CHEMISTRY AND TECHNOLOGY OF MEDICINAL COMPOUNDS AND BIOLOGICALLY ACTIVE SUBSTANCES

ХИМИЯ И ТЕХНОЛОГИЯ ЛЕКАРСТВЕННЫХ ПРЕПАРАТОВ И БИОЛОГИЧЕСКИ АКТИВНЫХ СОЕДИНЕНИЙ

ISSN 2686-7575 (Online)

https://doi.org/10.32362/2410-6593-2022-17-4-311-322

UDC 547.792.6

RESEARCH ARTICLE

Synthesis of 5-oxymethyl-1,2,4-triazole-3-carboxamides

Lyubov E. Grebenkina✉, Alexander N. Prutkov, Andrey V. Matveev, Mikhail V. Chudinov

MIREA – Russian Technological University (M.V. Lomonosov Institute of Fine Chemical Technologies), Moscow, 119571 Russia

✉*Corresponding author, e-mail: LEGrebenkina@mail.ru*

Abstract

Objectives. *A key step in the synthesis of natural nucleoside analogs is the formation of a glycosidic bond between the carbohydrate fragment and the heterocyclic base. Glycosylation methods differ in terms of regio- and stereoselectivity. A promising method for the highly specific synthesis of new pharmacologically active compounds involves an enzymatic reaction catalyzed by genetically engineered nucleoside phosphorylases. This study is devoted to the synthesis of a library of analogs of nucleoside heterocyclic bases—5-oxymethyl-1,2,4-triazole-3-carboxamides—in order to investigate the substrate specificity of genetically engineered nucleoside phosphorylases.*

Methods. *A method of cyclization of acylamidrazones obtained from the single synthetic precursor β-N-tert-butyloxycarbonyl-oxalamidrazone was used to parallel-synthesize new 5-alkoxy/aryloxymethyl-1,2,4-triazole-3-carboxamides. Silica gel column chromatography was used to isolate and purify the synthesized compounds. A complex of physicochemical analysis methods (nuclear magnetic resonance spectroscopy, chromatography, and mass spectrometry) confirmed the structure of the compounds obtained in the work.*

Results. *5-alkoxy/aryloxymethyl-1,2,4-triazole-3-carboxamides were obtained to study the substrate specificity of genetically engineered nucleoside phosphorylases. The possibility of obtaining new nucleoside analogs by the chemico-enzymatic method was demonstrated on the basis of preliminary assessment results.*

Conclusions. *The physicochemical characteristics of a series of novel 5-alkoxy/aryloxymethyl-1,2,4-triazole-3-carboxamides were studied along with their potential to act as substrates for the transglycosylation reaction catalyzed by nucleoside phosphorylases.*

Keywords: *nucleoside analogs, 5-oximethyl-1,2,4-triazole-3-carboxamides, parallel synthesis, nucleoside phosphorylase, substrate specificity*

For citation: Grebenkina L.E., Prutkov A.N., Matveev A.V., Chudinov M.V. Synthesis of 5-oxymethyl-1,2,4-triazole-3-carboxamides. *Tonk. Khim. Tekhnol. = Fine Chem. Technol.* 2022;17(4):311–322 (Russ., Eng.). https://doi.org/10.32362/2410-6593-2022-17-4-311-322

НАУЧНАЯ СТАТЬЯ

Синтез 5-оксиметил-1,2,4-триазол-3-карбоксамидов

Л.Е. Гребенкина, А.Н. Прутков, А.В. Матвеев, М.В. Чудинов

МИРЭА – Российский технологический университет (Институт тонких химических технологий им. М.В. Ломоносова), Москва, 119571 Россия
✉*Corresponding author, e-mail: LEGrebenkina@mail.ru*

Аннотация

Цели. *Ключевая стадия синтеза аналогов природных нуклеозидов – образование гликозидной связи между углеводным фрагментом и гетероциклическим основанием. Методы гликозилирования различаются по регио- и стереоселективности. Ферментативная реакция, катализируемая генно-инженерными нуклеозидфосфорилазами – перспективный метод высокоспецифичного синтеза новых фармакологически активных соединений. Данное исследование посвящено синтезу библиотеки аналогов гетероциклических оснований нуклеозидов – 5-оксиметил-1,2,4-триазол-3-карбоксамидов для изучения субстратной специфичности генно-инженерных нуклеозидфосфорилаз.*

Методы. *Для параллельного синтеза новых 5-алкокси/арилоксиметил 1,2,4-триазол-3-карбоксамидов применен метод циклизации ациламидразонов, получаемых из единого синтетического предшественника – β-N-третбутилоксикарбонил-оксаламидразона. Для выделения и очистки синтезированных соединений использована колоночная хроматография на силикагеле. Структура полученных в работе соединений подтверждена комплексом методов физико-химического анализа: спектроскопией ядерного магнитного резонанса и хромато-масс-спектрометрометрией.*

Результаты. *Получены 5-алкокси/арилоксиметил-1,2,4-триазол-3-карбоксамиды для изучения субстратной специфичности генно-инженерных нуклеозидфосфорилаз. По результатам предварительной оценки показана возможность получения из них новых аналогов нуклеозидов химико-ферментативным методом.*

Выводы. *Для серии новых 5-алкокси/арилоксиметил-1,2,4-триазол-3-карбоксамидов изучены физико-химические характеристики, а также их способность выступать в роли субстратов реакции трансгликозилирования, катализируемой нуклеозидфосфорилазами.*

Ключевые слова: *аналоги нуклеозидов, 5-оксиметил-1,2,4-триазол-3-карбоксамиды, параллельный синтез, нуклеозидфосфорилазы, субстратная специфичность.*

Для цитирования: Гребенкина Л.Е., Прутков А.Н., Матвеев А.В., Чудинов М.В. Синтез 5-оксиметил-1,2,4-триазол-3-карбоксамидов. *Тонкие химические технологии*. 2022;17(4):311–322. https://doi.org/10.32362/2410-6593-2022-17-4-311-322

INTRODUCTION

The work of many research groups in the field of medical chemistry, molecular biology and biotechnology is focused on the study of the biological properties of nucleoside analogs (NA) [1–4]. These compounds are mainly used for antiviral [5–10], anticancer drug [2, 11–13] and antibiotic [3, 14–15] purposes. Various approaches and their combinations used in the design of biologically active nucleoside preparations include modification of both the carbohydrate fragment of the molecule and the aglycone [2, 16–19]. At the same time, most nucleoside analogs that exhibit biological activity retain the β-*N*-glycoside bond of natural nucleosides.

To date, the need for drugs based on modified nucleoside analogs is mainly being met using effective chemical synthesis protocols. However, the chemico-enzymatic method of glycosylation of heterocyclic bases using recombinant enzymes is also a promising alternative approach to implementing the creation of a β-*N*-glycoside bond, comprising one of the key stages of nucleoside production [20]. The variety of nucleoside analogs obtained via classical chemical synthesis is limited by the stereo- and regioselectivity of glycosylation strategies, as well as the stability of intermediates during chemical transformations. The chemico-enzymatic method, conversely, is strictly stereo- and regiospecific, but the variety of target compounds obtained by this pathway is limited by the substrate specificity of the enzyme used.

Genetically engineered bacterial nucleoside phosphorylases (NP) of the pentosyltransferase group (CF 2.4.2) are often used for the chemico-enzymatic production of nucleoside analogs [21–23]. These enzymes catalyze several reactions: the synthesis of nucleotides and their phosphorolysis, as well as the transfer of a carbohydrate fragment of nucleosides to heterocyclic bases (transglycosylation reaction). Due to their rather low substrate specificity, they are suitable for use in the synthesis of a number of drugs [24–28]. Chemico-enzymatic transglycosylation becomes a convenient tool for obtaining new nucleoside analogs with modified heterocyclic bases and different carbohydrate fragments on account of the low selectivity of bacterial nucleoside phosphorylases [28–31]. In order to study the potential of nucleoside phosphorylases as a research and technological tool, an important task is the synthesis of heterocyclic bases of nucleoside analogs—potential substrates of these enzymes. In particular, derivatives of 1,2,4-triazole-3-carboxamide can form substrates for the biotechnological production of analogs of the antiviral drug ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide, virazole). In the present study, we developed a synthetic approach and obtained a series of 5-alkoxy/aryloxymethyl-1,2,4-triazole-3-carboxamides, substrates for the chemico-enzymatic synthesis of new ribavirin analogs.

RESULTS AND DISCUSSION

Although isosteric to purine bases 1,2,4-triazole-3-carboxamide is an excellent substrate of genetically engineered purine nucleoside phosphorylases (PNP) and uridine phosphorylases (UrP), the introduction of substituents in the 5 position of the 1,2,4-triazole ring significantly affects the ability of such derivatives to react with enzymatic transglycosylation. Studies of the substrate properties of heterocyclic bases synthesized in our laboratory, which were carried out in the Laboratory of Biotechnology of the Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences (IBCh RAS), initially led us to believe that steric difficulties in

the active center of NP limit substrate properties with an increase in the size of the substituent. With the exception of 5-methyl-1,2,4-triazole-3-carboxamide, 1,2,4-triazole-3-carboxamides containing a halogen, a nitro group, a hydroxyl, or simple alkyl substituents at position 5 were not found to be suitable for use as substrates for NP [32]. However, recently obtained results have contradicted this point of view. Some of the compounds synthesized by us, which contained an oxymethyl fragment at the 5 position of the 1,2,4-triazole ring, unexpectedly entered into an enzymatic transglycosylation reaction. In order to establish the limits of the substrate specificity of NP with respect to homologs of 5-oxymethyl-1,2,4-triazole-3-carboxamide, we synthesized a library of derivatives of 1,2,4-triazole-3-carboxamide **6a-i** with alkoxymethyl substituents increasing along the length of the carbon backbone, up to *n*-decyloxymethyl, as well as bulk 5-isopropoxy-, 5-phenoxy-, and 5-benzyloxymethyl substituents.

The main methods of synthesis of 1,2,4-triazole-3-carboxylic acid derivatives are based on the cyclization of amidrazones [17–20]. In order to obtain a homologous library of derivatives of 5-substituted 1,2,4-triazole-3-carboxamide, we considered two previously developed methods (Scheme 1). Although the first [33] can be used to synthesize a number of compounds in parallel from a single precursor of β-*N*-Boc-oxalamidrazone (**a**), (where Boc is the *tert*–butyloxycarbonyl protective group), it has the disadvantage of relatively low yields of target compounds. Conversely, while the second method [34] produces high yields of 5-substituted 1,2,4-triazole-3-carboxylic acids, it requires the production of individual synthetic precursors for each target compound, thus increasing the time of the experiment [35]. Therefore, in order to solve the problem of combinatorial synthesis, we chose a parallel method for obtaining derivatives of 1,2,4-triazole-3-carboxylic acids **c** (method I in Scheme 1).

A single synthetic precursor of β-*N*-Boc-oxalamidrazone (**3**) was obtained by the interaction of thiooxamic acid ethyl ester (**2**) with Boc-hydrazine (**1**) (Scheme 2).

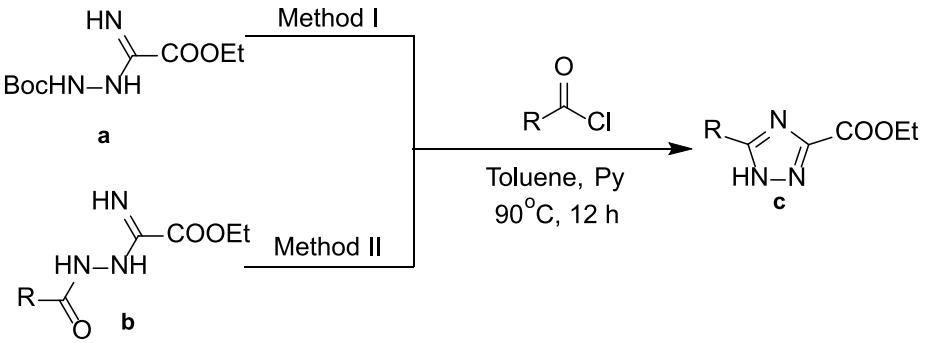
Ethyl esters of 5-alkoxy/aryloxymethyl-1,2,4-triazole-3-carboxylic acids **5a-h** were obtained by acylation of amidrazone **3** with **4a-h** hydroxyacetic acids chlorides followed by cyclization in pyridine at a boiling point (115°C). Further, the **5a-h** esters were converted into the corresponding **6a-h** amides by treatment with an aqueous-methanolic solution of ammonia (Scheme 3).

Compound **6j** was also prepared according to the above procedure. However, it was difficult to isolate the ethyl ether of 5-acetoxymethyl-1,2,4-triazole-3-carboxylic acid **5i** following cyclization in this case since the labile acetyl group was partially hydrolyzed during the treatment of the reaction mixture. A mixture of ethyl esters of 5-acetoxymethyl-**5i** and 5-hydroxymethyl-1,2,4-triazole-3-carboxylic acid **5j** was received. This mixture was treated with an ammonia solution, resulting in the complete removal of the acetyl group to obtain amide **6j** (Scheme 4).

The structure and purity of all final and intermediate compounds are proved by methods ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy and high-performance liquid chromatography (HPLC) with a mass spectrometric (MS) detector, the outputs are shown in Table 1.

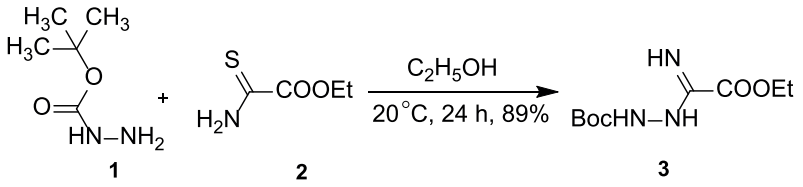
The primary results of a preliminary study of the substrate specificity of NP in relation to synthesized amides **6a-h**, **6j** carried out in the Laboratory of Biotechnology of the IBCh RAS by the previously described method [32] are given in Table 2.

As can be seen from the data, the chemico-enzymatic method can be used to obtain

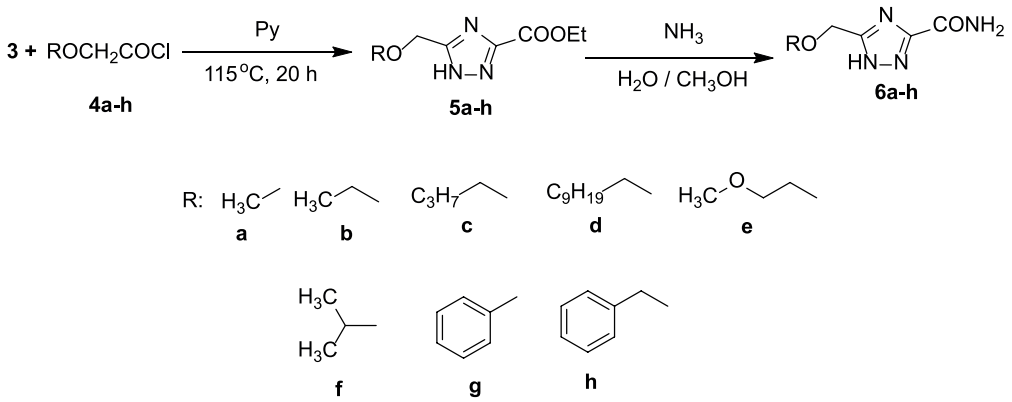


R = alkyl, aryl

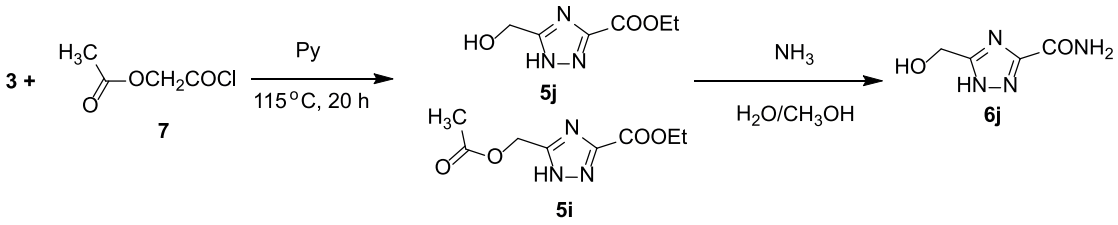
Scheme 1. 5-Substituted 1,2,4-triazole-3-carboxamide derivatives main synthesis methods (Boc is *tert*-butyloxycarbonyl; Et is ethyl; Py is pyridine).



Scheme 2. Synthesis of ethyl β-*N*-Boc-oxalamidrazone (Boc is *tert*-butyloxycarbonyl; Et is ethyl).



Scheme 3. Synthesis of 5-alkoxy/aryloxymethyl-1,2,4-triazole-3-carboxamides **6a-h** (Et is ethyl; Py is pyridine).



Scheme 4. Synthesis of 5-hydroxymethyl-1,2,4-triazole-3-carboxamide **6j** (Et is ethyl; Py is pyridine).

deoxyribosides from almost the entire range of synthesized compounds **6a-h**, **6j** (Schemes 3 and 4). Conversely, it seems to be impossible to obtain ribosides of 5-hydroxymethyl-1,2,4-triazole-3-carboxamides using this method.

EXPERIMENTAL

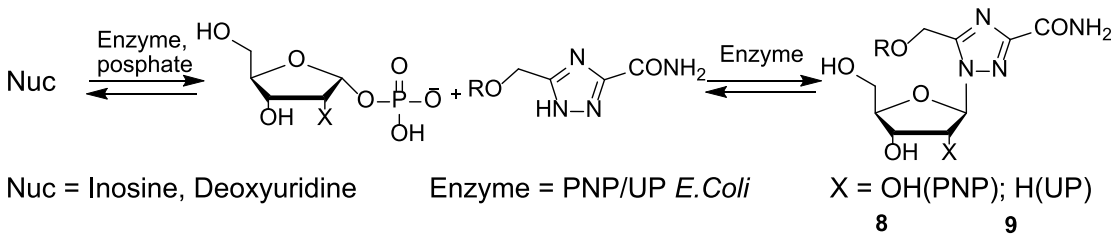
The solvents used in the work were purified by standard methods, with absolution being carried out in the usual way [32]. The reactions were monitored using thin-layer chromatography (TLC) on PTSH-AF-A–ultra violet (UV) plates (aluminum base; fractionated silica gel particle size is 5–17 μm; sorbent layer thickness is 90–120 μm; phosphor is 254 nm) (*Sorbfil*, Russia). The substances were visualized in UV light at 254 nm; phosphoric-molybdenum acid, ninhydrin, and iodine were additionally used. Kiselgel 60 Å (0.040–0.063 mm) silica gel (*Merck*, Germany) was used for column chromatography.

^{1}H and ^{13}C NMR spectra were recorded on a DPX-300 instrument (*Bruker*, Germany) with a resonant frequency for protons of ^{1}H = 300 MHz, ^{13}C = 75 MHz. Notation in ^{1}H NMR spectra as follows: s is a singlet, d is a doublet, dd is a doublet of doublets, t is a triplet, q is a quartet, m is a multiplet, w is a widened signal. Chromatography-mass spectrometry studies were performed on the LCMS-2020 device (*Shimadzu*, Japan) on the Luna C18 column (*Phenomenex*, USA) in gradient mode with electrospray ionization (150 × 4.6 mm, particle size is 3 μm, pore size is 100 Å. Mobile phases: A is 0.1% formic acid solution in water, B is acetonitrile. Gradient: up to 0.5 min—5% V, from 0.5 to 10.5 min—from 5 to 100% V, from 10.5 to 12 min—100% V, from 12 to 14 min—from 100% to

Table 1. Yields of synthesized compounds

Compound	5a	5b	5c	5d	5e	5f	5g	5h	5i+5j
Yield, %	57	22	45	36	31	14	63	59	–
Compound	**6a**	**6b**	**6c**	**6d**	**6e**	**6f**	**6g**	**6h**	**6j**
Yield, %	64	84	21	50	60	33	89	77	79

Table 2. Specificity of uridine phosphorylase towards 5-alkoxy/aryloxymethyl-1,2,4-triazole-3-carboxamides **6a-h, 6j**



No.	R	PNP	UP
6a	Me	–	+
6b	*n*-Pr	–	+
6c	*n*-Bu	–	+
6d	*n*-$C_{10}H_{21}$	–	–
6e	–CH_2CH_2OMe	–	+
6f	*i*-Pr	–	+
6g	Ph	–	–
6h	Bn	–	+
6j	H	+	+

Note: Minus sign indicates, that products of the enzymatic reaction **8** and **9** were not detected in the reaction mass by the HPLC–MS method. Plus sing indicates, that the products of the enzymatic reaction **8** and **9** were found in the reaction mass by the HPLC–MS method. PNP is purine nucleoside phosphorylase; UP is uridine phosphorylase; Me is methyl; *n*-Pr is *n*-propyl; *n*-Bu is *n*-butyl; *i*-Pr is isopropyl; Ph is phenyl; Bn is benzyl; H is hydrogen; Nuc is inosine, deoxyuridine; *E. coli* is *Escherichia coli.*

5% V. The volume of the injected sample is 2–10 μL. Mass spectra were recorded in the positive ion mode (range from 160 to 2000 Da). Parameters of the ionization source: heater temperature is 40°C, capillary temperature is 25°C, desiccant gas flow is 15 L/min, spray gas is 1.5 L/min, ionization voltage is 4.5 kV).

Ethyl-β-*N*-Boc-oxalamidrazone (3)

A mixture of 18.23 g (137 mmol) of thiooxamic acid ethyl ether **2** and 18.10 g (137 mmol) of Boc-hydrazine **1** was dissolved in 80 mL of ethyl alcohol. The reaction mixture was stirred for 24 h. The resulting precipitate was filtered, washed with 10 mL of ethyl alcohol and dried in air. Product yield: 28.25 g (89%), $T_{m.p.}$ = 178–180°C.

^{1}H NMR spectrum (dimethyl sulfoxide (DMSO)-d_6), δ, ppm: 1.23 (3H, t, J = 7.14 Hz, $CH_2C\underline{H}_3$); 1.43 (9H, s, $C(CH_3)_3$); 4.19 (2H, q, J = 7.14 Hz, $C\underline{H}_2CH_3$); 6.21 (2H, s, NHNH); 9.22 (1H, s, NH). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 13.90; 28.01; 61.31; 79.12; 136.70; 152.51; 162.09. For $C_9H_{18}N_3O_4$, m/z $[M+H]^+$ calculated: 232.13; found: 232.12.

General procedure for synthesis of ethyl esters of 5-alkoxy/aryloxymethyl-1,2,4-triazole-3-carboxylic acids 5a-j

Carboxylic acid chlorohydride (2.15 eq.) was added drop-by-drop to a suspension of **1** eq. of ethyl-β-*N*-Boc-oxalamidrazone **3** in absolute pyridine when cooled to 0°C. The reaction mass was heated to boiling point and stirred for 20 h. After the end of the reaction (reaction controlled by TLC; chloroform-methanol 5% system), the solvent was removed on a vacuum rotary evaporator. A 1 M aqueous HCl solution was added to the residue and extracted 3 times with ethyl acetate in equal portions. The organic phases were combined and dried Na_2SO_4, the solvent was removed on a vacuum rotary evaporator. The product was isolated using column chromatography on silica gel, the chloroform eluent was methanol (with a methanol gradient from 0 to 7%).

Ethyl ether of 5-(methoxymethyl)-1,2,4-triazole-3-carboxylic acid (5a)

1.00 g (4.32 mmol) of ethyl-β-*N*-Boc-oxalamidrazone **3**, 10 mL of absolute pyridine, 0.85 mL (9.31 mmol) of methoxyacetic acid chlorangidride. R_f = 0.58. Product yield: 0.46 g (57%).

^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.40 (3H, t, J = 7.14 Hz, $CH_2C\underline{H}_3$); 3.47 (3H, s, $C\underline{H}_3OCH_2$); 4.46 (2H, q, J = 7.14 Hz, $C\underline{H}_2CH_3$); 4.72 (2H, s, $CH_3OC\underline{H}_2$). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 13.91; 58.86; 61.96; 65.69; 153.24; 156.19; 159.52. For $C_7H_{11}N_3O_3$, m/z $[M+H]^+$ calculated: 186.19; found: 186.17.

Ethyl ether of 5-(ethoxymethyl)-1,2,4-triazole-3-carboxylic acid (5b)

1.00 g (4.3 mmol) ethyl-β-*N*-Boc-oxalamidrazone **3**, 10 mL of absolute pyridine, 1.11 g (9.3 mmol) ethoxyacetic acid chlorangidride. R_f = 0.55. Product yield: 0.19 g (22%).

^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.16 (3H, t, J = 7.00 Hz, $CH_2O–CH_2C\underline{H}_3$); 1.35 (3H, t, J = 7.14 Hz, $COO–CH_2C\underline{H}_3$); 3.59 (2H, q, J = 7.01 Hz, $CH_2O–C\underline{H}_2CH_3$); 4.42 (2H, q, J = 7.14 Hz, $C\underline{H}_2CH_3$); 4.73 (2H, s, $C\underline{H}_2O–CH_2CH_3$). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 14.09; 14.84; 62.08; 64.13; 67.08; 153.92; 156.57; 159.65. For $C_8H_{13}N_3O_3$, m/z $[M+H]^+$ calculated: 200.22; found: 200.20.

Ethyl ether of 5-(*n*-butoxymethyl)-1,2,4-triazole-3-carboxylic acid (5c)

1.16 g (5 mmol) of ethyl-β-*N*-Boc-oxalamidrazone **3**, 10 mL of absolute pyridine, 1.61 g (13 mmol) of *n*-butoxyacetic acid chlorangidride. R_f = 0.45. Product yield: 0.51 g (45%).

^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.90 (3H, t, J = 7.32 Hz, $O(CH_2)_3–C\underline{H}_3$); 1.30–1.43 (2H, m, $O(CH_2)_2–C\underline{H}_2CH_3$); 1.41 (3H, t, J = 7.14 Hz, $CO–CH_2C\underline{H}_3$); 1.54–1.64 (2H, m, $OCN_2C\underline{H}_2CH_2CH_3$); 3.58 (2H, t, J = 6.63 Hz, $O–C\underline{H}_2(CH_2)_2CH_3$); 4.46 (2H, q, J = 7.14 Hz, $CO–C\underline{H}_2CH_3$); 4.73 (2H, s, $CH_3(CH_2)_3OC\underline{H}_2–$). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 13.80; 14.20; 19.11; 29.66; 31.44; 62.13; 64.71; 71.74; 154.10; 156.77; 159.60. For $C_{10}H_{17}N_3O_3$, m/z $[M+H]^+$ calculated: 228.27; found: 228.26.

Ethyl ether of 5-(*n*-decyloxymethyl)-1,2,4-triazole-3-carboxylic acid (5d)

0.40 g (1.7 mmol) ethyl-β-*N*-Boc-oxalamidrazone **3**, 10 mL of absolute pyridine, 0.84 g (3.6 mmol) decyloxyacetic acid chlorangidride. R_f = 0.62. Product yield: 0.19 g (36%).

^{1}H NMR specrtum ($CDCl_3$), δ, ppm: 0.83–0.87 (3H, m, $CH_2CH_2–C\underline{H}_3$); 1.23 (14H, s, $OCH_2CH_2–(C\underline{H}_2)_7CH_3$); 1.40 (3H, t, J = 7.14 Hz, $COOCH_2–C\underline{H}_3$); 1.54–1.63 (2H, m, $OCH_2–C\underline{H}_2(CH_2)_7CH_3$); 3.56 (2H, t, J = 6.72 Hz, $O–C\underline{H}_2(CH_2)_8CH_3$); 4.46 (2H, q, J = 7.14 Hz, $COO–C\underline{H}_2CH_3$); 4.73 (2H, s, $CH_3(CH_2)_9OC\underline{H}_2–$). ^{13}C NMR spectrum, ($CDCl_3$), δ, ppm: 29.27; 29.37; 29.40; 29.52; 29.51; 14.08; 14.18; 22.63; 25.91; 31.84; 62.14; 64.62; 65.33; 67.78; 72.05; 126.98; 127.65; 128.53; 153.96; 156.71; 159.58. For $C_{16}H_{29}N_3O_3$, m/z $[M+H]^+$ calculated: 312.43; found: 312.41.

Ethyl ether 5-[1-(2-methoxy)ethoxymethyl]-1,2,4-triazole-3-carboxylic acid (5e)

1.00 g (4.3 mmol) of ethyl-β-*N*-Boc-oxalamidrazone **3**, 10 mL of absolute pyridine, 1.40 g (9.3 mmol) of 1-(2-methoxy) ethoxyacetic acid chlorohydride. R_f = 0.47. Product yield: 0.30 g (31%).

^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.39 (3H, t, *J* = 7.11 Hz, COO–$CH_2\underline{CH_3}$); 3.45 (3H, s, CH_2O–$\underline{CH_3}$); 3.60–3.63 (2H, m, CH_2–$\underline{CH_2}OCH_3$); 3.76–3.79 (2H, m, O–$C\underline{H_2}CH_2OCH_3$); 4.45 (2H, q, *J* = 7.14 Hz, COO–$C\underline{H_2}CH_3$); 4.82 (2H, s, –$\underline{CH_2}OCH_2CH_2OCH_3$). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 14.19; 59.01; 61.91; 65.42; 70.74; 71.82; 154.86; 156.51; 159.83. For $C_9H_{16}N_3O_4$, *m*/*z* $[M+H]^+$ calculated: 230.24; found: 230.22.

Ethyl ether of 5-(isopropyloxymethyl)-1,2,4-triazole-3-carboxylic acid (5f)

6.74 g (27 mmol) ethyl-β-*N*-Boc-oxalamidrazone **3**, 15 mL of absolute pyridine, 7.74 g (57 mmol) isopropyloxyacetic acid chlorangidride. R_f = 0.45. Product yield: 0.78 g (14%).

^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.22 (6H, d, *J* = 6.17 Hz $(C\underline{H_3})_2CHO$–); 1.42 (3H, t, *J* = 7.14 Hz, COO–$CH_2C\underline{H_3}$); 3.73–3.81 (1H, m, $(CH_3)_2$–$C\underline{H}OCH_2$); 4.47 (2H, q, *J* = 7.12 Hz, COO–$C\underline{H_2}CH_3$); 4.74 (2H, s, $(C\underline{H_3})_2CHO$–$C\underline{H_2}$). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 14.19; 21.84; 62.09; 62.23; 73.03; 154.26; 156.95; 159.69. For $C_9H_{16}N_3O_3$, *m*/*z* $[M+H]^+$ calculated: 214.24; found: 214.21.

Ethyl ether of 5-(phenoxymethyl)-1,2,4-triazole-3-carboxylic acid (5g)

1.19 g (5.15 mmol) ethyl-β-*N*-Boc-oxalamidrazone **3**, 10 mL of absolute pyridine, 1.88 g (11.02 mmol) phenoxyacetic acid chlorangidride. R_f = 0.46. Product yield: 0.80 g (63%).

^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.34 (3H, t, *J* = 7.14 Hz, CH_3Et); 4.38 (2H, q, *J* = 7.14 Hz, CH_2Et); 5.29 (2H, s, 5-CH_2); 6.88 (2H, d, *J* = 8.19 Hz, 2-CH, and 6-CH Ph); 6.97 (1H, t, *J* = 7.39 Hz, 4-CH Ph); 7.25 (2H, t, *J* = 7.98 Hz, 3-CH, and 5-CHPh). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 14.03; 61.98; 62.31; 114.46; 121.90; 129.62; 153.42; 155.78; 157.41; 159.47. For $C_{12}H_{14}N_3O_3$, *m*/*z* $[M+H]^+$ calculated: 248.26; found: 248.24.

Ethyl ether 5-[(benzyloxy)methyl]-1,2,4-triazole-3-carboxylic acid (5h)

16.00 g (69 mmol) of ethyl-β-N-Boc-oxalamidrazone **3**, 30 mL of absolute pyridine, 23.50 mL (149 mmol) of benzyloxyacetic acid chlorangidride. R_f = 0.63. Product yield: 10.71 g (59%).

^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.40 (3H, t, *J* = 7.14 Hz, CH_3Et); 4.45 (2H, q, *J* = 7.1 Hz, CH_2Et); 4.61 (2H, s, 5-CH_2); 4.77 (2H, s, CH_2Bn); 7.28–7.36 (5H, m, CHBn). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 14.15; 62.13; 62.35; 72.35; 128.00; 128.25; 128.56; 136.57; 153.79; 156.45; 159.54. For $C_{13}H_{16}N_3O_3$, *m*/*z* $[M+H]^+$ calculated: 262.29; found: 262.27.

Ethyl ether of 5-(acetoxymethyl)-1,2,4-triazole-3-carboxylic acid (5i) and ethyl ether of 5-(hydroxymethyl)-1,2,4-triazole-3-carboxylic acid (5j)

1.00 g (4.32 mmol) of ethyl-β-*N*-Boc-oxalamidrazone **3**, 10 mL of absolute pyridine, 1.00 mL (9.29 mmol) of acetoxyacetic acid chlorangidride. R_f = 0.65 (main product); R_f = 0.55 (byproduct). 0.52 g of a mixture of esters of 5-acetoxymethyl- (main product) and 5-hydroxymethyl-1,2,4-triazole-3-carboxylic acid (byproduct) was isolated, which was used at the next stage without additional separation.

^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.37 (3H, t, *J* = 7.13 Hz, CH_3Et, impurity); 1.38 (3H, t, *J* = 7.13 Hz, CH_3Et, main product); 2.09 (3H, s, CH_3 acetyl group, main product); 4.44 (2H, q, *J* = 7.16 Hz, CH_2Et, main product); 4.45 (2H, q, *J* = 7.16 Hz, CH_2Et, impurity); 4.65 (2H, s, 5-CH_2, impurity); 5.32 (2H, s, 5-CH_2, main product).

^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 13.98 (CH_3Et, impurity); 14.03 (CH_3Et, main product); 20.48 (CH_3 acetyl group, main product); 57.54 (5-CH_2, main product); 60.54 (5-CH_2, impurity); 62.41 (CH_2Et, main product); 62.70 (CH_2Et, impurity); 151.61 (C^3, impurity); 152.36 (C^3, main product); 155.44 (C^5, impurity); 155.44 (C^5, main product); 158.16 (3-COO, impurity); 158.94 (3-COO, main product); 170.88 (COO of the acetyl group, main product).

General procedure for obtaining amides of 5-alkoxy/aryloxymethyl-1,2,4-triazole-3-carboxylic acids 6a-h, 6j

Ethyl ether of 5-hydroxymethyl-1,2,4-triazole-3-carboxylic acid **5a-i** was dissolved in 1.50 mL of 10 M methanol ammonia solution and heated to boiling point in a reflux flask with 0.50 mL of 14 M aqueous ammonia solution being added every 12 h. At the end of the reaction (complete conversion of ether, TLC control, eluent of 5% methanol in chloroform), the solvent was removed on a vacuum rotary evaporator. The product was suspended in anhydrous acetone, filtered and dried in a desiccator at reduced pressure above NaOH for 12 h.

Amide of 5-(methoxymethyl)-1,2,4-triazole-3-carboxylic acid (6a)

0.52 g (2.81 mmol) of 5-(methoxymethyl)-1,2,4-triazole-3-carboxylic acid ethyl ether, reaction time 72 h. Product yield: 280 mg (64%).

^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 3.30 (3H, s, CH_3 MeO); 4.49 (2H, s, 5-CH_2); 7.71 and 8.01 (2H, 2s, NH_2).^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 57.96; 65.82; 153.38; 157.41; 159.60. For $C_5H_9N_4O_2$, *m/z* $[M+H]^+$ calculated: 157.15; found: 157.13.

Amide of 5-ethoxymethyl-1,2,4-triazole-3-carboxylic acid (6b)

70 mg (3.5 mmol) ethyl ether of 5-(ethoxymethyl)-1,2,4-triazole-3-carboxylic acid, reaction time 48 h. Product yield: 50 mg (84%). $T_{m.p.}$ = 161–163°C.

^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.12 (3H, t, *J* = 6.98 Hz, –$CH_2C\underline{H}_3$); 3.51 (2H, q, *J* = 6.98 Hz, $C\underline{H}_2CH_3$); 4.5 (2H, s, –O–$C\underline{H}_2$); 7.69 and 7.99 (2H, 2s, $CON\underline{H}_2$). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 14.94; 63.89; 65.52; 153.40; 157.58; 159.61. For $C_6H_{11}N_4O_2$, *m/z* $[M+H]^+$ calculated: 171.18; found: 171.15.

Amide of 5-(butoxymethyl)-1,2,4-triazole-3-carboxylic acid (6c)

215 mg (0.8 mmol) ethyl ether of 5-(butoxymethyl)-1,2,4-triazole-3-carboxylic acid, reaction time 48 h. Product yield: 40 mg (21%). $T_{m.p.}$ = 125°C (partially), 135–136°C (completely).

^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 0.84 (3H, t, *J* = 6.47 Hz, CH_2–$C\underline{H}_3$); 1.25–1.50 (4H, m, CH_2–$C\underline{N}_2C\underline{H}_2CH_3$); 3.44 (2H, t, *J* = 5.99 Hz, –$C\underline{H}_2$O); 4.34 and 4.5 (2H, s, O$C\underline{H}_2$–); 7.68 and 7.99 (2H, 2s, $CON\underline{H}_2$). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 13.74; 18.74; 31.10; 31.25; 64.08; 65.30; 69.25; 69.81; 153.40; 155.12; 157.54; 159.62; 160.04. For $C_8H_{15}N_4O_2$, *m/z* $[M+H]^+$ calculated: 199.23; found: 199.20.

Amide of 5-(decyloxymethyl)-1,2,4-triazole-3-carboxylic acid (6d)

110 mg (0.4 mmol) ethyl ether of 5-(decyloxymethyl)-1,2,4-triazole-3-carboxylic acid, 48 h. Product yield: 50 mg (50%). $T_{m.p.}$ = 140°C (partially), 153–155°C (completely).

^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 0.84 (3H, t, *J* = 6.24 Hz, $(CH_2)_9$–$C\underline{H}_3$); 1.22 (14H, s, CH_2–$(C\underline{H}_2)_7CH_3$); 1.49 (2H, m, –$C\underline{H}_2CH_2$O); 3.43 (2H, t, *J* = 6.52 Hz, –$C\underline{H}_2$O); 4.49 (2H, s, O–$C\underline{H}_2$); 7.69 and 7.99 (2H, 2s, $CON\underline{H}_2$). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 13.97; 22.11; 25.55; 28.71; 28.84; 29.02; 31.30; 64.10; 70.13; 153.32; 157.61; 159.57. For $C_{14}H_{27}N_4O_2$, *m/z* $[M+H]^+$ calculated: 283.39; found: 283.37.

Amide of 5-(1-(2-methoxy) ethoxymethyl)-1,2,4-triazole-3-carboxylic acid (6e)

250 mg (0.8 mmol) of 5-(1-(2-methoxy) ethyl etherethoxymethyl)-1,2,4-triazole-3-carboxylic acid, reaction time 48 h. Product yield: 150 mg (60%). $T_{m.p.}$ = 109–112°C.

^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 3.22 (3H, d, O–$C\underline{H}_3$); 3.43–3.46 (2H, m, O–$C\underline{H}_2CH_2$); 3.57–3.60 (2H, m, OCH_2–$C\underline{H}_2$); 4.54 (2H, d, O$C\underline{H}_2$–); 7.70 and 8.01 (2H, 2d, $CON\underline{H}_2$).^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 58.07; 64.38; 69.35; 71.09; 153.25; 157.55; 159.53. For $C_7H_{13}N_4O_3$, *m/z* $[M+H]^+$ calculated: 201.20; found: 201.17.

Amide of 5-isopropyloxymethyl-1,2,4-triazole-3-carboxylic acid (6f)

310 mg (1.5 mmol) of 5-(isopropyloxymethyl)-1,2,4-triazole-3-carboxylic acid ethyl ether, reaction time 48 h. Product yield: 90 mg (33%). $T_{m.p.}$ = 160–161°C, T_{subl} = 144°C.

^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.11 (6H, d, *J* = 6.08 Hz, $(C\underline{H}_3)$–CH_2); 3.63–3.73 (1H, m, (CH_3)–$C\underline{H}_2$–O); 4.50 (2H, s, –O–$C\underline{H}_2$); 7.66 and 7.96 (2H, s, $CON\underline{H}_2$). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 21.85; 61.52; 70.96; 153.50; 157.80; 159.67. For $C_7H_{13}N_4O_2$, *m/z* $[M+H]^+$ calculated: 185.20; found: 185.19.

Amide of 5-(phenoxymethyl)-1,2,4-triazole-3-carboxylic acid (6g)

400 mg (1.62 mmol) ethyl ether of 5-(phenoxymethyl)-1,2,4-triazole-3-carboxylic acid, reaction time 96 h. Product yield: 310 mg (89%).

^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 5.16 (2H, s, 5-CH_2); 6.96 (1H, t, *J* = 7.30 Hz, 4CH, Ph); 7.04 (2H, d, *J* = 8.00 Hz, 2CH and 6CH, Ph); 7.30 (2H, t, *J* = 7.91 Hz, 3CH, and 5CH, Ph); 7.79 and 8.10 (2H, 2s, NH_2). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 62.17; 114.68; 121.16; 129.57; 152.69; 157.34; 157.89; 159.08. For $C_{10}H_{11}N_4O_2$, *m/z* $[M+H]^+$ calculated: 219.22; found: 219.20.

Amide 5-[(benzyloxy)methyl]-1,2,4-triazole-3-carboxylic acid (6h)

350 mg (2.45 mmol) of 5-[(benzyloxy)-ethyl ethermethyl]-1,2,4-triazole-3-carboxylic acid, reaction time 72 h. Product yield: 240 mg (77%).

^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 4.57 (2H, s, 5-CH_2); 4.60 (2H, s, CH_2Bn); 7.28–7.37 (5H,

m, CHBn); 7.72 and 8.02 (2H, 2s, NH_2). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 63.68; 71.75; 127.63; 127.77; 128.28; 137.74; 153.21; 157.50; 159.48. For $C_{11}H_{13}N_4O_2$, *m*/*z* $[M+H]^+$ calculated: 233.25; found: 233.22.

Amide of 5-hydroxymethyl-1,2,4-triazole-3-carboxylic acid (6j)

0.52 g (2.44 mmol) mixtures of ethyl esters of 5-acetoxymethyl- and 5-hydroxymethyl-1,2,4-triazole-3-carboxylic acid, reaction time 72 h. Product yield: 270 mg (77%).

^1H NMR spectrum (DMSO-d_6), δ, ppm: 4.55 (2H, s, 5-CH_2); 7.49 and 7.76 (2H, 2s, NH_2). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 55.98; 154.83; 159.70; 160.69. For $C_4H_7N_4O_2$, *m*/*z* $[M+H]^+$ calculated: 143.12; found: 143.09.

CONCLUSIONS

By applying the method of parallel synthesis to the solution of the problem of obtaining 5-substituted 1,2,4-triazole-3-carboxamides, it was possible to expand the range of their synthetic availability. The physicochemical characteristics of the obtained series of new 5-alkoxy/aryloxymethyl-1,2,4-triazole-3-carboxamides were studied. In addition, the demonstrated possibility for these compounds to be used as substrates of a transglycosylation reaction catalyzed by genetically engineered nucleoside phosphorylases allowing the synthesis new potentially pharmacologically active analogs of deoxynucleosides by a chemico-enzymatic method. The esters of 5-alkoxy/aryloxymethyl-1,2,4-triazole-3-carboxylic acids obtained in the course of this work can also be used for the synthesis of ribonucleosides by chemical glycosylation methods. As a result of the conducted research, a method for obtaining 5-alkoxy/aryloxymethyl-1,2,4-triazole-3-carboxamides, comprising heterocyclic bases of nucleoside analogs and esters of 5-alkoxy/aryloxymethyl-1,2,4-triazole-3-carboxylic acids as convenient precursors of chemical ribosylation, was introduced into the synthesis procedure. Thus, a necessary synthetic base has been created for the study of ribavirin analogs with 5-alkoxy/aryloxymethyl substituents.

Acknowledgments

We are grateful to I.D. Konstantinova, O.S. Smirnova, I.V. Fateev, and other employees of the Laboratory of Biotechnology of the IBCh RAS for conducting primary tests of the NP substrate properties in relation to synthesized compounds. NMR spectra were registered using the equipment of the RTU MIREA Collective Use Center (Agreement No. 075-15-2021-689 dated 01.09.2021, unique identification number 2296.61321X0010).

Authors' contributions

L.E. Grebenkina – conducting experiments;
A.N. Prutkov – conducting experiments;
A.V. Matveev – creating a research concept, processing experimental data, adjustment of experimental studies;
M.V. Chudinov – creating a research concept.

The authors declare no conflicts of interest.

REFERENCES

1. Jordheim L.P., Durantel D., Zoulim F., Dumontet C. Advances in the development of nucleoside and nucleotide analogues for cancer and viral diseases. *Nat. Rev. Drug Discov.* 2013;12(6):447–464. https://doi.org/10.1038/nrd4010

2. 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

3. Thomson J.M., Lamont I.L. Nucleoside analogues as antibacterial agents. *Front. Microbiol.* 2019;10:952. https://doi.org/10.3389/fmicb.2019.00952

4. Geraghty R.J., Aliota M.T., Bonnac L.F. Broad-spectrum antiviral strategies and nucleoside analogues. *Viruses.* 2021;13(4):667. https://doi.org/10.3390/v13040667

5. De Clercq E., Li G. Approved antiviral drugs over the past 50 years. *Clin. Microbiol. Rev.* 2016;29(3):695–747. https://doi.org/10.1128/CMR.00102-15

6. De Clercq E. New Nucleoside Analogues for the Treatment of Hemorrhagic Fever Virus Infections. *Chem. Asian J.* 2019;14(22):3962–3968. https://doi.org/10.1002/asia.201900841

7. Kasianenko K.V., Lvov N.I., Maltsev O.V., Zhdanov K.V. Nucleoside analogues for the treatment of influenza: History and experience. *Journal Infektology.* 2019;11(3):20–26 (in Russ.). https://doi.org/10.22625/2072-6732-2019-11-3-20-26

8. Pruijssers A.J., Denison M.R. Nucleoside analogues for the treatment of coronavirus infections. *Curr. Opin. Virol.* 2019;35:57–62. https://doi.org/10.1016/j.coviro.2019.04.002

9. Borbone N., Piccialli G., Roviello G.N., Oliviero G. Nucleoside analogs and nucleoside precursors as drugs in the fight against SARS-CoV-2 and other coronaviruses. *Molecules.* 2021;26(4):986. https://doi.org/10.3390/molecules26040986

10. Yoshida Y., Honma M., Kimura Y., Abe H. Structure, Synthesis and Inhibition Mechanism of Nucleoside Analogues as HIV-1 Reverse Transcriptase Inhibitors (NRTIs). *ChemMedChem*. 2021;16(5):743–766. https://doi.org/10.1002/cmdc.202000695

11. Zhang Y., Liu X., Lin Y., Lian B., Lan W., Iovanna J.L., *et al.* Novel triazole nucleoside analogues promote anticancer activity: Via both apoptosis and autophagy. *Chem. Commun*. 2020;56(69):10014–10017. https://doi.org/10.1039/D0CC04660D

12. Wang D., Yu C., Xu L., Shi L., Tong G., Wu J., *et al*. Nucleoside Analogue-Based Supramolecular Nanodrugs Driven by Molecular Recognition for Synergistic Cancer Therapy. *J. Am. Chem. Soc*. 2018;140(28):8797–8806. https://doi.org/10.1021/jacs.8b04556

13. Zeng X., Hernandez-Sanchez W., Xu M., Whited T.L., Baus D., Zhang J., *et al.* Administration of a Nucleoside Analog Promotes Cancer Cell Death in a Telomerase-Dependent Manner. *Cell Reports.* 2018;23(10):3031–3041. https://doi.org/10.1016/j.celrep.2018.05.020

14. Sun R., Wang L. Inhibition of Mycoplasma pneumoniae growth by FDA-approved anticancer and antiviral nucleoside and nucleobase analogs. *BMC Microbiol.* 2013;13:184. https://doi.org/10.1186/1471-2180-13-184

15. Pastuch-Gawolek G., Gillner D., Krol E., Walczak K., Wandzik I. Selected nucleos(t)ide-based prescribed drugs and their multi-target activity. *Eur. J. Pharmacol*. 2019;865:172747. https://doi.org/10.1016/j.ejphar.2019.172747

16. Seley-Radtke K.L., Yates M.K. The evolution of nucleoside analogue antivirals: A review for chemists and non-chemists. Part 1: Early structural modifications to the nucleoside scaffold. *Antiviral Res.* 2018;154:66–86. https://doi.org/10.1016/j.antiviral.2018.04.004

17. Yates M.K., Seley-Radtke K.L. The evolution of antiviral nucleoside analogues: A review for chemists and non-chemists. Part II: Complex modifications to the nucleoside scaffold. *Antiviral Res.* 2019;162:5–21. https://doi.org/10.1016/j.antiviral.2018.11.016

18. 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

19. Merino P. (Ed.). *Chemical Synthesis of Nucleoside Analogues*. NY: John Wiley & Sons, Inc.; 2013. 912 p. ISBN: 978-1-118-49808-8

20. Mikhailopulo I.A., Miroshnikov A.I. Biologically important nucloesides: modern trends in biotechnology and application. *Mendeleev Commun*. 2011;21(2):57–68. https://doi.org/10.1016/j.mencom.2011.03.001

21. Yehia H., Kamel S., Paulick K., Wagner A., Neubauer P. Substrate spectra of nucleoside phosphorylases and their potential in the production of pharmaceutically active compounds. *Curr. Pharm. Des.* 2017;23(45):6913–6935. http://dx.doi.org/10.2174/1381612823666171024155811

22. Kamel S., Yehia H., Neubauer P., Wagner A. Enzymatic Synthesis of Nucleoside Analogues by Nucleoside Phosphorylases. In: Lucas F.J., Rius M.-J.C. (Eds.). *Enzymatic and Chemical Synthesis of Nucleic Acid Derivatives*. Wiley-VCH Verlag GmbH & Co; 2018. P. 1–28. https://doi.org/10.1002/9783527812103.ch1

23. Barai V.N., Zinchenko A.I., Eroshevskaya L.A., Kalinichenko E.N., Kulak T.I., Mikhailopulo I.A. A Universal Biocatalyst for the Preparation of Base- and Sugar-Modified Nucleosides via an Enzymatic Transglycosylation. *Helv. Chim. Acta.* 2002;85(7):1901–1908. https://doi.org/10.1002/1522-2675(200207)85:<1901::AID-HLCA1901>3.0.CO;2-C

24. Konstantinova I.D., Leont'eva N.A., Galegov G.A., Ryzhova O.I., Chuvikovskii D.V., Antonov K.V., *et al*. Ribavirin: Biotechnological synthesis and effect on the reproduction of Vaccinia virus. *Russ. J. Bioorg. Chem*. 2004;30(6):553–560. https://doi.org/10.1023/B:RUBI.0000049772.18675.34

25. Konstantinova I.D., Esipov R.S., Muravieva T.I., Taran S.A., Verevkina K.N., Gurevich A.I., *et al*. *Method for preparing 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxyamide (ribavirin)*: RF Pat. 2230118. Publ. 10.03.2004 (in Russ.).

26. Sakharov V., Baykov S., Konstantinova I., Esipov R., Dorogov M. An Efficient Chemoenzymatic Process for Preparation of Ribavirin. *International Journal of Chemical Engineering*. 2015;2015:734851. https://doi.org/10.1155/2015/734851

27. Rabuffetti M., Bavaro T., Semproli R., Cattaneo G., Massone M., Morelli C.F., *et al*. Synthesis of Ribavirin, Tecadenoson, and Cladribine by Enzymatic Transglycosylation. *Catalysts*. 2019;9(4):355. https://doi.org/10.3390/catal9040355

28. Fateev I.V., Antonov K.V., Konstantinova I.D., Muravyova T.I., Seela F., Esipov R.S., *et al*. The chemoenzymatic synthesis of clofarabine and related 2'-deoxyfluoroarabinosyl nucleosides: the electronic and stereochemical factors determining substrate recognition by E. coli nucleoside phosphorylases. *Beilstein J. Org. Chem*. 2014;10:1657–1669. https://doi.org/10.3762/bjoc.10.173

29. Zhou X., Szeker K., Jiao L.-Y., Oestreich M., Mikhailopulo I.A., Neubauer P. Synthesis of 2,6-Dihalogenated Purine Nucleosides by Therm stable Nucleoside Phosphorylases. *Adv. Synth. Catal*. 2015;357(6):1237–1244. https://doi.org/10.1002/adsc.201400966

30. Vichier-Guerre S., Dugue L., Bonhomme F., Pochet S. Expedient and generic synthesis of imidazole nucleosides by enzymatic transglycosylation. *Org. Biomol. Chem*. 2016;14(14):3638–3653. https://doi.org/10.1039/C6OB00405A

31. Hatano A., Wakana H., Terado N., Kojima A., Nishioka C., Iizuka Y., *et al*. Bio-catalytic synthesis of unnatural nucleosides possessing a large functional group such as a fluorescent molecule by purine nucleoside phosphorylase. *Catal. Sci. Technol*. 2019;9(18):5122–5129. https://doi.org/10.1039/C9CY01063G

32. Konstantinova I.D., Chudinov M.V., Fateev I.V., Matveev A.V., Zhurilo N.I., Shvets V.I., Miroshnikov A.I. Nucleosides of 1,2,4-triazole: Possibilities and limitations of the chemical enzymatic method of preparation. *Bioorganicheskaya Khimiya*. 2013;39(1):61–80 (in Russ.). https://doi.org/10.7868/S0132342313010053

[Konstantinova I.D., Chudinov M.V., Fateev I.V., *et al*. 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]

33. Matveev A.V., Prutkov A.N., Chudinov M.V. *Method of producing 5-substituted 1,2,4-triazole-3-carboxylic acids and derivatives thereof from universal precursor*: RF pat. 2605414. Publ. 20.12.2015 (in Russ.).

34. Chudinov M.V., Matveev A.V., Zhurilo N.I., Prutkov A.N., Shvets V.I. An Efficient Route to Ethyl 5-Alkyl-(Aryl)-1*H*-1,2,4-triazole-3-carboxylates. *J. Heterocycl. Chem*. 2015;52(5):1273–1277. https://doi.org/10.1002/jhet.1934

35. Matveev A.V., Grebenkina L.E., Prutkov A.N., Chudinov M.V. 5-Substituted 1,2,4-Triazole-3-Carboxylates and 5-Substituted Ribavirin Analogs Synthesis. *Curr. Protoc*. 2021;1(11):e281 https://doi.org/10.1002/cpz1.281

About the authors:

Lyubov E. Grebenkina, Assistant, Department 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: LEGrebenkina@mail.ru. Scopus Author ID 57189663430, RSCI SPIN-code 6518-1280, https://orcid.org/0000-0002-5995-5608

Alexander N. Prutkov, Postgraduate Student, Department 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: alex_prutkov@mail.ru. ResearcherID G-4025-2016, Scopus Author ID 56228508300, RSCI SPIN-code 2965-1335, https://orcid.org/0000-0001-9522-7387

Andrey V. Matveev, Cand. Sci. (Chem.), Associate Professor, Department 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: 4motya@gmail.com. Scopus Author ID 7102723461, RSCI SPIN-code 7420-3188, https://orcid.org/0000-0002-0830-3036

Mikhail V. Chudinov, Cand. Sci. (Chem.), Associate Professor, Department 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, Scopus Author ID 6602589900, RSCI SPIN-code 3920-8067, https://orcid.org/0000-0001-9735-9690

Об авторах:

Гребенкина Любовь Евгеньевна, ассистент 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: LEGrebenkina@mail.ru. Scopus Author ID 57189663430, SPIN-код РИНЦ 6518-1280, https://orcid.org/0000-0002-5995-5608

Прутков Александр Николаевич, аспирант 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: alex_prutkov@mail.ru. ResearcherID G-4025-2016, Scopus Author ID 56228508300, SPIN-код РИНЦ 2965-1335, https://orcid.org/0000-0001-9522-7387

Матвеев Андрей Валерьевич, к.х.н., 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: 4motya@gmail.com. Scopus Author ID 7102723461, SPIN-код РИНЦ 7420-3188, https://orcid.org/0000-0002-0830-3036

Чудинов Михаил Васильевич, к.х.н., 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, Scopus Author ID 6602589900, SPIN-код РИНЦ 3920-8067, https://orcid.org/0000-0001-9735-9690

The article was submitted: February 17, 2022; approved after reviewing: March 11, 2022; accepted for publication: July 25, 2022.

Translated from Russian into English by N. Isaeva
Edited for English language and spelling by Thomas Beavitt