Synthesis and antiviral activity of nonannulated tetrazolylpyrimidines

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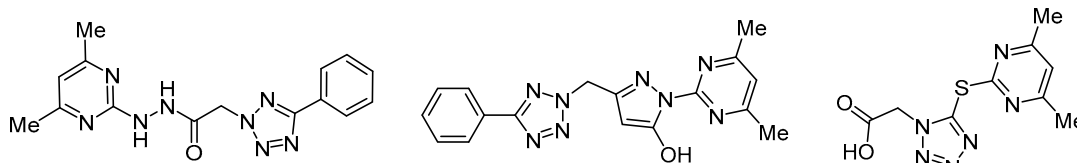
Nonannulated tetrazolylpyrimidines in the structure of which the heterocyclic fragments are separated by hydrazinocarbonylmethyl, methylpyrazolyl groups or a sulfur atom were synthesized. Some of these compounds showed moderate *in vitro* activity against H1N1 subtype of influenza A virus. The selectivity index of the anti-influenza action of {5-[(4,6-dimethylpyrimidin-2-yl)sulfanyl]-1*H*-tetrazol-1-yl}acetic acid, which has very low cytotoxicity, was twice as high as the selectivity index of the reference drug rimantadine.

Keywords: pyrimidines, tetrazoles, biological activity, linkers, properties, structure, synthesis.

The relevance of the development of new antiviral drugs, as a rule, is determined by the presence or absence of prophylactic vaccines against newly emerging infections, as well as the ability of viruses to escape the host immune response and develop drug-resistant strains.[1] The current situation has sharply exacerbated the problem of the availability of drugs against highly pathogenic variants of the influenza virus and the SARS2 coronavirus, the cause of COVID-19. The solution of this problem is determined by the effectiveness of the search for new drugs for the treatment of influenza and associated infectious diseases. Compounds of various structures are used in the treatment of this viral disease (Fig. 1). In this context, drugs that contain fragments of heterocyclic compounds, azoles and azines, in their active pharmaceutical ingredients (API) are of particular interest.

In 2014, riamilovir (Triazavirin) was registered as a drug for the treatment of influenza in the Russian Federation.[2] Triazavirin has recently been proven effective in treating the new coronavirus infection and is well tolerated by patients with COVID-19.[3] Triazavirin API, 7-(methylsulfanyl)-3-nitro[1,2,4]triazolo[5,1-*c*]triazin-4(1*H*)-one belongs to annulated azoloazines, which indirectly indicates the prospect of searching for new agents for influenza chemotherapy in the ranks of biannulated ("hybrid") heterocyclic compounds based on azoles and azines. Derivatives of tetrazole and pyrimidine are among the most sought after scaffolds in modern medicinal chemistry.[4,5] In particular, it was shown that some tetrazole derivatives exhibit pronounced activity against rhinoviruses[6a] and various strains of influenza A virus.[6b] In this regard, the synthesis and study of biological activity of binuclear
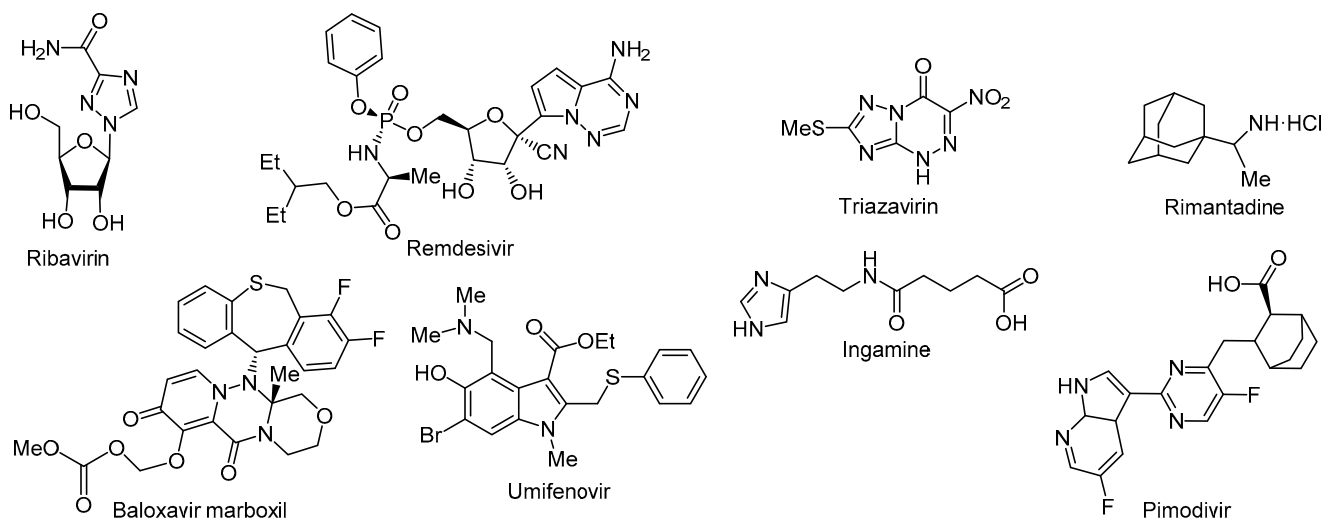
Figure 1. Active pharmaceutical ingredients of drugs used for influenza chemotherapy.

heterocyclic compounds containing both tetrazolyl and pyrimidyl fragments in their structure are of interest. In the case of annulated tetrazolylpyrimidines, the possibility of azidoazomethine-tetrazole isomerism should be considered.[7,8] For some represantives of this series, the equilibrium can be shifted toward the open-chain azide form with significant cytotoxicity.

In this study, a series of nonannulated tetrazolyl-pyrimidines was synthesized, and their structure and anti-influenza activity were investigated as well. Fragments of different nature were chosen as linker groups connecting the tetrazole and pyrimidine heterocycles – methylene, hydra-zinylcarbonylmethyl, pyrazolyl groups and a sulfur atom. This set of linkers would allow us to assess the prospects of given structural types in regards to biological activity.

At the first stage of our study, we attempted to synthesize 4,6-dimethyl-2-(5-phenyl-2H-tetrazol-2-yl)pyri-midine (**3**) by direct hetarylation of the sodium salt of 5-phenyltetrazole (**1**) by 2-chloro-4,6-dimethylpyrimidine (**2**). However, it was not possible to isolate the target tetrazolylpyrimidine **3** from the reaction mixture. Instead of nonannulated tetrazolylpyrimidine **3**, a compound was formed the properties of which were identical to those of 5,7-dimethyl-3-phenyl[1,2,4]triazolo[4,3-a]pyrimidine (**4**)
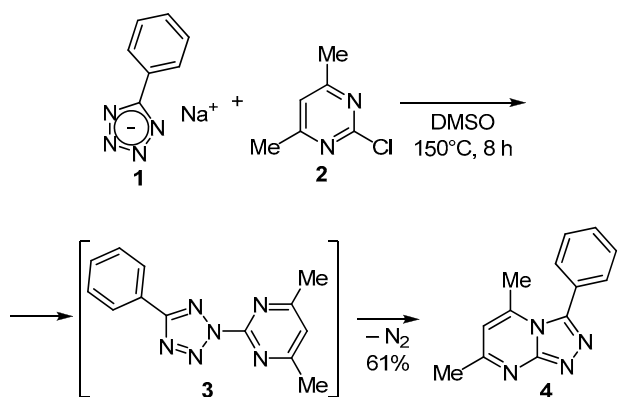
described earlier.[9] A possible reason for the discovered phenomenon is the thermolytic recyclization of interme-diate **3** accompanied by the elimination of an N_2 molecule and the formation of annulated triazolylpyrimidine **4** (Scheme 1).

Considering this experience, we focused our efforts on the synthesis of nonannulated tetrazolylpyrimidines in the structure of which heterocyclic fragments are linked by linker groups such as hydrazinocarbonylmethyl, pyrazolyl, as well as a sulfur atom. To form a linking bridge in the target compounds, we used classical methods such as acylation of the terminal nitrogen atom of the $NHNH_2$ group with carboxylic acid halides, hydrazinolysis of esters, and the substitution of the chlorine atom in the corresponding hetaryls for a sulfur atom by the action of hetarylthiones.[10] Hydrazides **7a,b** were obtained by acylation of 2-hydrazinylpyridines (**5a**) or 2-hydrazinyl-4,6-dimethylpyrimidine (**5b**) with 5-phenyltetrazol-2-yl-acetic acid chloride (**6**) (Scheme 2).

1-(4,6-Dimethylpyrimidin-2-yl)-3-[(5-phenyl-2H-tetrazol-2-yl)methyl]-1H-pyrazol-5-ol (**9**) was accessed by hydra-zinolysis of ethyl 3-oxo-4-(5-phenyl-2H-tetrazol-2-yl)-

Scheme 1

Scheme 2

7a R = H (48%)
b R = Me (78%)
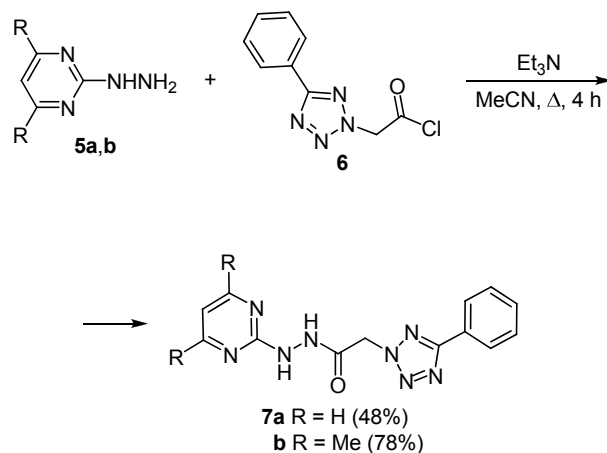
butanoate (**8**) by 2-hydrazinyl-4,6-dimethylpyrimidine (**5b**) (Scheme 3). According to X-ray structural analysis data, the pyrazole fragment of compound **9** exists in the aromatic form – a hydroxy group is present at position 5. The rendering of the structures of the molecules of compounds **7b** and **9** according to X-ray structural analysis data is shown in Figure 2.

Scheme 3
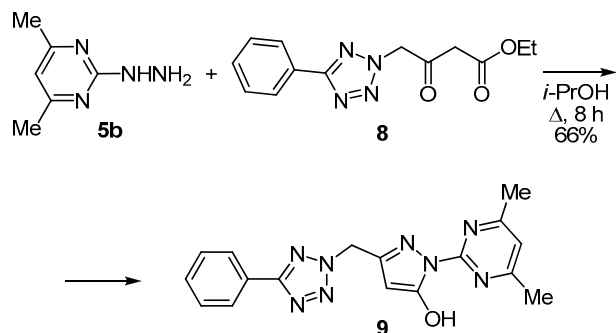
According to the results of X-ray structural analysis, compounds **7b** and **9** are nonplanar systems. The torsion angle HN(5)N(4)H of the hydrazine fragment is 110°. The nitrogen atom in the pyrimidine ring has a trigonal pyramid configuration. The phenyl substituent lies practically in the same plane as the azole fragment, while the planes of the heterocycles intersect at a significant (81°) angle to each other. The peculiarities of molecular packing in the solid state are determined by the presence of intermolecular hydrogen bonds with the participation of protons of the hydrazine fragment. A feature of the structure of compound **9** is its existence in the 5-hydroxypyrazole form, rather than that of 2,3-dihydropyrazol-3-one. In this case, the conformation of the compound turned out to be locked by
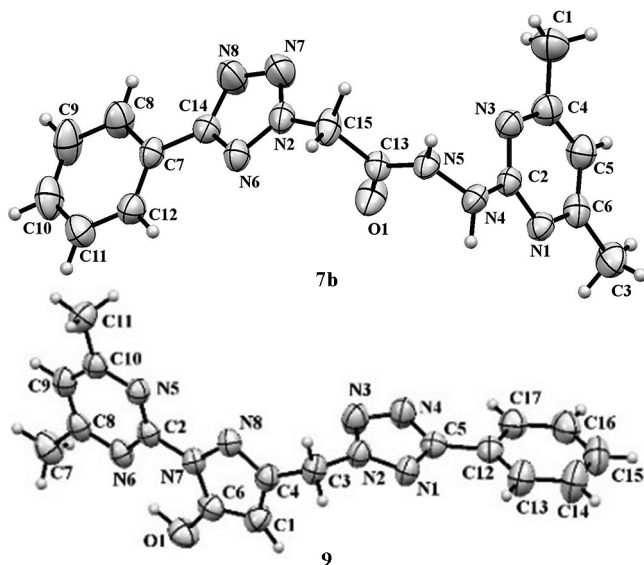

Figure 2. The molecular structures of compound **7b** and **9** with atoms represented as thermal vibration ellipsoids of 50% probability.

the intramolecular hydrogen bond O(1)–H(1)···N(6) with the distance O(1)···N(6) 2.599 Å. At the same time, the tetrazole and pyrazole fragments of the molecule at the tetrahedral carbon atom are turned in relation to each other at an angle of 88.4° which makes the conformation of this compound propeller-like. A solid-state structural feature is the packing of molecules in the form of stacks along the 0a axis in such a way that the pyrimidine fragments of neighboring molecules form interatomic π–π contacts at a distance that is 0.05 Å less than the sum of the van der Waals radii of the atoms of these fragments.

2-[(1-Phenyl-1*H*-tetrazol-5-yl)sulfanyl]pyrimidine (**12a**) was obtained by hetarylation of 1-phenyl-5-sulfanyl-tetrazole (**10a**) with 2-chloropyrimidine (**11**). Likewise, {5-[(4,6-dimethylpyrimidin-2-yl)sulfanyl]-1*H*-tetrazol-1-yl}-acetic acid (**12b**) was synthesized from 1-carboxymethyl-5-sulfanyltetrazole (**10b**) and 2-chloro-4,6-dimethyl-pyrimidine (**2**) (Scheme 4).

Scheme 4
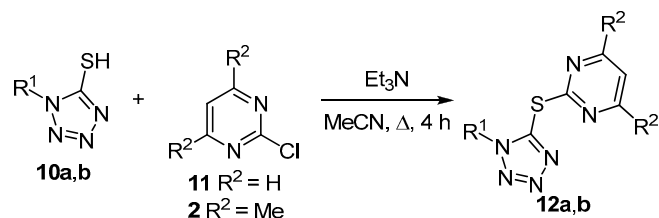
12 a R¹ = Ph, R² = H (46%), **b** R¹ = CH₂CO₂H, R² = Me (66%)

The prediction of the activity of nonannulated tetrazolylpyrimidines **7a**,**b**, **9**, and **12a**,**b**, performed using the PASS computer system,[11] indicates low (*Pa* 0.28–0.33) probability of antiviral activity for the compounds of this sample. However, low values of *Pa* factor are not always a verdict since they may indirectly indicate the novelty of the evaluated object.[12] In this study, we investigated the *in vitro* antiviral activity of nonannulated tetrazolyl-pyrimidines **7a**,**b**, **9**, and **12a**,**b** in the Laboratory of Experimental Virology of Saint Petersburg Pasteur Research Institute of Epidemiology and Microbiology. The study was carried out using the MDCK cell culture (ATCC CCL-34) against influenza virus strain A/Puerto Rico/8/34 (H1N1). Table 1 shows the values of 50% cytotoxic concentration (CC$_{50}$), 50% inhibitory concentration (IC$_{50}$), and selectivity index SI (the ratio of CC$_{50}$ to IC$_{50}$). Rimantadine was used as the reference drug. Compounds with a selectivity index of 10 and higher were considered promising.

The data in Table 1 illustrate that compounds **7a**,**b**, **9**, and **12a** did not show activity against influenza virus strain A/Puerto Rico/8/34 (H1N1). The best results were obtained for compound **12b**, the SI of which is twice that of the reference drug (rimantadine), but, in contrast to it, compound **12b** has significantly lower cytotoxicity. This result indirectly indicates the promise of further study of compounds of this group and an expanded search for substances with pronounced antiviral activity among non-annulated tetrazolylpyrimidines the heterocyclic fragments

Table 1. Antiviral properties of nonannulated tetrazolyl-
pyrimidines **7a**,**b**, **9**, and **12a**,**b** against
influenza A H1N1 virus in MDCK cell cultures

Compound	CC_{50}, µg/ml	IC_{50}, µg/ml	SI
7a	>300	>300	1
7b	>300	>300	1
9	53.9	>30	2
12a	>300	170	2
12b	>300	31	10
Rimantadine	60	12	5

of which are separated by a sulfur atom. The synthesis and
study of the antiviral activity of analogs of compound **12b**
containing various functional groups at the nitrogen atom
in position 1 of the tetrazole ring deserve further
development. Importantly, the influenza virus used in the
study is resistant to rimantadine (SI 5). The results of the
study of tetrazolylpyrimidine **12b** indicate that this
compound may have a different mechanism of interaction
with the target as compared to adamantane derivatives.
This makes the evaluated group of compounds promising
as prototypes of anti-influenza drugs with an alternative
mechanism of action in comparison with existing drugs for
influenza therapy.

To conclude, nonannulated tetrazolylpyrimidines can be
recommended for *in vivo* evaluation of antiviral activity.

Experimental

IR spectra were registered on a Shimadzu 8400-FTIR
spectrometer in KBr pellets. ^{1}H and ^{13}C NMR spectra were
acquired on a Bruker Avance III spectrometer (400 and
100 MHz, respectively) in DMSO-d_6 (compounds **5a**,**b**, **6**,
7a,**b**, **8**, **9**, **12a**,**b**) and CDCl$_3$ (compound **4**) at 25°C with
TMS as internal standard. Elemental analysis was
performed on a Leco CHNS-932 elemental analyzer. Mass
spectra were recorded on a Bruker maXis impact ultra-high
resolution quadrupole time-of-flight mass spectrometer
(electrospray ionization, MeOH solvent). Melting points
were determined by the capillary method on a Büchi
M-560 apparatus with a heating rate of 1°C/min in the
melting range. Assessment of the purity of synthesized
compounds was done by TLC on Macherey-Nagel
Alugram Xtra SIL G plates, visualization in UV light
(254 nm).

The physiochemical properties of 1-phenyl-5-sulfanyl-
tetrazole (**10a**) and other commercial reagents, solvents,
and materials correspond to the data given in the catalogs
of Sigma-Aldrich, Merck, Fluka. 5-Phenyltetrazole sodium
salt (**1**),[13] 2-chloro-4,6-dimethylpyrimidine (**2**),[14] 1-carb-
oxymethyl-5-sulfanyltetrazole (**10b**),[15] and 2-chloro-
pyrimidine (**11**)[16] were obtained following known or
modified methods. The properties of compounds **1**, **2**, **10b**,
and **11** correspond to those described previously.[13–16]

**5,7-Dimethyl-3-phenyl[1,2,4]triazolo[4,3-*a*]pyrimidine
(4)**. 2-Chloro-4,6-dimethylpyrimidine (**2**) (0.86 g, 6.0 mmol)
was added with stirring to a suspension of 5-phenyl-
tetrazole sodium salt (**1**) (1.0 g, 6.0 mmol) in DMSO
(20 ml). The reaction mixture was heated to 150°C and

kept at this temperature for 8 h. The mixture was then
cooled, the formed precipitate was filtered off and dried in
a stream of air. Yield 0.81 g (61%), colorless crystals,
mp 259°C (decomp., PhMe–EtOH, 1:1) (decomp. temp.
258°C (PhMe–EtOH, 1:1)[9]). R_f 0.47 (CCl$_4$–*i*-PrOH, 4:1,
25°C). IR spectrum, ν, cm^{-1}: 3048 (C–H), 1630 (C$_6$H$_5$),
1518 (C$_4$N$_2$H), 1448(CN$_4$), 764 (C$_4$N$_2$H). ^{1}H NMR
spectrum, δ, ppm: 2.17 (3H, s, 5-CH$_3$); 2.56 (3H, s, 7-CH$_3$);
6.83 (1H, s, H pyrimidine); 7.51–7.65 (5H, m, H Ph).
^{13}C NMR spectrum, δ, ppm: 19.9 (C-5 CH$_3$); 24.8 (C-7
CH$_3$); 111.7 (C-6); 128.3 (C Ph); 129.7 (C Ph); 130.5
(C Ph); 131.5 (C Ph); 144.9 (C-3); 145.8 (C-9); 154.8
(C-7); 164.6 (C-5). Found, *m/z*: 225.1135 [M+H]$^+$.
C$_{13}$H$_{13}$N$_4$. Calculated, *m/z*: 225.1135. Found, %: C 69.22;
H 5.28; N 24.80. C$_{13}$H$_{12}$N$_4$. Calculated, %: C 69.62;
H 5.39; N 24.98.

2-Hydrazinopyrimidine (5a). N$_2$H$_4$·H$_2$O (1.1 ml,
14.0 mmol) was added with stirring at 10°C to a solution of
2-chloropyrimidine (**11**) (3.6 g, 25 mmol) in EtOH (15 ml).
The precipitate that formed after a few hours was filtered
off and dried in a stream of air. Yield 1.23 g (80%),
colorless crystals, mp 112–113°C (EtOH–H$_2$O, 1:1)
(mp 112–113°C (EtOH–H$_2$O, 1:1)[17]). R_f 0.11
(CCl$_4$–*i*-PrOH, 4:1, 25°C). IR spectrum, ν, cm^{-1}: 3255
(N–H val), 1602, 1577, 798 (C$_4$N$_2$H def). ^{1}H NMR
spectrum, δ, ppm: 3.52 (1H, br. s, NH); 6.58–6.60 (1H, m,
H Ar); 6.97 (2H, br. s, NH$_2$); 8.27–8.29 (2H, m, H Ar).
^{13}C NMR spectrum, δ, ppm: 110.9 (C-5); 158.3 (C-2);
164.8 (C-4,6). Found, *m/z*: 111.0665 [M+H]$^+$. C$_4$H$_7$N$_4$.
Calculated, *m/z*: 111.0665. Found, %: C 44.28; H 5.12;
N 51.02. C$_4$H$_6$N$_4$. Calculated, %: C 44.63; H 5.49; N 50.88.

2-Hydrazino-4,6-dimethylpyrimidine (5b). N$_2$H$_4$·H$_2$O
(6 ml, 124 mmol) was added with stirring at 10°C to a
solution of 2-chloro-4,6-dimethylpyrimidine (**2**) (3.6 g,
25 mmol) in EtOH (40 ml). The precipitate that formed
after a few hours was filtered off and dried in a stream of
air. Yield 2.4 g (70%), colorless crystals, mp 164–165°C
(EtOH–H$_2$O, 1:1) (mp 165°C (EtOH–H$_2$O, 1:1)[18]). R_f 0.54
(CCl$_4$–*i*-PrOH, 4:1, 25°C). IR spectrum, ν, cm^{-1}: 3307 (N–H,
val), 1371 (C–CH$_3$), 1596, 1467, 794 (C$_4$N$_2$H def).
^{1}H NMR spectrum, δ, ppm: 2.22 (6H, s, 2CH$_3$); 3.36 (1H,
br. s, NH); 6.38 (1H, s, H Ar); 7.88 (2H, br. s, NH$_2$).
^{13}C NMR spectrum, δ, ppm: 23.9 (2CH$_3$); 109.7 (C-5);
164.8 (C-2); 167.2 (C-4,6). Found, %: C 52.33; H 7.80;
N 40.54. C$_6$H$_{10}$N$_4$. Calculated, %: C 52.16; H 7.30;
N 40.55.

2-(5-Phenyltetrazol-2-yl)acetic acid chloride (6).
Crushed PCl$_5$ (6.75 g, 32 mmol) was added to crushed
2-(5-phenyltetrazol-2-yl)acetic acid (6.0 g, 29 mmol). The
reaction mixture was heated in a boiling water bath
(reaction flask was closed by a distillation drip adapter with
a calcium chloride tube) until the mixture was homogenized
and gas evolution stopped. Upon completion of the
reaction, boiling hexane (75 ml) was added to the hot
reaction mixture, and the contents of the flask was cooled
to 10°C. Acid chloride **6** was filtered off, washed on a filter
with chilled hexane, dried for 1 h under reduced pressure
over P$_2$O$_5$ in a pistol dryer heated with CHCl$_3$ vapor. Yield
6.43 g (96%), colorless crystals, mp 103–104°C (mp 103–

104°C (hexane)[19]). IR spectrum, ν, cm^{-1}: 2985, 2945 (C–H), 1777 (C=O), 1450, 1278, 1148, 1073, 1039, 1022, 923 (CN$_4$), 729, 688 (C$_6$H$_5$). ^{1}H NMR spectrum, δ, ppm: 5.84 (2H, s, CH$_2$); 7.53–7.49 (3H, m, H Ph); 8.17–8.14 (2H, m, H Ph). ^{13}C NMR spectrum, δ, ppm: 60.7 (CH$_2$); 126.6 (C Ph); 127.2 (C Ph); 129.2 (C Ph); 131.0 (C Ph); 166.2 (C tetrazole); 166.5 (C=O). Found, %: C 48.31; H 3.20; N 25.60. C$_9$H$_7$ClN$_4$O. Calculated, %: C 48.55; H 3.17; N 25.17.

N'-(Pyrimidin-2-yl)-2-(5-phenyl-2*H*-tetrazol-2-yl)aceto-hydrazide (7a), a mixture of isomers *E*/*Z* in a 3.6:1 ratio. Et$_3$N (0.97 g, 9.60 mmol) was added with stirring to a solution of 2-hydrazinopyrimidine (**5a**) (1.0 g, 9.08 mmol) in MeCN (20 ml). The reaction mixture was cooled to 0–5°C, and 2-(5-phenyltetrazol-2-yl)acetic acid chloride (**6**) (2.0 g, 8.98 mmol) was added with stirring. The reaction mixture was heated under reflux for 4 h. At the end heating, the solvent was removed under reduced pressure. The residue was washed with distilled H$_2$O (2×20 ml) and dried in a stream of air. Yield 1.3 g (48%), colorless crystals, mp 235–236°C (DMF–H$_2$O, 1:1). R_f 0.52 (CCl$_4$–*i*-PrOH, 4:1, 25°C). IR spectrum, ν, cm^{-1}: 3278 (NH val), 3033 (C–H val), 1689 (C=O val), 1448, 1043 (CN$_4$), 819, 788 (C$_4$N$_2$H def). ^{1}H NMR spectrum, δ, ppm: 5.64* (2H, s, CH$_2$); 5.66** (2H, s, CH$_2$); 6.80–6.82** (1H, m, H pyrimidine); 6.95–6.97* (1H, H pyrimidine); 7.55–7.60** (5H, m, H Ar); 8.02–8.09* (5H, m, H Ar); 8.40–8.42** (2H, m, H Ar); 8.53–8.54* (2H, m, H Ph); 9.17** (1H, s, N$\underline{H}$C$_4$N$_2$H$_4$); 9.32* (1H, s, N$\underline{H}$C$_4$N$_2$H$_4$); 9.84* (1H, s, NHC(O)); 10.45** (1H, s, NHC(O)). ^{13}C NMR spectrum, δ, ppm: 53.9* (CH$_2$); 54.1** (CH$_2$); 113.3** (C-5 pyrimidine); 114.4* (C-5 pyrimidine); 126.8 (C Ph); 127.2 (C Ph); 129.8 (C Ph); 131.1 (C Ph); 158.6 (C-2 pyrimidine); 163.0** (C=O); 163.2* (C=O); 164.6** (C-4,6 pyrimidine); 164.7* (C-4,6 pyrimidine); 169.4 (C tetrazole). Found, *m/z*: 297.1219 [M+H]$^+$. C$_{13}$H$_{13}$N$_8$O. Calculated, *m/z*: 297.1207. Found, %: C 52.77; H 4.12; N 37.49. C$_{13}$H$_{12}$N$_8$O. Calculated, %: C 52.70; H 4.08; N 37.82.

N'-(4,6-Dimethylpyrimidin-2-yl)-2-(5-phenyl-2*H*-tetra-zol-2-yl)acetohydrazide (7b), a mixture of isomers *E*/*Z* in a 2.9:1 ratio. Et$_3$N (0.44 g, 4.31 mmol) and 2-hydrazino-4,6-dimethylpyrimidine (**5b**) (0.6 g, 4.35 mmol) were added with stirring to a solution of 2-(5-phenyltetrazol-2-yl)acetic acid chloride (**6**) (0.96 g, 4.31 mmol) in MeCN (20 ml). The reaction mixture was heated under reflux for 4 h. The mixture was concentrated to half the original volume by evaporation under reduced pressure. The remaining mixture was cooled, the formed precipitate was filtered off, washed on a filter with distilled H$_2$O (2×20 ml), and dried in a stream of air. Yield 1.1 g (78%), colorless crystals, mp 228°C (DMF–H$_2$O, 1:1). R_f 0.54 (CCl$_4$–*i*-PrOH, 4:1, 25°C). IR spectrum, ν, cm^{-1}: 3307 (N–H val), 3033 (C–H val), 1683 (C=O val), 1450, 1041 (CN$_4$), 819, 788 (C$_4$N$_2$H def). ^{1}H NMR spectrum, δ, ppm: 2.26** (6H, s, 2CH$_3$); 2.33* (6H, s, 2CH$_3$); 5.61* (2H, s, CH$_2$); 5.62**

(2H, s, CH$_2$); 6.57** (1H, s, H pyrimidine); 6.71* (1H, s, H pyrimidine); 7.57–7.60 (3H, m, H Ph); 8.06–8.09 (2H, m, H Ph); 8.90** (1H, s, N$\underline{H}$C$_4$N$_2$H$_4$); 9.09* (1H, s, N$\underline{H}$C$_4$N$_2$H$_4$); 9.79* (1H, s, NHC(O)); 10.40** (1H, s, NHC(O)). ^{13}C NMR spectrum, δ, ppm: 23.9** (2CH$_3$); 24.0* (2CH$_3$); 53.9* (CH$_2$); 54.1** (CH$_2$); 111.9** (C-5 pyrimidine); 112.9* (C-5 pyrimidine); 126.8 (C Ph); 127.3 (C Ph); 129.8 (C Ph); 131.1 (C Ph); 162.9 (C-2 pyrimidine); 164.5** (C=O); 164.7 (C tetrazole); 167.6** (C-4,6 pyrimidine); 168.1 (C-4,6 pyrimidine); 169.2* (C=O). Found, *m/z*: 325.1530 [M+H]$^+$. C$_{15}$H$_{17}$N$_8$O. Calculated, *m/z*: 325.1520. Found, %: C 55.43; H 4.82; N 34.25. C$_{15}$H$_{16}$N$_8$O. Calculated, %: C 55.55; H 4.97; N 34.55.

Ethyl 3-oxo-4-(5-phenyl-2*H*-tetrazol-2-yl)butanoate (8). Ethyl 4-chloro-3-oxobutanoate (10.0 g, 61 mmol) and benzyltriethylammonium chloride (0.01 g, 0.044 mmol) were added with stirring at 80°C to a suspension of 5-phenyltetrazole sodium salt (**1**) (10.2 g, 61 mmol) in MeCN (150 ml). The reaction mixture was stirred at 80°C for 3 h and cooled to room temperature. The formed precipitate was filtered off and dried in a stream of air. Yield 10.65 g (56%), beige crystals, mp 75–76°C (PhMe–EtOH, 1:1). R_f 0.8 (CCl$_4$–*i*-PrOH, 4:1, 25°C). IR spectrum, ν, cm^{-1}: 1715, 1749 (C=O), 1530 (C$_6$H$_5$), 1452, 1044 (CN$_4$). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.22 (3H, t, *J* = 6.9, CH$_3$); 3.92 (2H, s, 2-CH$_2$); 4.15 (2H, q, *J* = 6.9, CH$_2$); 6.10 (2H, s, 4-CH$_2$); 7.56–7.59 (2H, m, H Ph); 8.06–8.10 (3H, m, H Ph). ^{13}C NMR spectrum, δ, ppm: 14.4 (CH$_3$); 46.6 (C-2); 61.2 (CH$_2$); 61.3 (C-4); 126.8 (C Ph); 127.1 (C Ph); 129.8 (C Ph); 131.1 (C Ph); 164.7 (C tetrazole); 166.8 (C(1)=O); 195.9 (C(3)=O). Found, *m/z*: 297.0958 [M+Na]$^+$. C$_{13}$H$_{14}$N$_4$NaO$_3$. Calculated, *m/z*: 297.0958. Found, %: C 56.89; H 5.01; N 20.27. C$_{13}$H$_{14}$N$_4$O$_3$. Calculated, %: C 56.93; H 5.15; N 20.43.

1-(4,6-Dimethylpyrimidin-2-yl)-3-[(5-phenyl-2*H*-tetrazol-2-yl)methyl]-1*H*-pyrazol-5-ol (9). Ethyl 3-oxo-4-(5-phenyl-2*H*-tetrazol-2-yl)butanoate (**8**) (1.16 g, 4.23 mmol) was added to a solution of 2-hydrazino-4,6-dimethylpyrimidine (**5b**) (0.58 g, 4.23 mmol) in *i*-PrOH (50 ml). The reaction mixture was heated under reflux for 8 h. The mixture was concentrated to half the original volume by evaporation under reduced pressure, the remaining mixture was cooled to room temperature, and 10% aqueous NaOH (20 ml) was added. The formed precipitate was filtered off, the filtrate was acidified with HCl to pH 2–3. The formed precipitate was filtered off and dried in a stream of air. Yield 0.97 g (66%), beige crystals, mp 175–176°C (PhMe). R_f 0.58 (CCl$_4$–*i*-PrOH, 4:1, 25°C). IR spectrum, ν, cm^{-1}: 1448, 1040 (CN$_4$), 784 (C$_4$N$_2$H def). ^{1}H NMR spectrum, δ, ppm: 2.50 (6H, s, 2CH$_3$); 5.71 (1H, s, H Ar); 5.93 (2H, s, CH$_2$); 7.28 (1H, s, H Ar); 7.56–7.58 (3H, m, H Ph); 8.06–8.08 (2H, m, H Ph); 12.27 (1H, s, OH). ^{13}C NMR spectrum, δ, ppm: 23.8 (2CH$_3$); 51.8 (CH$_2$); 87.0 (C-4 pyrazole); 118.2 (C-5 pyrimidine); 126.8 (C Ph), 127.3 (C Ph); 129.8 (C Ph); 131.1 (C Ph); 147.4 (C-3 pyrazole); 156.6 (C-2 pyrimidine); 158.1 (C-5 pyrazole); 164.7 (C tetrazole); 168.8 (C-4,6 pyrimidine). Found, *m/z*: 349.1527 [M+H]$^+$. C$_{17}$H$_{17}$N$_8$O. Calculated, *m/z*: 349.1520.

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* Hereinafter in the Experimental, one asterisk (*) denotes the signals of the Z-isomer, two asterisks (**) denote the signals of the E-isomer.

Found, %: C 58.35; H 4.71; N 32.36. $C_{17}H_{16}N_8O$. Calculated, %: C 58.61; H 4.63; N 32.17.

2-[(1-Phenyl-1*H*-tetrazol-5-yl)sulfanyl]pyrimidine (12a). Et_3N (0.85 g, 8.42 mmol) and 2-chloropyrimidine (**11**) (1.1 g, 9.60 mmol) were added with stirring to a solution of 1-phenyl-1*H*-tetrazole-5-thiol (**10a**) (1.5 g, 8.42 mmol) in MeCN (10 ml). The reaction mixture was heated under reflux for 4 h. The mixture was concentrated to half the original volume by evaporation under reduced pressure, the remaining reaction mixture was cooled, and H_2O (50 ml) was added. The formed precipitate was filtered off and dried in a stream of air. Yield 1.0 g (46%), beige crystals, mp 76°C (PhMe–EtOH, 1:1). R_f 0.17 (CCl_4–*i*-PrOH, 4:1, 25°C). IR spectrum, ν, cm^{-1}: 1554 (C_6H_5), 1444, 1045 (CN_4), 765 (C_4N_2H def). ^1H NMR spectrum, δ, ppm: 7.30–7.32 (1H, m, H pyrimidine); 7.56–7.57 (3H, m, H Ph); 7.69–7.71 (2H, m, H pyrimidine); 8.58–8.59 (2H, m, H Ph). ^{13}C NMR spectrum, δ, ppm: 119.8 (C-5); 125.7 (C Ph); 130.0 (C Ph); 131.2 (C Ph); 133.9 (C Ph); 148.3 (C-4,6); 159.3 (C-2); 167.1 (C tetrazole). Found, *m/z*: 257.0616 $[M+H]^+$. $C_{11}H_9N_6S$. Calculated, *m/z*: 257.0604. Found, %: C 51.59; H 3.28; N 32.44; S 12.62. $C_{11}H_8N_6S$. Calculated, %: C 51.55; H 3.15; N 32.79; S 12.51.

{5-[(4,6-Dimethylpyrimidin-2-yl)sulfanyl]-1*H*-tetrazol-1-yl}acetic acid (12b). Et_3N (1.27 g, 12.57 mmol) and chloro-4,6-dimethylpyrimidine (**2**) (0.89 g, 6.24 mmol) were added with stirring to a solution of 2-(5-sulfanyl-1*H*-tetrazol-1-yl)acetic acid (**10b**) (1.0 g, 6.25 mmol) in MeCN (25 ml). The reaction mixture was heated under reflux with stirring for 4 h. The solvent was removed under reduced pressure. The oily residue was dissolved in H_2O (20 ml). The resulting solution was acidified with HCl to pH 2–3 and extracted with EtOAc (3×20 ml). The combined extracts were washed with H_2O and dried over $MgSO_4$. EtOAc was removed under reduced pressure. Yield 1.1 g (66%), beige crystals, mp 92°C (PhMe). R_f 0.2 (CCl_4–*i*-PrOH 4:1, 25°C). IR spectrum, ν, cm^{-1}: 2995 (O–H), 1743 (CH_2–COOH), 1423, 1037 (CN_4 val), 883 (C_4N_2H def). ^1H NMR spectrum, δ, ppm: 2.31 (6H, s, $2CH_3$); 5.38 (2H, s, CH_2); 7.15 (1H, s, H pyrimidine); 13.72 (1H, br. s, OH). ^{13}C NMR spectrum, δ, ppm: 23.7 ($2CH_3$); 49.2 (CH_2); 118.8 (C-5); 148.5 (C-2); 165.3 (C tetrazole); 167.5 (C-4,6); 169.1 (C=O). Found, *m/z*: 267.0681 $[M+H]^+$. $C_9H_{11}N_6O_2S$. Calculated, *m/z*: 267.0659. Found, %: C 40.97; H 4.03; N 31.35; S 12.05. $C_9H_{10}N_6O_2S$. Calculated, %: C 40.60; H 3.79; N 31.56; S 12.04.

The study of the biological activity of compounds 7a,b, 9, 12a,b. A series of threefold dilutions (300–3.7 μg/ml) were prepared from the studied compounds. The dilutions were introduced into the wells of plates with a monolayer of MDCK cells. The plates were incubated for 72 h at 36°C in 5% CO_2. The analysis of cell survival was carried out using the methyl tetrazolium (MTT) assay: MTT solution was added to the wells, wherein by the action of mitochondrial enzymes it transforms into an insoluble violet formazan derivative.[20] The plates with cells were kept at 36°C in an atmosphere of 5% CO_2 for 2 h. The precipitate was dissolved in DMSO (0.1 ml per well). The optical density in the wells was measured on a Thermo Multiskan FC photometer (Thermo Fisher Scientific, USA) at a wavelength of 540 nm. Based on the obtained data, the CC_{50} value was calculated, that is, the concentration of the compound leading to a decrease in optical density by half compared to the wells without addition of compounds.

The study of the antiviral activity of compounds 7a,b, 9, 12a,b was carried by determining the reduction of the degree of cytopathic effect. The experiments used influenza virus strains A/Puerto Rico/8/34 (H1N1) and B/Florida/4/2006. The studied compounds in various concentrations were added to the cells in the wells of the plate, incubated for 1 h at 36°C in 5% CO_2, then the cells were infected with the virus at a dose of 0.01 $TCID_{50}$ per cell. The cells were incubated for 72 h at 36°C in 5% CO_2, after which the cell viability assay was performed using the methyl tetrazolium assay as described above. Based on the obtained data, a 50% inhibitory concentration was calculated for each compound, that is, the concentration that reduces the degree of viral cell destruction by 50%.

X-ray structural analysis of compounds 7b and 9 was carried out on an Xcalibur 3 automatic 4-circle diffracto-meter equipped with a CCD detector. All calculations were carried out in the Olex software shell using the SHELX software package.[21] The full set of X-ray structural data for compounds **7b** and **9** was deposited at the Cambridge Crystallographic Data Center (deposits CCDC 1818619 and CCDC 2047135, respectively).

References

1. Kiselev, O. I. *Khimiopreparaty i khimioterapiya grippa* (*Chemo Drugs and Influenza Chemotherapy* [In Russian]); Saint Petersburg: Rostok, 2011, p. 9.
2. Rusinov, V. L.; Charushin, V. N.; Chupakhin, O. N. *Russ. Chem. Bull., Int. Ed.* **2018**, *67*, 573. [*Izv. Akad. Nauk, Ser. Khim.* **2018**, 573.]
3. Sabitov, A. U.; Belousov, V. V.; Edin, A. S.; Oleinichenko, E. V.; Gladunova, E. P.; Tikhonova, E. P.; Kuzmina, T. Yu.; Kalinina, Yu. S.; Sorokin, P. V. *Antibiotiki i khimioterapiya* **2020**, *65*(7–8), 27.
4. Popova, E. A.; Trifonov, R. E.; Ostrovskii, V. A. *Russ. Chem. Rev.* **2019**, *88*, 644. [*Usp. Khim.* **2019**, *88*, 644.]
5. Rewcastle, G. W. *Comprehensive Heterocyclic Chemistry III*; Elsevier: Oxford, 2008, Vol. 8, p. 120.
6. (a) Egorova, A. P.; Makarov, V. A. *Russ. Chem. Bull., Int. Ed.* **2020**, *69*, 635. [*Izv. Akad. Nauk, Ser. Khim.* **2020**, 635.] (b) Zarubaev, V. V.; Golod, E. L.; Anfimov, P. M.; Shtro, A. A.; Saraev, V. V.; Gavrilov, A. S.; Logvinov, A. V.; Kiselev, O. I. *Bioorg. Med. Chem.* **2010**, *18*, 839.
7. Pochinok, V. Ya.; Avramenko L. F.; Grigorenko, P. S.; Skopenko, V. N. *Russ. Chem. Rev.* **1975**, *44*, 481. [*Usp. Khim.* **1975**, *44*, 1028.]

8. Scapin, E.; Salbego, P. R. S.; Bender, C. R.; Meyer, A. R.; Pagliari, A. B.; Orlando, T.; Zimmer, G. C.; Frizzo, C. P.; Bonacorso, H. G.; Zanatta, N.; Martins, M. A. P. *Beilstein J. Org. Chem.* **2017**, *13*, 2396.

9. Bower, J. D.; Doyle, F. P. *J. Chem. Soc.* **1957**, 727.

10. Ostrovskii, V. A.; Popova, E. A.; Trifonov R. E. *Adv. Heterocycl. Chem.* **2017**, *123*, 1.

11. Poroikov, V. V. *Biomed. Chem.* **2020**, *14*, 3, 216. [*Biomeditsinskaya Khimiya* **2020**, *66*, 30].

12. Filimonov, D. A.; Druzhilovskiy, D. S.; Lagunin, A. A.; Gloriozova, T. A.; Rudik, A. V.; Dmitriev, A. V.; Pogodin, P. V.; Poroikov, V. V. *Biomedical Chemistry*: *Research and Methods* **2018**, *1*(1), 1.

13. Tsentovskii, V. M.; Bashkirtseva, V. E.; Evgen'ev, M. I.; Ivanova, Z. P.; Poplavskii, V. S.; Ostrovskii, V. A.; Koldobskii, G. I. *Chem. Heterocycl. Compd.* **1983**, *19*, 1238. [*Khim. Geterotsikl. Soedin.* **1983**, 1556.]

14. Kosolapoff, G. M.; Roy, C. H. *J. Org. Chem.* **1961**, *26*, 1895.

15. DeSelms, R. Ch.; Kapecki, J. A. EP Patent 0299725.

16. Singh, J. N.; Lal, A. B. *J. Indian Chem. Soc.* 1**966**, *43*, 308.

17. Brown, D. J.; Ford, P. W. *J. Chem Soc. C* **1967**, 568.

18. Brady, L. E.; Herbst, R. M. *J. Org. Chem.* **1959**, *24*, 922.

19. Putis, S. M.; Zubarev, V. Yu.; Poplavskii, V. S.; Ostrovskii, V. A. *Chem. Heterocycl. Compd.* **2004**, *40*, 854. [*Khim. Geterotsikl. Soedin.* **2004**, 997.]

20. Mosmann, T. *J. Immunol. Methods* **1983**, *16*, 55.

21. Dolomanov, O. V.; Bourhis, L. J.; Gildea, R. J.; Howard, J. A. K.; Puschmann, H. *J. Appl. Crystallogr.* **2009**, *42*, 339.