.Y., Yao J.H., Peng L., Xia Y. Acyclonucleosides bearing coplanar arylethynyltriazole nucleobases: synthesis, structural analysis, and biological evaluation. *New Journal of Chemistry.* 2017;41(16):8509-8519. https://doi.org/10.1039/C7NJ01406F

116. Xia Y., Wang M., Beraldi E., Cong M., Zoubeidi A., Gleave M., Peng L. A novel triazole nucleoside suppresses prostate cancer cell growth by inhibiting heat shock factor 1 and androgen receptor. *Anticancer Agents Med. Chem.* 2015;15(10):1333-1340. https://doi.org/10.2174/1871520615666150617110943

117. Konstantinova I.D., Chudinov M.V., Prutkov A.N., Matveev A.V., Grebenkina L.E., Dorofeeva E.V. 5-(Tetrahydrofuran-2-yl)-1,2,4-triazole-3-carboxylic acid amide with antiviral activity, and method for production: pat. RU 2624018. Appl. 09/26/2016; publ. 06/30/2017. 8 p. (in Russ.).

About the author:

Mikhail V. Chudinov, Cand. of Sci. (Chemistry), Associate Professor of the Chair of Biotechnology and Industrial Pharmacy, M.V. Lomonosov Institute of Fine Chemical Technologies, MIREA – Russian Technological University (86, Vernadskogo pr., Moscow 119571, Russia). E-mail: chudinov@mirea.ru. ResearcherID L-5728-2016, https://orcid.org/0000-0001-9735-9690

Об авторе:

Чудинов Михаил Васильевич, кандидат химических наук, доцент кафедры биотехнологии и промышленной фармации Института тонких химических технологий им. М.В. Ломоносова ФГБОУ ВО «МИРЭА – Российский технологический университет» (119571, Россия, Москва, пр-т Вернадского, д. 86). E-mail: chudinov@mirea.ru. ResearcherID L-5728-2016, https://orcid.org/0000-0001-9735-9690

For citation: Chudinov M.V. Ribavirin and its analogs: Can you teach an old dog new tricks? *Tonkie Khimicheskie Tekhnologii = Fine Chemical Technologies.* 2019;14(4):7-23 (in Russ.). https://doi.org/10.32362/2410-6593-2019-14-4-7-23

Для цитирования: Чудинов М.В. Рибавирин и его аналоги: можно ли старую собаку научить новым фокусам? // Тонкие химические технологии. 2019. Т. 14. № 4. С. 7–23. https://doi.org/10.32362/2410-6593-2019-14-4-7-23

Translated by R. Trikin