2-(5-Phenyl-2*H*-tetrazol-2-yl)acetyl chloride as a key reagent in the synthesis of non-annulated polynuclear tetrazole-containing compounds with potential antidiabetic activity*

V. A. Ostrovskii,[a,b]★ N. T. Shmaneva,[a,b] I. S. Ershov,[a,b] D. V. Antonenko,[b] M. A. Skryl´nikova,[a,b] A. V. Khramchikhin,[b] E. N. Chernova,[a] A. Yu. Grishina,[c] N. A. Anisimova,[c] S. M. Napalkova,[c] O. V. Buyuklinskaya,[c] V. S. Mazhai,[b] Yu. N. Pavlyukova,[b] and R. E. Trifonov[b]

[a]*St. Petersburg Federal Research Center of the Russian Academy of Sciences, 39 14-ya liniya V.O., 199178 St. Petersburg, Russian Federation. Email: va_ostrovskii@mail.ru*
[b]*St. Petersburg State Institute of Technology (Technical University), 24—26/49 Moskovsky prosp., 190013 St. Petersburg, Russian Federation*
[c]*St. Petersburg State Chemical and Pharmaceutical University, lit. A, 14 ul. Professora Popova, 197022 St. Petersburg, Russian Federation*

Non-annulated tetrazole-containing polynuclear compounds, in which heterocyclic fragments are bound by an acetamide linker were synthesized. The synthesis was accomplished by acylation of 2-hydrazinyl-4,6-dimethylpyrimidine, 4-amino-4*H*-1,2,4-triazol-3-thiol, 2-[(1-amino-1*H*-tetrazol-5-yl)thio]-*N*-*tert*-butylacetamide, 1-methyl-1*H*-tetrazol-5-amine, and 2-methyl-2*H*-tetrazol-5-amine with one key reagent, (5-phenyltetrazol-2-yl)acetyl chloride. Preliminary *in silico* studies showed the presence of the *in vivo* antidiabetic (diabetes 2 type) activity in *N*´-(4,6-dimethylpyrimidin-2-yl)-2-(5-phenyl-2*H*-tetrazol-2-yl)acetohydrazide. *N*-(3-Mercapto-4*H*-1,2,4-triazol-4-yl)-2-(5-phenyl-2*H*-tetrazol-2-yl)acetamide demonstrated minimal hypoglycemic activity in *in vivo* studies, at the same time, it showed pronounced activity as a drug for combating obesity.

Key words: 2-(5-phenyl-2*H*-tetrazol-2-yl)acetyl chloride, acylation, synthesis, non-annulated heterocyclic compounds, biological activity, diabetes, post-diabetic disorders.

One of the important areas of medicinal chemistry is the search and study of the biological activity of tetrazole-containing non-annulated polynuclear (hybrid) compounds, in which the tetrazole ring is bound by a linker group to another, at least one, heterocyclic fragment.[1,2] The modern strategy for obtaining promising active ingredients of drugs is based on the development of rational schemes for the synthesis of target products using a minimum number of chemical steps.[3] We hypothesized that the key reagent for the synthesis of such compounds could be (5-phenyltetrazol-2-yl)acetyl chloride (**1**). Previously,[4] it was shown that the acylation of carbocyclic and heterocyclic amines with acyl chloride **1** proceeds smoothly and allows one to obtain the corresponding amides in good yields. It is important that, in contrast to (5-phenyltetrazol-2-yl)acetic acid ester, acylation with acyl chloride **1** is effective even in the case of low-basic amines.[4—6] In the development of rational methods for the synthesis of tetrazole-containing polynuclear compounds, the use of acyl chloride **1** can solve several problems at once: the introduction of a tetrazole ring in the design of molecules and the synthesis of drug candidates,[1] as well as a linker, in this case an acetamide group, binding the tetrazole ring with other heterocyclic fragments of the hybrid molecule.[4—6] As we have shown previously,[7] promising substrates for the acylation with acyl chloride **1** are 2-hydrazine derivatives of pyrimidine and 4,6-dimethylpyrimidine. The acylation products of these heterocyclic substrates showed pronounced antiviral activity.[7]

However, *N*-amino derivatives of 1,2,4-triazoles,[8] *N*-amino and *C*-amino derivatives of tetrazoles[1,9]

* Dedicated to Academician of the Russian Academy of Sciences O. N. Chupakhin on the occasion of his 90th birthday.

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* Vol. 73, No. 7, pp. 1977—1983, July, 2024.
1066-5285/24/7307-1977

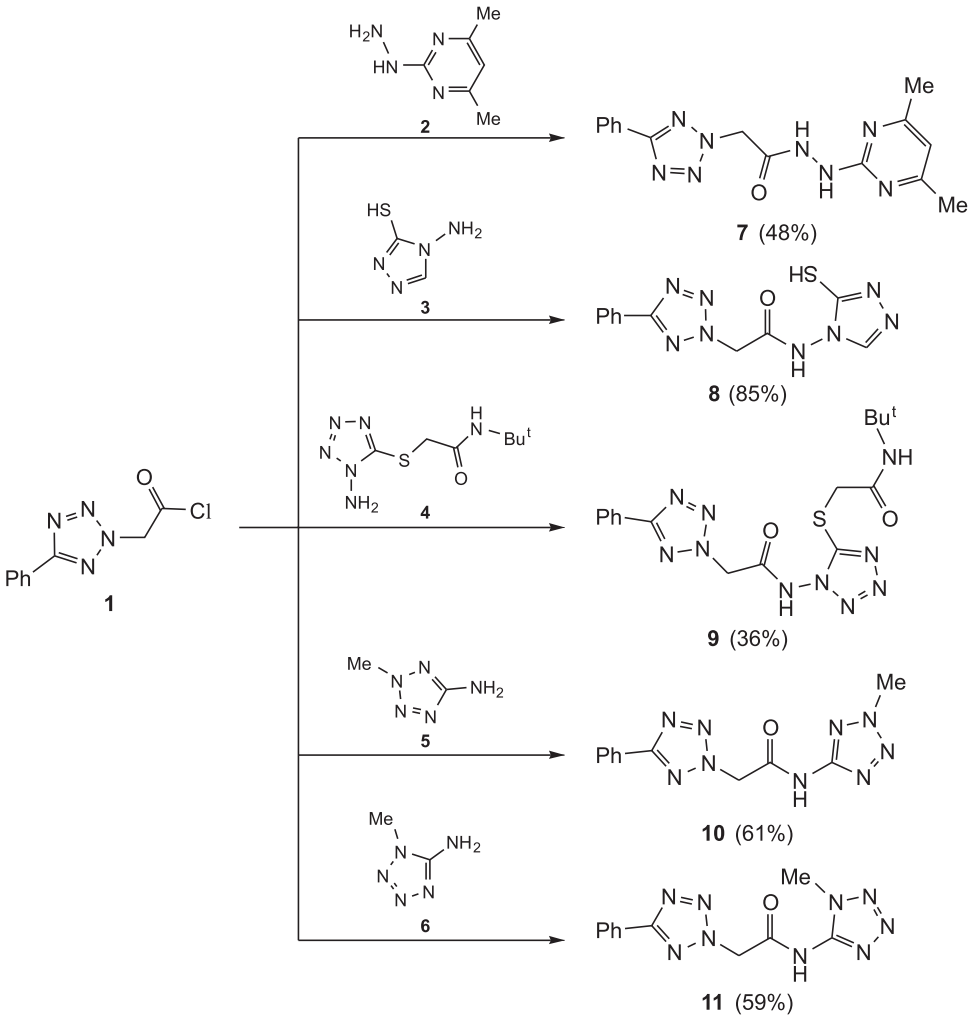
can be no less interesting and promising substrates for acylation.

Results and Discussion

In the present work, we used acyl chloride **1** to acylate 2-hydrazinyl-4,6-dimethylpyrimidine (**2**), 4-amino-4*H*-1,2,4-triazole-3-thiol (**3**), 2-[(1-amino-1*H*-tetrazol-5-yl)thio]-*N*-*tert*-butylacetamide (**4**), 2-methyl-2*H*-tetrazol-5-amine (**5**), and 1-methyl-1*H*-tetrazol-5-amine (**6**) and obtain the target products **7**—**11** (Scheme 1).

N′-(4,6-Dimethylpyrimidin-2-yl)-2-(5-phenyl-2*H*-tetrazol-2-yl)acetohydrazide (**7**) was obtained according to the previously described procedure,[7] *N*-(3-mercapto-4*H*-1,2,4-triazol-4-yl)-2-(5-phenyl-2*H*-tetrazol-2-yl)acetamide (**8**), *N*-*tert*-butyl-2-({1-[2-(5-phenyl-2*H*-tetrazol-2-yl)acetamido]-1*H*-tetrazol-5-yl}thio)acetamide (**9**), *N*-(2-methyl-2*H*-tetrazol-5-yl)-2-(5-phenyl-2*H*-tetrazol-2-yl)acetamide (**10**), and *N*-(1-methyl-1*H*-tetrazol-5-yl)-2-(5-phenyl-2*H*-tetrazol-2-yl)acetamide (**11**) were synthesized by us for the first time. Compounds **7**, **8**, **10**, and **11** were obtained in good yields (48—85%). The relatively low yield of compound **9** (36%) may be explained by a decrease in the rate of the main reaction, the nucleophilic substitution of the halogen atom, due to steric hindrances caused by the bulky substituent at the sulfur atom at position 5 of the tetrazole ring in compound **4**. In fact, during the synthesis of compound **9** a spot of (5-phenyltetrazol-2-yl)acetic acid (R_f 0.32, $CHCl_3$—MeOH (85 : 15, v/v) + 1 drop

Scheme 1



Reagents and conditions: Et_3N, MeCN, reflux, 4 h.

of AcOH) appeared on the TLC plate of the reaction mixture. This compound is formed in the reaction mixture as a result of hydrolysis of acyl chloride **1**, which competes with the main process of acylation of substrate **4**. Taking into account the presence of two exocyclic nucleophilic centers in compound **3**, one could have expected the formation of two acylation products: both at the nitrogen atom of the amino group and at the sulfur atom of the thiol group. Under the experimental conditions (see Scheme 1), the formation of an acylation product only at the amino group was observed, which is consistent with the data of the work.[10]

As is known, the tetrazole ring is a metabolically stable, bioisosteric analog of *cis*-amide and carboxy groups.[11] The most important role in the design of compounds with potential biological activity is played by functional groups capable of non-covalent interactions with receptors of living systems.[12] It was shown that tetrazoles, being weak bases, nevertheless exhibit the ability to effectively form hydrogen bonds involving both "pyridine" and "pyrrole" nitrogen atoms of the ring.[13]

Study of biological activity *in silico*. Before testing *in vivo*, we carried out computational prediction of biological activity of tetrazole-containing non-annealed polynuclear heterocyclic compounds **7—11**. At the first stage of the *in silico* study, we assessed the likelihood of various types of activity using the PASS Online 2022 program (https://www.way2drug.com/all/).[14—16]

For compounds **9—11**, the probability values for the presence of antidiabetic activity are moderate ($Pa \approx 0.400$—0.500), but, at the same time, compounds **9—11** are considered promising for testing antiviral activity. For compounds **7** and **8**, the obtained *Pa* values indicate a high probability of manifestation of the properties of an alpha-glucosidase inhibitor: $Pa = 0.751$ and 0.757, respectively. These data indicate the possibility of the presence of activity against type 2 diabetes. Type 2 diabetes mellitus (T2DM) is an incurable disease characterized by metabolic disorders and accompanied by hyperglycemia, as well as by the development of a number of dangerous diseases: hypertension, thrombosis, neurodegenerative disorders, and other complications.[17] Our analysis of the bibliography showed that compounds which are very potent in the treatment of type 2 diabetes can be found in the series of tetrazole derivatives and related heterocycles.[18,19]

In this regard, based on the above prediction (PASS), we carried out molecular docking, as well as *in vivo* studies of the antidiabetic activity of compounds **7** and **8**.

High-throughput virtual screening based on molecular docking is a molecular modeling method that allows one to position a low-molecular-weight compound (ligand) in the active center of an enzyme (target protein) and estimate the free energy of binding of the target protein to this ligand. The greater (in absolute value) the value of such energy, the stronger the ligand binds to the active site of the target protein, and therefore potentially has a stronger effect on the enzyme. Currently, one of the most used docking programs in the scientific community is the AutoDock Vina. As output information, the AutoDock Vina program provides the value of the interaction energy (free binding) of the ligand with the receptor, the "docking score" (evaluation function). In this case, a satisfactory result should be taken as values lying in the range from -6 kcal mol^{-1} and higher in absolute value.[20,21]

We chose a representative of the PPAR-γ group as the target protein. Peroxisome proliferator-activated receptor gamma (PPAR-γ or PPARG) is a popular target in predicting antidiabetic activity.[22] We performed the docking analysis of positioning of molecules **7** and **8** in the active site of the enzyme (target protein) PPAR-γ (the AutoDock Vina code sc-PDB 1i7i). As can be seen from Fig. 1, *a*, for molecule **7**, effective non-covalent interactions are possible involving heterocyclic fragments (tetrazolyl, 4,6-dimethylpyrimidinyl) and the active site of the enzyme.[11] A similar conclusion can be drawn by visual analysis of the positioning of molecule **8** in the active center of the same enzyme (Fig. 1, *b*). These data confirm the high (in absolute value) values of non-covalent binding energies (scoring) with the active site of PPAR-γ. For compound **7**, a high binding energy value was obtained (-8.8 kcal mol^{-1}) (see Fig. 1, *a*), it is lower for compound **8** (-7.8 kcal mol^{-1}) (see Fig. 1, *b*).

The results of *in silico* prediction of biological activity gave us grounds to recommend compounds **7** and **8** for *in vivo* study of antidiabetic activity.

Studies of biological activity *in vivo*. Existing scientific evidence and established guidelines do not provide acceptable *in vitro* alternatives to the use of laboratory animals as a test system in this work.[23] The *in vivo* study of antidiabetic activity was carried out

Table 1. Change in blood glucose levels over time after epinephrine injection (t = 0—3 h) in rats

Group of animals	Concentration of blood glucose (mmol L^{-1}) at different t			
	0	1	2	3
Control	6.32±0.37	13.44±1.76*	16.42±2.02*	20.36±3.73*
Metformin (300 mg kg^{-1})	6.52±0.49	7.20±1.65*	8.60±2.40*	6.90±1.07*
7 (100 mg kg^{-1})	5.56±0.34	7.78±2.46*	8.00±2.25*	8.92±1.78*

* Value of p compared to control group ($p < 0.01$).

in rats (compound **7**) and mice (compound **8**). A model of adrenaline hyperglycemia was used for rats.[24]

The results of the *in vivo* experiment for compound **7** on the model of adrenaline hyperglycemia in rats are presented in Table 1.

The results of the biochemical blood test in mice after administration of metformin or compound **8** for 14 days demonstrate minimal hypoglycemic activity (Table 2).

Impedancemetry analysis revealed comparable results for the group receiving compound **8** and the reference drug group. The content of adipose tissue in the body of animals of both groups differed: 49.40±2.95 g in mice of the control group and 40.26±2.34 (p = 0.0379) in mice treated with compound **8** (Fig. 2).

Table 2. Results of biochemical analysis of blood in mice

Group of animals	Concentration of blood glucose/mmol L^{-1}
Control	41.32±2.50
Metformin (300 mg kg^{-1})	28.93±1.75*
8 (100 mg kg^{-1})	35.83±2.56

* Value of p compared to control group ($p < 0.01$).

This phenomenon requires further study; it is planned to continue research with a reorientation from type 2 diabetes mellitus to obesity in this substance. Of interest is the fact that, apparently, the effectiveness in obesity is not related to the hypoglycemic effect, in contrast to the sensitizer metformin.

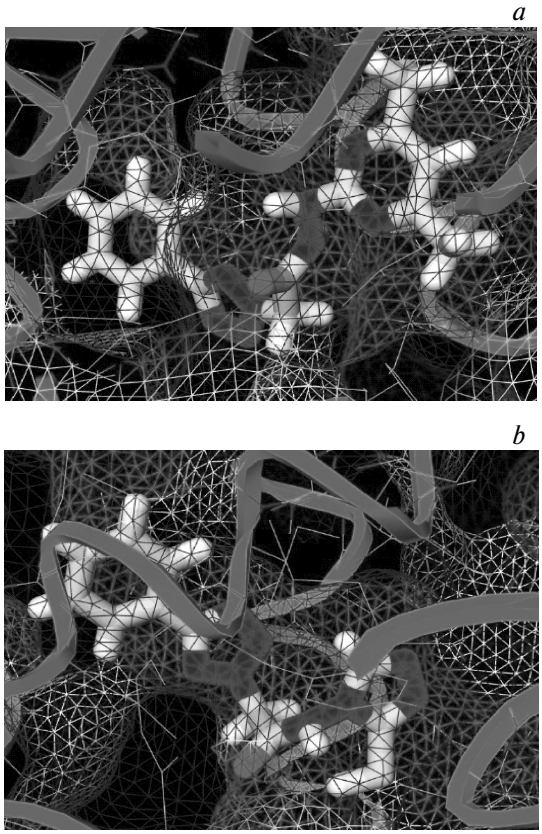


Fig. 1. Positioning of ligands, molecules **7** (*a*) and **8** (*b*), in the active site of PPAR-γ (code sc-PDB 1i7i) (docking), a model of a biological target macromolecule; binding energy −8.8 and −7.8 kcal mol^{-1}, respectively.

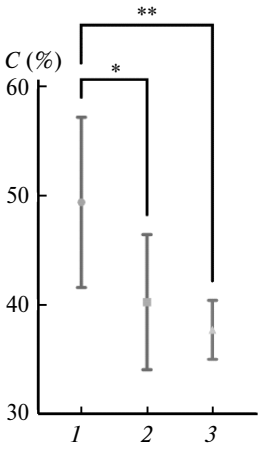


Fig. 2. Final values of fat percentage (*C*) in animals: control (*1*), compound **8** (100 mg kg^{-1}) (*2*), metformin (300 mg kg^{-1}) (*3*); the values of statistical significance threshold (p): *$p < 0.05$, **$p < 0.01$.

***

In conclusion, we developed a rational and unified method for the synthesis of tetrazole-containing polynuclear heterocyclic systems as promising platform compounds for the design, synthesis, and screening in the field of antidiabetic biological activity, including those with possible multi-target action.

Experimental

IR spectra were recorded on a Shimadzu 8400-FTIR spectrometer in KBr pellets, ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III spectrometer (^{1}H, 400 MHz; ^{13}C, 100 MHz) in DMSO-d_6 at 25 °C, using residual signals of the solvent DMSO-d_6 as references (δ_H 2.50, δ_C 39.51). Mass spectra were obtained on a Bruker maXis impact quadrupole time-of-flight ultrahigh resolution mass spectrometer (electrospray ionization, solvent MeOH). Melting points were measured on a Büchi M-560 apparatus at heating rate of 1 °C min^{-1} in the melting range. Purity of the synthesized compounds was monitored by TLC on Macherey-Nagel Alugram Xtra SIL G plates, visualizing under the UV light (254 nm).

(5-Phenyltetrazol-2-yl)acetyl chloride (**1**), 2-hydrazinyl-4,6-dimethylpyrimidine (**2**), 4-amino-4*H*-1,2,4-triazole-3-thiol (**3**), 2-methyl-2*H*-tetrazol-5-amine (**5**), 1-methyl-1*H*-tetrazol-5-amine (**6**), and *N′*-(4,6-dimethylpyrimidin-2-yl)-2-(5-phenyl-2*H*-tetrazol-2-yl)acetohydrazide (**7**) were obtained and purified as described previously.[7,25,26] Physicochemical properties of the synthesized compounds **1—3** and **5—7** are consistent with literature data. Due to the high hygroscopicity of acyl chloride **1**, it is necessary to store it over P_2O_5 in a drying pistol under vacuum.

2-[(1-Amino-1*H*-tetrazol-5-yl)thio]-*N*-*tert*-butylacetamide (4). *N*-*tert*-Butyl-2-chloroacetamide (1.0 g, 6.7 mmol) and sodium 1-amino-1*H*-tetrazole-5-thiolate (0.93 g, 6.7 mmol) were added to acetonitrile (40 mL). The reaction mixture was stirred for 2 h. The solvent was evaporated on a rotary evaporator at reduced pressure, the residue was diluted with water (50 mL) and stirred for 15 min, the precipitate was collected by filtration. The resulting compound was recrystallized from aqueous ethanol, m.p. 188—190 °C, R_f 0.38 ($CHCl_3$—MeOH (90 : 10, v/v), 24 °C). ^{1}H NMR, δ: 7.93 (s, 1 H, NH); 6.96 (s, 2 H, NH_2); 3.98 (s, 2 H, S—CH_2); 1.24 (s, 9 H, Me). ^{13}C NMR, δ: 165.48 (C=O), 154.03 (C—N_4), 50.61 (CH_2), 36.32 ($\underline{C}Me_3$), 28.33 (Me). IR, ν/cm^{-1}: 3321, 3197 (NH_2), 3085, 2935, 2885 (C—H), 1662 (C=O), 1550, 1458, 1272, 1218, 1103, 983 (CN_4). MS, found *m/z* 231.1025 $[M + H]^+$, calculated for $C_7H_{15}N_6OS$ 231.1023.

Synthesis of compounds 8—11 (general procedure). Amine **3—6** (4 mmol) was added to a solution of acyl chloride **1** (1.0 g, 4.5 mmol) in acetonitrile (50 mL) with stirring. The reaction mixture was cooled to 0—5 °C, followed by a dropwise addition of triethylamine (0.4 g, 4.0 mmol) and reflux for 4 h. The reaction progress was monitored by TLC ($CHCl_3$—MeOH (95 : 5, v/v)). After cooling the mixture to room temperature, the solvent was evaporated at reduced pressure on a rotary evaporator, the residue was washed with water (3×20 mL) and recrystallized from aqueous ethanol.

***N*-(3-Mercapto-4*H*-1,2,4-triazol-4-yl)-2-(5-phenyl-2*H*-tetrazol-2-yl)acetamide (8).** The yield was 0.56 g (85%), m.p. 272 °C (with decomp), R_f 0.32 ($CHCl_3$—MeOH (95 : 5, v/v)). ^{1}H NMR, δ: 14.01 (s, 1 H, SH); 12.14 (s, 1 H, NH); 8.67 (s, 1 H, CH); 8.10—8.08 (m, 2 H, CH_{Ph}); 7.59—7.57 (m, 3 H, CH_{Ph}); 5.88 (s, 2 H, CH_2). ^{13}C NMR, δ: 166.89, 164.86, 164.74, 142.96, 131.25, 129.83, 127.08, 126.88, 53.78 (CH_2). IR, ν/cm^{-1}: 3150 (NH), 1737 (C=O), 1280, 1180, 1072, 1044, 1026 (CN_4), 1326 (Me), 1610, 733, 665 (Ph), 553 (C—S). MS, found *m/z* 325.0591 $[M + Na]^+$, calculated for $C_{11}H_{10}N_8NaOS$ 325.0593.

***N*-*tert*-Butyl-2-({1-[2-(5-phenyl-2*H*-tetrazol-2-yl)-acetamido]-1*H*-tetrazol-5-yl}thio)acetamide (9).** The yield was 0.15 g (36%), colorless crystals, m.p. 168—169 °C, R_f 0.82 ($CHCl_3$—MeOH (95 : 5, v/v)). ^{1}H NMR, δ: 13.21 (s, 1 H, NH); 8.10—8.08 (m, 2 H, CH_{Ph}); 7.98 (s, 1 H, NH); 7.59—7.56 (m, 3 H, CH_{Ph}); 6.08 (s, 2 H, CH_2); 4.12 (s, 2 H, N(2)—CH_2); 1.25 (s, 9 H, Me). ^{13}C NMR, δ: 165.34 (C=O), 156.01 (C=O), 164.97, 164.84 (CN_4), 131.25, 129.80, 127.03, 126.92 (C_{Ph}), 53.80 (N(2)—CH_2), 51.19 (CH_2), 37.60 ($\underline{C}Me_3$), 28.76 (Me). IR, ν/cm^{-1}: 3401, 3117 (N—H), 2972, 2875 (C—H), 1649 (C=O), 1549, 1452, 1278, 1194, 1027, 965 (CN_4). MS, found *m/z* 417.1582 $[M + H]^+$, calculated for $C_{16}H_{21}N_{10}O_2S$ 417.1570.

***N*-(2-Methyl-2*H*-tetrazol-5-yl)-2-(5-phenyl-2*H*-tetrazol-2-yl)acetamide (10).** The yield was 0.7 g (61%), colorless crystals, m.p. 204—207 °C, R_f 0.46 ($CHCl_3$—MeOH (95 : 5, v/v), 24 °C). ^{1}H NMR, δ: 11.84 (s, 1 H, NH); 8.10–8.06 (m, 2 H, CH_{Ph}); 7.59—7.53 (m, 3 H, CH_{Ph}); 6.00 (s, 2 H, N(2)—CH_2); 3.92 (s, 3 H, Me). ^{13}C NMR, δ: 164.35 (C=O, CN_4); 149.25 (CN_4), 130.77, 129.37, 126.65, 126.40 (C_{Ph}), 54.81 (N(2)—CH_2), 34.13 (Me). IR, ν/cm^{-1}: 3255 (N—H), 2989, 2924 (C—H), 1726 (C=O), 1573, 1450, 1288, 1110, 1073, 1026, 972 (CN_4). MS, found *m/z* 286.1158 $[M + H]^+$, calculated for $C_{11}H_{12}N_9O$ 286.1159.

***N*-(1-Methyl-1*H*-tetrazol-5-yl)-2-(5-phenyl-2*H*-tetrazol-2-yl)acetamide (11).** The yield was 0.65 g (59%), colorless crystals, m.p. 195—199 °C, R_f 0.65 ($CHCl_3$—MeOH (95 : 5, v/v), 24 °C). ^{1}H NMR, δ: 11.79 (s, 1 H, NH); 8.10—8.06 (m, 2 H, CH_{Ph}); 7.59—7.53 (m, 3 H, CH_{Ph}); 5.88 (s, 2 H, N(2)—CH_2); 4.32 (s, 3 H, Me). ^{13}C NMR, δ: 164.31 (C=O), 162.93, 158.92 (CN_4), 130.72, 129.35, 126.73, 126.39 (C_{Ph}), 54.99 (N(2)—CH_2),

39.86 (Me). IR, ν/cm^{-1}: 3255 (N—H), 2935 (C—H), 1693 (C=O), 1573, 1450, 1226, 1199, 1041, 972 (CN_4). MS, found *m/z* 286.1156 $[M + H]^+$, calculated for $C_{11}H_{12}N_9O$ 286.1159.

Study of biological activity. An adrenaline hyperglycemia model was used for rats.[24] The experiment was performed on 15 white male rats weighing from 139 to 254 g, randomized in groups of five individuals. The fasting blood glucose concentration was preliminarily determined (mmol L^{-1}). Rats in the experimental group were administered a finely dispersed aqueous suspension (Tween-80 stabilizer) of compound **7** at a dose of 100 mg kg^{-1} intragastrically. As a reference drug,[27] we used the original metformin drug, Siofor 500®, series 0821021. Animals in the control group were administered purified water. After 30 min, a solution of adrenaline hydrochloride (1 mg mL^{-1}) was administered once at a dose of 1 mg kg^{-1} subcutaneously. The glucose concentration in the blood was measured again 1, 2, and 3 h after the administration of adrenaline using an Accu-Chek Active glucometer (Roche Diagnostics, Switzerland) and test strips. To do this, the animal´s gum was cut between the lower incisors and the blood was applied to a test strip. The results were recorded and the average value was calculated for each time period.

The study of the hypoglycemic activity of compound **8** was carried out on black mice of the C57BL/Ks-db+/+m line. This test system can be characterized as follows: coat color is black; inbreeding Fn+8, genotype a, db+/+m, animals carry the recessive diabetes gene db (8th linkage group, 4th chromosome), the db gene in the homozygous state causes diabetes, accompanied by abnormal obesity. Glycemia is very high, the level of glucose utilization is reduced, there is no insulin deficiency, males and females are infertile, the recessive m-misty gene, a color-lightening marker of the opposite chromosome, not carrying the db gene.[28] Animals were kept during the period of adaptation and experiment in groups of three to five individuals. The test sample was administered intragastrically for 14 days daily in a volume of 0.5 mL per animal. Animals in the control group received purified water in equivolume quantities. We also used a comparison drug, metformin (Siofor®) at a dose of 300 mg kg^{-1}, it was administered as a suspension. Blood was collected from the retro-orbital sinus, after which glucose levels were measured using an ERBA XL biochemical analyzer. Additionally, bioimpedance analysis was performed at the end of the treatment period using a ImpediVET® BIS1 spectroscopic impedance meter (ImpediMed Inc., USA) after preliminary anesthesia with zolazepam/tiletamine (Zoletil®, Virbac, France; 25 mg kg^{-1} intramuscular) with xylazine (Xyla®, Interchemie, Netherlands; 10 mg kg^{-1} intramuscularly). Fat mass (%) was determined by performing three consecutive measurements for each animal at an interval of 3 s at the given parameters: body proportion 1.0, body density 1.05 g cm^{-3}, hydration constant 0.732, resistance coefficients ρi = 1220.2 and ρe = 998.9.[29] Statistical analysis of the obtained data was performed using the GraphPad Prism 8 software package using the nonparametric Mann—Whitney statistical test.

Funding

This work was financially supported by the Russian Science Foundation (Project No. 23-13-00224, https://rscf.ru/project/23-13-00224/).

Animal Testing and Ethics

All animals were obtained from the "Rappolovo" Federal State Unitary Enterprise Laboratory Animal Nursery (Leningrad region) and kept under conditions of a 12/12-hour light-dark regime, receiving standard food and drinking water *ad libitum*. All manipulations with animals were carried out in accordance with the principles of the European Convention for the Protection of Vertebrate Animals Used for Experiments and Other Scientific Purposes (Strasbourg, 1986), Directive 2010/63/EU of the European Parliament and Council of the European Union dated 22.09.2010 on the protection of animals used for scientific purposes, Recommendation of the Board of the Eurasian Economic Commission dated November 14, 2023 No. 33 "On the Guidelines for working with laboratory (experimental) animals during preclinical (non-clinical) studies". All procedures with animals in the study were approved by the bioethical commission of the Saint-Petersburg State Chemical and Pharmaceutical University (SPCPU) of the Ministry of Health of the Russian Federation (minutes of meeting No. Rats-A/D-01/Diab-23, No. Mice-A/D-01/Diab-23 dated 15.09.2023). Manipulations with animals were carried out in accordance with the documents of the quality management system of the Center for Experimental Pharmacology of the SPCPU, each stage of the study was regulated by established Standard operating procedures.

Conflict of Interest

The authors declare no competing interests.

References

1. V. A. Ostrovskii, E. A. Popova, R. E. Trifonov, in *Advances in Heterocyclic Chemistry*, Eds C. F. V.

Scriven, Ch. A. Ramsden, Academic Press (An imprint of Elsevier), Cambridge—San Diego—London—Oxford, 2017, **123**, pp. 1—62; DOI: 10.1016/bs.aihch.2016.12.003.
2. V. A. Ostrovskii, E. A. Popova, R. E. Trifonov, in *Comprehensive Heterocyclic Chemistry IV*, Eds D. Black, J. Cossy, C. Stevens, Elsevier, Oxford, 2022, **6**, pp. 182—232; DOI: 10.1016/B978-0-12-818655-8.00131-1.
3. V. A. Ostrovskii, S. B. Miron, Yu. N. Pavlyukova, *Russ. Chem. Bull.*, 2023, **72**, 3037; DOI: 10.1007/s11172-023-4116-3.
4. S. M. Putis, V. Yu. Zubarev, V. S. Poplavskii, V. A. Ostrovskii, *Chem. Heterocycl. Compd.*, 2004, **40**, 854; DOI: 10.1023/B:COHC.0000044567.39872.b0.
5. V. A. Ostrovskii, V. S. Poplavskii, V. Yu. Zubarev, G. B. Erussalimsky, *Mendeleev Commun.*, 1996, **6**, 24; DOI: 10.1070/MC1996v006n01ABEH000562.
6. L. I. Vereshchagin, O. N. Verkhozina, A. G. Proidakov, A. I. Smirnov, V. N. Kizhnyaev, *Chem. Heterocycl. Compd.*, 2008, **44**, 1158; DOI: 10.1007/s10593-008-0163-9.
7. V. A. Ostrovskii, G. G. Danagulyan, O. M. Nesterova, Yu. N. Pavlyukova, V. V. Tolstyakov, O. S. Zarubina, P. A. Slepukhin, Y. L. Esaulkova, A. A. Muryleva, V. V. Zarubaev, R. E. Trifonov, *Chem. Heterocycl. Compd.*, 2021, **57**, 448; DOI: 10.1007/s10593-021-02922-6.
8. T. Opsomer, W. Dehaen, in *Comprehensive Heterocyclic Chemistry IV*, Eds D. Black, J. Cossy, C. Stevens, Elsevier, Oxford, 2022, **5**, pp. 78—118; DOI: 10.1016/B978-0-12-409547-2.14854-1.
9. D. Moderhack, *Heterocycles*, 2011, **83**, 1435; DOI: 10.3987/REV-11-693.
10. M. M. Kamel, N. Y. Megally Abdo, *Eur. J. Med. Chem.*, 2014, **86**, 75; DOI: 10.1016/j.ejmech.2014.08.047.
11. E. A. Popova, R. E. Trifonov, V. A. Ostrovskii, *Russ. Chem. Rev.*, 2019, **88**, 644; DOI: 10.1070/RCR4864.
12. *Nekovalentnye vzaimodeystviya v dizayne i sinteze novykh soedineniy* [*Noncovalent Interactions in the Design and Synthesis of New Compounds*], Eds A. M. Magerramov, K. T. Makhmudov, M. N. Kovylovich, A. J. L. Pombeiro, Tekhnosfera, Moscow, 2016, 624 pp. (in Russian).
13. M. V. Kaplanskiy, O. E. Faizullina, R. E. Trifonov, *J. Phys. Chem. A*, 2023, **127**, 5572; DOI: 10.1021/acs.jpca.3c02931.
14. V. V. Poroikov, D. A. Filimonov, T. A. Gloriozova, A. A. Lagunin, D. S. Druzhilovskiy, A. V. Rudik, L. A. Stolbov, A. V. Dmitriev, O. A. Tarasova, S. M. Ivanov, P. V. Pogodin, *Russ. Chem. Bull.*, 2019, **68**, 2143; DOI: 10.1007/s11172-019-2683-0.
15. D. A. Filimonov, D. S. Druzhilovsky, A. A. Lagunin, T. A. Gloriozova, A. V. Rudik, A. V. Dmitriev, P. V. Pogodin, V. V. Poroikov, *Biomedical Chemistry: Research and Methods*, 2018, **1**, e00004; DOI: 10.18097/BMCRM00004.
16. A. Lagunin, A. Stepanchikova, D. Filimonov, V. Poroikov, *Bioinformatics*, 2000, **16**, 747; DOI: 10.1093/bioinformatics/16.8.747.
17. S. Chatterjee, K. Khunti, M. J. Davies, *Lancet*, 2017, **389**, 2239; DOI: 10.1016/S0140-6736(17)30058-2.
18. R. E. Trifonov, V. A. Ostrovskii, *Int. J. Mol. Sci.*, 2023, **24**, 17190; DOI: 10.3390/ijms242417190.
19. V. L. Rusinov, I. M. Sapozhnikova, A. A. Spasov, O. N. Chupakhin, *Russ. Chem. Bull.*, 2022, **71**, 2561; DOI: 10.1007/s11172-022-3687-8.
20. O. Trott, A. J. Olson, *J. Comput. Chem.*, 2010, **31**, 455; DOI: 10.1002/jcc.21334.
21. V. V. Poroikov, *Biochemistry (Moscow), Supplement Ser. B: Biomed. Chem.*, 2020, **14**, 216; DOI: 10.1134/S1990750820030117.
22. P. Tiwari, R. Suravajhala, S. Gupta, P. Chaturvedi, S. K. Mathur, P. S. Solanki, B. Malik, *Int. J. Comput. Biol.*, 2019, **8**, 2; DOI: 10.34040/IJCB.8.1.2019.01.
23. *NIH Guide: Position Statement on the Use of Animals in Research,* 1993, **22**; https://grants.nih.gov/grants/guide/notice-files/not93-071.html.
24. *Rukovodstvo po provedeniyu doklinicheskikh issledovaniy lekarstvennykh sredstv* [*Guidelines for Conducting Preclinical Studies of Medicinal Products*], Part 1, Grif I K, Moscow, 2012, 944 pp. (in Russian).
25. A. Ismael, J. A. Paixão, R. Fausto, M. L. S. Cristiano, *J. Mol. Struct.*, 2012, **1023**, 128; DOI: 10.1016/j.molstruc.2012.04.018.
26. *Sintezy geterotsiklicheskikh soedineniy. Vypusk 6* [*Syntheses of Heterocyclic Compounds. Issue 6*], Ed. A. L. Mndzhoyan, AN SSSR, Moscow, 1964, 91 pp. (in Russian).
27. *Algoritmy spetsializirovannoy meditsinskoy pomoshchi bol'nym sakharnym diabetom*, v zhurn. *Sakharnyi diabet* [*Algorithms for Specialized Medical Care for Patients with Diabetes Mellitus,* in the journal *Diabetes Mellitus*], Eds I. I. Dedov, M. V. Shestakova, A. Yu. Mayorova, 9th issue (added), Endokrinologicheskiy Nauchnyi Tsentr, Moscow, 2019, **22**, 214 pp.; DOI: 10.14341/DM221S1 (in Russian).
28. F. Suriano, S. Vieira-Silva, G. Falony, M. Roumain, A. Paquot, R. Pelicaen, M. Régnier, N. M. Delzenne, J. Raes, G. G. Muccioli, M. Van Hul, P. D. Cani, *Microbiome*, 2021, **9**, 147, DOI: 10.1186/s40168-021-01097-8.
29. N. V. Demakova, M. V. Krasnova, G. A. Plisko, E. D. Semivelichenko, D. Yu. Ivkin, S. V. Okovity, *Laboratornye zhivotnye dlya nauchnykh issledovaniy* [*Laboratory Animals for Scientific Research*], 2021, **2**, 54; DOI: 10.29296/2618723X-2021-02-07 (in Russian).

Received April 4, 2024;
in revised form April 23, 2024;
accepted May 3, 2024