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Evaluation of *N*-Hydroxy-, *N*-Metoxy-, and *N*-Acetoxybenzoyl-Substituted Derivatives of Thymine and Uracil as New Substances for Prevention and Treatment of Long-Term Complications of Diabetes Mellitus

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Abstract—New uracil and thymine derivatives, N^1-, N^3- and N^1,N^3-(RO-benzoyl)-(1*H*,3*H*)-pyrimidine-2,4-diones, were synthesized (RO- is hydroxy, acetoxy- or methoxy-group). The compounds were studied in a complex of in vitro tests for the ability to inhibit the development of long-term complications of diabetes. Their ability to cleave cross-links of proteins has been evaluated. The most significant ways of pharmacological correction of thrombosis, angio-, nephro-, encephalo-, and cardiopathy, antiglycation, chelating, and antiplatelet activities, have been established. The most active compound in terms of antiplatelet action, N^1-hydroxybenzoyluracil, exceeded acetylsalicylic acid by ~44%. In terms of their ability to chelate copper (II) cations, all compounds (with the exception of 1,3-bis(3-hydroxybenzoyl)-(1*H*,3*H*)-pyrimidine-2,4-dione that was not not studied in this test) showed the activity, whose IC_{50} fell in the range between that for pioglitazone (44.1 μM) and pyridoxamine (136.7 μM) comparison drugs. The best antiglycation effect at the 1 mM concentration was observed for N^1,N^3-bismethoxy- and N^1,N^3-bisacetoxybenzoyl derivatives of thymine. The maximum activity to cleave cross-links of proteins (C = 1 mM), comparable to that of alagebrium, was established for 1,3-bis(4-methoxybenzoyl)uracil, for which also high rates of other estimated activities were noted. Thus, the N^1-, N^3- and N^1,N^3-(RO-benzoyl) derivatives of uracil and thymine are promising basics for creating drugs that suppress the development of long-term complications of diabetes.

Keywords: diabetes mellitus, long-term complications of diabetes mellitus, pyrimidine, thymine, uracil
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INTRODUCTION

DM is a complex medical and social problem worldwide, including in the Russian Federation. The primary disease is accompanied by the development of numerous complications. According to global medical and economic analyses, the annual cost of combating DM and the complex of its complications, including nephropathy, cardiopathy, angiopathy, polyneuropathy, encephalopathy, etc., account for about 12% of total public health financing [1]. Most of the funds are indirect costs due to the disability of the population associated with the development of long-term complications, the largest contribution among which is made by cardiovascular diseases [2]. However, there are no reliable drugs that can prevent the development of long-term effects of DM, the treatment and prevention of which for a long time faded into insignificance compared to the development of means for correcting glucose levels [3].

The basis of the pathogenesis of the most life-threatening distant complications of DM (damage to the cardiovascular system with the development of acute ischemia), as well as a number of polysystemic pathologies, lies in the general mechanism of disrupting the structural organization of proteins (collagens, elastin, crystallins, beta-amyloids, etc.) due to the formation of covalent bonds between them [4–8]. The process of formation of protein-protein cross-links actively occurs in the stroma of organs and vessels in addition to prolonged hyperglycemia and oxidative stress, observed in DM [4, 5, 9]. Similar cross-linking

Abbreviations: DM, diabetes mellitus; ROS, reactive oxygen species; GEP, glycation-end products; NMR, nuclear magnetic resonance; BSA, bovine serum albumin.

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processes are noted during aging of the body, Alzheimer's, Parkinson's and other diseases [6]. The formation of cross-linked proteins proceeds along the glucose-dependent pathway (glycation of amino acid residues ⇒ Schiff bases ⇒ Amadori products ⇒ Suyama or Namiki, or Hodge reaction ⇒ formation of aminoadipate ⇒ cross-linking) or the oxidative pathway (formation of ROS ⇒ Stadtman path ⇒ formation of aminoadipate (or other product of oxidative degradation of amino acids) ⇒ cross-linking) [7]. Both pathways include redox reactions catalyzed directly by transition metal cations (Cu^{2+}, Fe^{2+}, Fe^{3+}, etc.) or accelerated by ROS formed by metals [7, 8]. Thus, ROS and the ways of their formation, transition metals, including the effect on their electronic properties causing catalysis, the stages of reactions of formation of GEP, as well as covalent protein-protein bonds available for cleavage can serve as new targets of potential medicinal substances capable of slowing down the development of DM complications. It can be assumed that the effect on the listed targets and reactions can slow the development of long-term complications of DM. Moreover, taking into account the interrelation of the process of oxidative degradation of amino acids, dependent on the production of ROS and the presence of transition metals, with the formation of GEP, as mentioned above, the quantitative expression of the activity of compounds in their influence on the catalytic and ROS-producing functions of transition metals will likely be correlated with the ability of the same substances to prevent the glycation reaction from proceeding, i.e., with their antiglycation activity. That is, the ability of substances to bind transition metal cations and their antiglycation action should probably be related quantitatively. According to some literature data [10], the ability of compounds to bind transition metals and simultaneously inactivate the ROS they produce is called "multifunctional antioxidant action with chelating". Drawing a conclusion from the above, it can be assumed that the ability of compounds to be antioxidants and/or bind transition metals can be used in the fight against long-term complications of DM.

Many researches in modern medical chemistry are associated with the search for new biologically active structures as the basis for the creation of medicines and the development of effective methods for their synthesis. Pyrimidine derivatives, thymine and uracil, are of interest because some of their derivatives have the abovementioned properties. For example, nitrazolopyrimidine derivatives destroy cross-links of glycated proteins, inhibit the formation of GEP, and also bind copper ions [11].

Hemodynamics under conditions of the development of angiopathy in DM is prevented by increased atherogenesis and increased thrombus formation in addition to a decrease in fibrinolysis [12]. Disruption of hemostasis is associated with dysglycemia, and atherogenesis, that complicates the course of the disease, is mediated by pathological inflammation, partly determined by the impaired antioxidant status of the organism. Currently, antiplatelet agents (including cyclooxygenase inhibitors, for example, acetylsalicylic acid) are widely used to combat pathological thrombosis. In opposition to the development of atherosclerosis, a certain efficacy in clinical practice, especially in DM, was achieved by using substances with an antioxidant effect, for example, probucol, which protects lipoproteins from peroxidation and suppresses the formation of foam cells [13]. Considering the potential for creating new compounds that affect the processes of thrombus formation, it should be noted that the presence of antithrombotic activity is established for isomers of hydroxybenzoic acid [14], therefore, its derivatives can be the basis for creating agents with antiplatelet activity. In addition, pyrimidine-based substances are also reported to exhibit antiplatelet effect. For example, this has been established for derivatives of 2-aminopyrimidine, 2-substituted derivatives of 4,6-diaminopyrimidine, as well as for polycyclic compounds based on it [15, 16]. And as shown in [11], nitrazolopyrimidines have signs of an antioxidant effect, which is expressed in their inhibition of the autoxidation reaction of ascorbic acid. These data give additional medical and pharmacological significance to pyrimidine derivatives, as well as compounds-analogues of hydroxybenzoic acid, and the development of new antidiabetic agents on their basis.

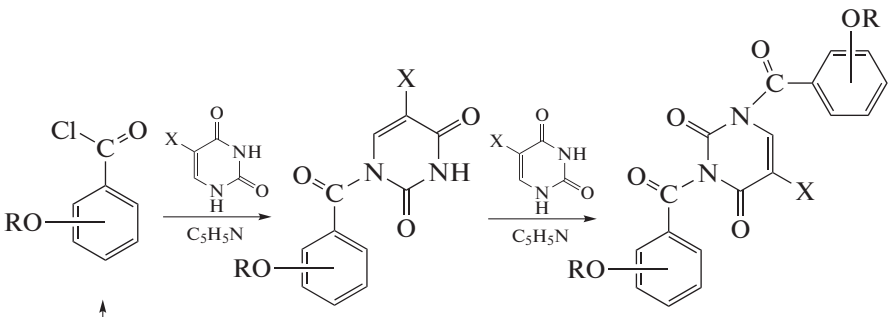
The purpose of our work is the synthesis and study of the biological properties of new *N*-(RO-benzoyl)pyrimidines (thymine and uracil derivatives, where RO is a methoxy, acetoxy or hydroxy group) as the basis for development of potential drugs for the treatment of complications of DM.

RESULTS AND DISCUSSION

The target compounds were synthesized by the acylation of the corresponding nitrogenous bases with substituted hydroxybenzoyl chlorides in pyridine at room temperature (Scheme).

Table 1. *N*-(RO-benzoyl)-derivatives of (1*H*,3*H*)-pyrimidine-2,4-diones (**I**)–(**XIV**) (Scheme).

Compound	5-X	RO-substituent in Bz		Name
		N^1	N^3	
(I)	H	3-OH	3-OH	1,3-Bis(3-hydroxybenzoyl)-(1*H*,3*H*)-pyrimidine-2,4-dione
(II)	H	4-OH	H	1-(4-Hydroxybenzoyl)-(1*H*,3*H*)-pyrimidine-2,4-dione
(III)	H	4-OH	4-OH	1,3-Bis(4-hydroxybenzoyl)-(1*H*,3*H*)-pyrimidine-2,4-dione
(IV)	H	2-OAc	H	1-(2-Acetoxybenzoyl)-(1*H*,3*H*)-pyrimidine-2,4-dione
(V)	H	4-OAc	H	1-(4-Acetoxybenzoyl)-(1*H*,3*H*)-pyrimidine-2,4-dione
(VI)	H	4-OMe	4-OMe	1,3-Bis(4-methoxybenzoyl)-(1*H*,3*H*)-pyrimidine-2,4-dione
(VII)	CH_3	3-OH	3-OH	1,3-Bis(3-hydroxybenzoyl)-5-methyl-(1*H*,3*H*)-pyrimidine-2,4-dione
(VIII)	CH_3	4-OH	H	1-(4-Hydroxybenzoyl)-5-methyl-(1*H*,3*H*)-pyrimidine-2,4-dione
(IX)	CH_3	4-OH	4-OH	1,3-Bis(4-hydroxybenzoyl)-5-methyl-(1*H*,3*H*)-pyrimidine-2,4-dione
(X)	CH_3	2-OAc	2-OAc	1,3-Bis(2-acetoxybenzoyl)-5-methyl-(1*H*,3*H*)-pyrimidine-2,4-dione
(XI)	CH_3	3-OAc	3-OAc	1,3-Bis(3-acetoxybenzoyl)-5-methyl-(1*H*,3*H*)-pyrimidine-2,4-dione
(XII)	CH_3	4-OAc	4-OAc	1,3-Bis(4-acetoxybenzoyl)-5-methyl-(1*H*,3*H*)-pyrimidine-2,4-dione
(XIII)	CH_3	4-OMe	H	1-(4-Methoxybenzoyl)-5-methyl-(1*H*,3*H*)-pyrimidine-2,4-dione
(XIV)	CH_3	4-OMe	4-OMe	1,3-Bis(4-methoxybenzoyl)-5-methyl-(1*H*,3*H*)-pyrimidine-2,4-dione



(II) X = H, OR = 4-OH
(IV) X = H, OR = 2-OAc
(V) X = H, OR = 4-OAc
(VIII) X = CH_3, OR = 4-OH
(XIII) X = CH_3, OR = 4-OMe

(I) X = H, OR = 3-OH
(III) X = H, OR = 4-OH
(VI) X = C, OR = 4-OMe
(VII) X = CH_3, OR = 3-OH
(IX) X = CH_3, OR = 4-OMe
(X) X = CH_3, OR = 2-OAc
(XI) X = CH_3, OR = 3-OAc
(XII) X = CH_3, OR = 4-OAc
(XIV) X = CH_3, OR = 4-OMe

Scheme.

N^1-Derivatives of uracil and thymine were synthesized by the acylation of the corresponding nitrogenous bases with an equimolar amount of a RO-substituted benzoyl chloride. To obtain the N^1,N^3-bis-derivatives, the initial reagents were used in a ratio of 2 : 1; the reaction was carried out in pyridine at room temperature. Thus, we synthesized the new compounds (Table 1), whose structure was confirmed by the ^{1}H NMR spectroscopy (Table 2).

The described experimental models for evaluation of biological activity imitate different stages of the pathogenesis of long-term complications of DM. Methods for assessing the copper-binding, antiglycation and cleaving cross-links activities of compounds

Table 2. Characterization of the obtained *N*-(RO-benzoyl)-substituted pyrimidines.

Compound	Yield,%	m.p., °C	Data of ^{1}H NMR spectra (DMSO-d_6), δ, ppm.
(I)	71	321–323	8.25–8.23 (1 H, d, Pyr), 7.86–7.82 (2 H, m, C_6H_4), 7.75–7.72 (2 H, m, C_6H_4), 7.30–7.26 (2 H, t, C_6H_4), 7.08 (2 H, s, OH), 6.95–6.94 (2 H, m, C_6H_4), 5.48–5.45 (1 H, d, Pyr)
(II)	81	355–356	6.86–7.89 (4 H, m, C_6H_4), 5.87–5.89 (1 H, d, Pyr), 7.90–7.92 (1H, d, Pyr), 1.86 (3H, s, CH_3), 10.27 (1 H, s, OH), 11.48 (1 H, s, NH)
(III)	76	348–350	8.16–8.14 (1H, d, Pyr), 8.19 (2H, s, OH), 7.39–7.37 (4H, d, C_6H_4), 6.82–6.80 (4H, m, C_6H_4), 5.47–5.45 (1H, d, Pyr)
(IV)	72	338–339	7.14–7.81 (4 H, m, C_6H_4), 5.86–5.88 (1 H, d, Pyr), 7.85–7.88 (1 H, d, Pyr), 2.38 (3H, s, $OC(O)CH_3$), 11.48 (1 H, s, NH)
(V)	77	319–320	7.28–7.85 (4 H, m, C_6H_4), 5.87–5.89 (1 H, d, Pyr), 7.87–7.89 (1 H, d, Pyr), 2.31 (3H, s, $OC(O)CH_3$), 11.48 (1 H, s, NH)
(VI)	82	319–322	7.92–7.88 (1H, d, Pyr), 7.73–7.68 (4H, m, C_6H_4), 7.33 (1H, d, Pyr), 7.05–7.01 (4H, m, C_6H_4), 3.85 (6H, s, $-CH_3$)
(VII)	80	313–314	6.93–8.22 (4 H, m, C_6H_4), 8.22 (1H, s, Pyr), 1.80 (3H, s, CH_3), 9.72 (2 H, s, OH)
(VIII)	73	382–383	6.84–7.62 (4 H, m, C_6H_4), 8.07 (1H, s, Pyr), 1.86 (3H, s, Pyr, CH_3), 10.27 (1 H, s, OH), 11.48 (1 H, s, NH)
(IX)	77	384–385	6.78–7.76 (8 H, m, C_6H_4), 8.06 (1H, s, Pyr), 1.80 (3H, s, CH_3), 10.27 (2H, s, OH)
(X)	86	331–332	7.16–7.96 (8 H, m, C_6H_4), 7.89 (1H, s, Pyr), 2.38 (6H, s, $OC(O)CH_3$), 1.91 (3H, s, CH_3)
(XI)	79	309–310	7.38–7.99 (8 H, m, C_6H_4), 8.04–8.05 (1H, d, Pyr) , 2.28 (6H, s, $OC(O)CH_3$), 1.80 (3H, s, CH_3)
(XII)	84	312–313	7.28–7.91 (8 H, m, C_6H_4), 8.06–8.07 (1H, d, Pyr), 2.31 (6H, s, $OC(O)CH_3$), 1.80 (3H, s, CH_3)
(XIII)	88	328–329	7.02–7.88 (4 H, m, C_6H_4), 8.07 (1H, s, Pyr), 3.86 (3H, s, OCH_3), 1.86 (3H, s, CH_3), 11.48 (1 H, s, NH)
(XIV)	69	323–324	6.94–7.90 (8 H, m, C_6H_4), 8.06 (1H, s, Pyr), 3.86 (6H, s, OCH_3), 1.80 (3H, s, CH_3)

Pyr – pyrimidine.

simulate the individual stages of the metabolic pathway for the cross-linked proteins formation. The summary data from studies of biological activities are given in Table 3.

As a result of evaluation of the antiplatelet effect of *N*-(RO-benzoyl)pyrimidine derivatives, we found that they are characterized by suppression of ADP-induced platelet functional activity at an inducer concentration of 5 μM. The analysis of results showed that the derivative **(I)** had shown the highest activity, significantly exceeding the result of acetylsalicylic acid (single factor analysis, Newman-Keuls posttest, $p < 0.05$). The compound is an uracil derivative, and is 1,3-bis(3-hydroxybenzoyl)-(1*H*,3*H*)-pyrimidine-2,4-dione. The antiplatelet activity of all other synthesized compounds was comparable to that of acetylsalicylic acid and, therefore, it can be concluded that the RO-benzoyl residue in the pyrimidine heterocycle is a promising substituent for targeted search of agents with antiplatelet activity. The hemodynamic disorder induced by the mechanism of increased thrombosis is one of the important parts in the pathogenesis of DM complications [17], and the pharmacological correction of this pathological process is an important contribution to the cumulative antidiabetic effect.

When evaluating copper-binding activity, we found that all compounds were sufficiently active in the copper-induced ascorbic acid autoxidation model. The exception was compound **(I)**, the activity level of which could not be determined due to the extremely high optical density of the substance at 265 nm, which is a limitation for the corresponding technique. Their IC_{50} values were in the range between those for the control substances, pyridoxamine (136.7 μM) and pioglitazone (44.1 μM). At the same time, none of the compounds statistically significantly exceeded pioglitazone in activity (Table 3). Thus, all the investigated substances can be considered as copper chelators, having a fairly high level of this activity, corresponding to the activity of pioglitazone and/or pyrodoxamine.

Table 3. Pharmacological activity of derivatives of uracil (**I**)–(**VI**) and thymine (**VII**)–(**XIV**)

Compounds/derivatives	Chelation, $IC_{50,}$ μM	Antiglycation, Δ% ($M \pm m$), $C = 1$ μM	Antiplatelet activity (platelets) Δ% ($M \pm m$), $C = 0.1$ μM	Cleavage of cross-links, Δ% ($M \pm m$), $C = 1$ μM
(I)	N.A.	−7.40 ± 2.40	25.10 ± 2.30	5.13 ± 9.69
(II)	117.5 ($R^2 = 0.98$)	24.40 ± 2.10*	21.70 ± 1.80	–
(III)	107.2 ($R^2 = 0.99$)	12.00 ± 4.90	18.12 ± 1.52	–
(IV)	148.2 ($R^2 = 0.99$)	−5.20 ± 4.00	15.90 ± 1.14	–
(V)	174.8 ($R^2 = 0.98$)	−9.70 ± 0.80	20.08 ± 1.64	6.32 ± 6.76
(VI)	67.5 ($R^2 = 0.93$)	13.50 ± 2.00*	18.68 ± 1.90	22.19 ± 3.28
(VII)	108.1 ($R^2 = 0.98$)	14.50 ± 4.00	17.21 ± 1.18	–
(VIII)	120.5 ($R^2 = 0.98$)	−18.50 ± 7.10	21.35 ± 2.70	–
(IX)	128.3 ($R^2 = 0.99$)	2.40 ± 7.80	15.28 ± 0.79	–
(X)	50.7 ($R^2 = 0.99$)	25.60 ± 6.90	19.38 ± 2.50	–
(XI)	73.9 ($R^2 = 0.98$)	27.80 ± 2.30*	13.59 ± 0.74	–
(XII)	71.0 ($R^2 = 0.96$)	33.70 ± 1.80*	14.14 ± 2.30	–
(XIII)	32.6 ($R^2 = 0.99$)	35.60 ± 1.10*	20.43 ± 1.43	–
(XIV)	45.6 ($R^2 = 0.98$)	8.00 ± 3.80	19.15 ± 2.10	–
Aminoguanidine	>1000.0	43.40 ± 2.40*	N. A.	N. A
Acetylsalicylic acid	N.A.	N.A.	17.38 ± 1.37	N.A.
EDTA	0.7 ($R^2 = 0.95$)	N.A.	N.A.	N.A.
Pioglitazone	44.1 ($R^2 = 0.98$)	N.A.	N.A.	N.A.
Lipoic acid	68.9 ($R^2 = 0.96$)	N.A.	N.A.	N.A.
Pyridoxamine	136.7 ($R^2 = 0.98$)	N.A.	N.A.	N.A.
Alagebrium (ALT-711)	N.A.	N.A.	N.A.	23.02 ± 8.79

N.A., the compound was not tested or, due to limitations of the method, the activity could not be established; "–", the compound did not exhibit activity in the test; Univariate analysis of variance, Newman-Keuls posttest, $p < 0.05$; $M \pm m$ correspond to the arithmetic mean (M) and the standard error of the arithmetic mean (m); R^2 value corresponds to the coefficient of determination in the IC_{50} estimation by the linear regression method and indicates how much the conditional dispersion of the model (line) differs from the dispersion of real values.

Evaluating the results established by this method, we carried out a structural and functional analysis of the compounds. As a result, we found a correspondence between the presence of a higher chelating activity and the presence of a methoxy group (compound (**VI**), (**XIII**) and (**XIV**)) as a substituent in the benzoyl radical, but not hydroxy or acetoxy groups whose carriers had lower activity. However, we also noted that the molecular core probably affects the manifestation of this activity. Uracil-based compounds were able to bind copper more actively in the autoxidation reaction of ascorbic acid than thymine derivatives, and the activity level of uracil-based compounds was higher in IC_{50}.

In general, taking into account the peculiarities of the ascorbic acid autoxidation model, the ability of compounds to exert antioxidant and chelating effects in a system in which the oxidation inducer is a transition metal cation makes it possible to additionally consider (RO-benzoyl)-containing pyrimidine derivatives as compounds capable of giving pharmacological correction of oxidative stress under conditions of DM, in particular, accompanied by disordered transition metals homeostasis. This approach is potentially promising in the treatment of long-term complications of DM, taking into account the characteristics of their pathogenesis [9].

Evaluating the antiglycation activity of *N*-(RO-benzoyl)-pyrimidine derivatives at a concentration of 1 mM, we noted the presence of statistically significant activity for compounds (**II**), (**VI**), (**XII**), (**XIII**), and (**XIV**). The compounds differ both in the substituent in the RO-benzoyl residue and in the number of *N*-substitutions (mono- and disubstituted) in the pyrimidine ring. However, by visual comparison, it was noted that higher antiglycation activity is inherent in compounds with uracil core than in ones with thymine core, similar to the fact that the chelating activity of the tested compounds was also more pronounced in uracil derivatives.

Based on the fact that the ability of compounds to eliminate the catalytic and ROS producing abilities of

copper cations correlates with the antiglycation activity [7, 8], the level of these activities was compared. Between the two activities, the Spearman's rank correlation coefficient $r(s) = -0.7$ was determined at a significance level of $p \le 0.05$ (the negative value of $r(s)$ is due to the comparison of the IC_{50} values of the chelating activity and the Δ indicator, % of the antiglycation action). Compound (**I**) was excluded from the correlation analysis, which is associated with its high light absorption at λ 265 nm and the associated inability to establish copper-binding activity.

The correlation of the two activities can be associated with:

a) The presence of the required target in both experimental models and its role in the key reaction processes.

b) Common functional groups of compounds involved in both reactions.

c) Common factors affecting the course of both reactions.

Discussing item *a*), it should be mentioned that the presence of copper (II) cations permanently bonded to BSA affects the glycation kinetics [18], so BSA itself can act as a source of Cu^{2+} during the reaction.

For item *b*) the commonality of the groups implies that the same pharmacophore groups of the compound are involved in the manifestation of both activities, that is, the fragments of the molecule responsible for the manifested action. In one case, these groups act as binding sites for the transition metal; in the other, they participate in the inactivation of carbonyl intermediates. At the same time, the submolecular mechanisms of the participation of functional groups in reactions are different, and the tendency for the realization of one or other of the two mentioned reactions may vary under the influence of environmental conditions. pH is one of the factors on which these pharmacophore groups depend on metal binding or inactivation of carbonyl intermediates. This is noted for the drug metformin, which is widely used in treatment of diabetes of the second type. The drug simultaneously exhibits antiglycation and chelating properties [8], and at the same time, it has two potential binding sites for metals, which under certain conditions are also involved in dicarbonyl binding reactions. At a neutral pH, metformin is in a partially protonated (monoprotonated) state [19], which determines its multiple action, the ability to act as an antiglycation substance, directly binding carbonyl intermediates, and the ability to bind transition metals. This is due to the biophysical features, the ability to participate in the ion interaction, to act as a donor/acceptor of protons, to enter into π-π interactions [19]. At the same time, a more acidic environment, favorable for the preservation of groups in the protonated state, is also favorable for the occurrence of exchange reactions with dicarbonyls, as described in [8]. It is known that the formation of long-term complications of DM is accompanied by a shift in the pH value of the interstitial fluid towards acidification [20]. Thus, the same groups, apparently, are able to realize several variants of activity, which is associated with their biophysical features.

Item *c*) implies that ascorbic acid, due to its sensitivity to the direct oxidative effect of transition metals, as well as the ROS formed by them, is very convenient in studying the effect of substances on the oxidative agents of the redox reactions, which are included in the metabolic pathway of the formation of cross-linked proteins (clearly presented in [7]) in order to determine whether substances are capable of inhibiting the oxidative action of metals or highly reactive oxygen compounds. GEP formation reactions are one of the steps in this metabolic pathway. Besides this, the autoxidation reaction of ascorbic acid seems to be similar to the autoxidation of glucose, since both reactions proceed in the presence of transition metals in a similar way. When the glycation reaction proceeds, glucose autoxidation, which is one of the stages of this reaction, serves as one of the sources of carbonyl intermediates with high reactivity [21], which contributes to an increase in the rate of the reaction process. However, due to the similarity of the two reactions, it can be assumed that substances inhibiting autoxidation of ascorbic acid may slow down the autoxidation of glucose, which will also contribute to slowing down the reaction of formation of GEP while reducing the pool of highly reactive carbonyl intermediates that will be formed from less glucose.

Thus, the three arguments presented allow us to interpret the possible causes of the ratio of the two pharmacological activities of the compounds, as well as the two inextricably connected pathways of pathogenesis of the development of long-term complications of DM.

We found that only one of the synthesized compounds is able to cleave effectively proteins' cross-links. Compound (**VI**) (Table 3) was comparable in level to the control compound, alagebrium (ALT-711). Two more compounds, (**I**) and (**V**), proved to be of low activity. For all other studied compounds, cleaving cross-links activity was not found. For compound (**VI**) and alagebrium, the IC_{50} values determined were of 1.32 and 1.89 mM, respectively (Fig. 1). Compounds (**I**), (**V**), and (**VI**) have a uracil core.

Summarizing the evaluation of the pharmacological properties of the studied compounds, we can conclude that this class of compounds is promising as a basis for developing substances capable of suppressing the development of diabetes-associated pathias. All compounds exhibit antiplatelet effect to varying degrees (the highest activity was observed in compounds (**I**), (**II**), and (**VII**). This property is possibly related to the structural features of the benzoyl substituent. All synthesized compounds with the exception of (**I**) possess copper (II) chelating activity with signs of

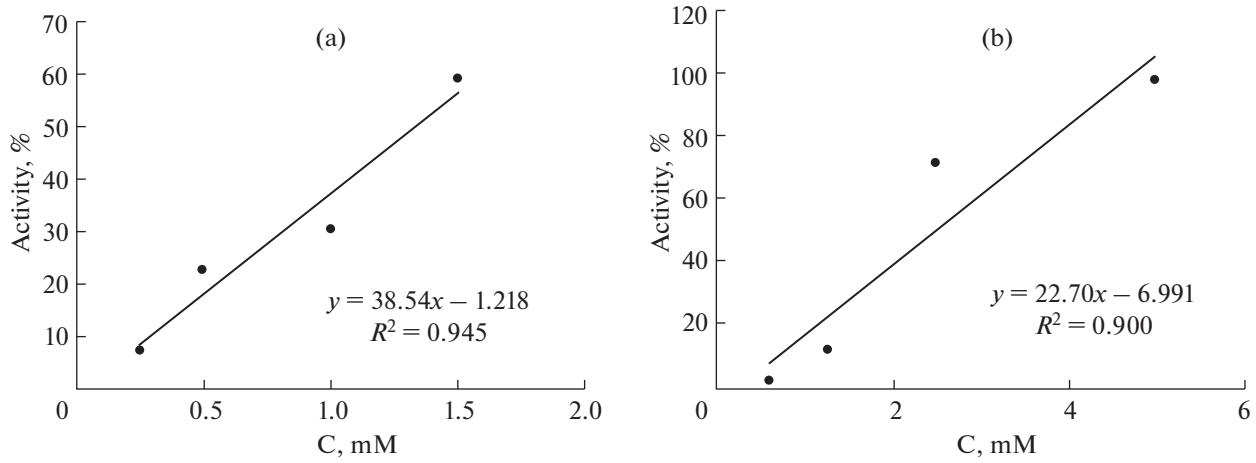


Fig. 1. The dependence of the activity to cleave cross-links of proteins of the compound (**VI**) (a) and alagebrium (b) on concentrations.

an antioxidant effect. Compound (**I**) had an extremely high optical density at λ 265 nm, which limited the opportunity in its study. The chelating activity is probably related and partially determines the presence of the antiglycation action (compound (**II**), (**VI**), (**XI**), (**XII**), (**XIII**)).

In the series of all considered thymine and uracil derivatives, the best activity profile was established for compound (**VI**), which is due to its ability to cleave protein-protein covalent cross-links. The formation of these cross-links is the final stage in the pathway of metabolic transformations occurring in the stroma in patients with DM, which is the basis of the pathogenesis of the development of some diabetes-associated pathias. In addition, giving an assessment of the potential medical significance of the established activity of the compound (**VI**), it can be said that among the activities of chelation of transition metals, suppression of the formation of GEP and cleavage of protein-protein cross-links, the latter is of particular importance.

Synthesized *N*-(RO-benzoyl)-substituted derivatives of thymine and uracil can be a promising basis for development of agents that slow down the development of diabetes-associated pathias and long-term complications of the disease. However, it is assumed that the additional cleaving cross-links activity of compound (**VI**), comparable to that of alagebrium, will not only slow down the development of long-term complications of DM, but also probably partially restore the biophysical properties of the finely organized stroma ultrastructure, which undergoes changes in this disease. Thus, the class of substances described has a high medical and pharmacological potential.

EXPERIMENTAL

Melting points were determined by the capillary method on a StuartSMP-30 instrument at a heating rate of 10°C/min. The purity and identity of the compounds was confirmed by thin-layer chromatography on Silufol UV-254 plates; the mobile phase was 2-propanol–water, 7 : 3; the spots were developed in iodine vapor and UV light. The ^{1}H NMR spectra of the derivatives were recorded on a Bruker DRX500 spectrometer in DMSO-d_6, hexamethyldisiloxane as an internal standard. To interpret the spectra, a licensed software product of the company Advanced Chemistry Development Inc. under the commercial name ACD/HNMR Predictor Pro v.3 was used.

For evaluation of the antiplatelet activity, a two-channel laser analyzer of platelet aggregation 230 LA (Biola, Russia) was used. For evaluation of antiglycation activity, the fluorescence of GEP was measured on a TECAN M 200 Pro spectrofluorometer ($\lambda_{(excitation)}$ 370 nm, $\lambda_{(emission)}$ 440 nm). The ability of compounds to cleave cross-links in proteins was determined using TECAN M 200 Pro in terms of light absorption (λ 450 nm). Chelating activity was determined on a spectrophotometer APEL PD 303 UV, Japan (λ 265 nm).

All solvents and reagents were obtained from commercial sources and were used without purification. **RO-substituted** benzoyl chlorides were synthesized according to the reported procedures [22, 23].

General procedure for the synthesis of 1,3-bis(RO-benzoyl)-(1*H*,3*H*)-pyrimidine-2,4-diones (I), (III), (VI), (VII), (IX), (X) derivatives), (XI), (XII), (XIV) [24]. A solution of 200 mmol of RO-benzoyl chloride in 10 mL of chloroform was added dropwise over 15 min to a solution of 100 mmol of uracil or thymine in 30 mL of dry pyridine. A solid product and pyridine hydrochloride were precipitated. The mixture was stirred for 1 h at room temperature. Then the solution

was filtered under vacuum; the precipitate was washed with cold water and recrystallized from benzene.

N^1-Derivatives of uracil and thymine (**II**), (**IV**), (**V**), (**VIII**), (**XIII**) were obtained similarly at a molar ratio of starting materials of 1 : 1.

Yields, melting points and NMR spectral data of compounds (**I**)–(**XIV**) are given in Table 1.

Experimental (biology)

Antiplatelet activity of substances was investigated at a concentration of 100 μM on a model of ADP–induced (Reanal, Hungary) aggregation of rabbit platelets by changing the degree of light transmission of plasma rich in platelets [25]. The activity of the substances was evaluated by a decrease in platelet aggregation relative to the control. Acetylsalicylic acid (Shandong Xinhua Pharmaceutical Co., Ltd, China) was used as a control drug. Statistical data processing was performed using Microsoft Excel 2007 software, as well as GraphPadPrism 5.0 software using univariate variational analysis with the Newman-Keuls posttest ($p < 0.05$) and preliminary verification of the normality of the distribution using the Kolmogorov–Smirnov criterion.

Antiglycation activity was determined by the method reported in [16]. The reaction medium for the glycation reaction contained 500 mM of glucose and 1 mg/mL of BSA (fraction V), dissolved in a phosphate buffer solution (pH 7.4). Test compounds were dissolved in 99% DMSO. The final concentration of substances after their addition to the reaction medium was 1 mM. Control samples contained an equivalent volume of DMSO. Samples were incubated for 24 h at 60°C. Aminoguanidine hydrochloride (Sigma-Aldrich) was used as a control substance. Statistical data processing was performed using Microsoft Excel 2007 software, as well as GraphPadPrism 5.0 software using univariate variational analysis with the Newman-Keuls posttest ($p < 0.05$) and preliminary verification of the normality of the distribution using the Kolmogorov–Smirnov criterion.

Chelating activity of the compounds was studied in a concentration range of 10–200 μM by the method of suppressing the copper-dependent autoxidation of ascorbic acid [26, 27]; the IC_{50} value were estimated. Lipoic acid, pioglitazone and pyridoxamine [8] were used as controls, for which a combination of chelating and antipathia properties was shown [28–30]. The compounds were dissolved in 99% DMSO. The indicator of the activity of copper (II) cations was 100 μM ascorbic acid solution; the source of Cu^{2+} was $CuSO_4 \cdot 5H_2O$ in a final concentration of 150 μM (95 μM in terms of the same mass of anhydrous salt). Complexation was carried out by preliminary mixing and incubation for 5 min at 37°C of a solution of copper sulfate and the test substance or DMSO. The mixture was added to a solution of ascorbic acid. Autoxidation was recorded spectrophotometrically in a low-temperature quartz cuvette (λ 265 nm). The IC_{50} of test substances were determined by the linear regression method (Microsoft Excel 2007). Statistical data processing was performed using Microsoft Excel 2007 software, as well as GraphPad Prism 5.0 software using the Mann-Whitney pairwise comparison criterion, as well as a single-factor variational analysis at $p < 0.05$.

The ability to cleave the cross-links of glycated proteins was investigated by measuring the optical absorption of solutions previously obtained by incubating a BSA solution (50 mg/mL) with glucose (0.5 M) for three months, introduced into the medium containing collagen to form cross-links with this protein [31]. Collagen was isolated from the tails of rats three to five months old weighing about 200 g, devitalized by decapitation under ether anesthesia. The material of the connective tissue of the tail, collected by the operational method, was immediately transferred to a 0.1% solution of acetic acid for acid dissolution of collagen and its extraction for seven days. Then the contents of the vessels were centrifuged at 8000 g (10 min) to precipitate residual fibers and the collagen-rich supernatant was collected. The resulting collagen-containing gel was used to coat the bottom of flat-bottomed, transparent 96-well microplates (70 μL of gel per well). Microplates were carefully treated with a phosphate buffer (pH 7.4) for 1 h to neutralize acidic collagen. The plates were blocked with Superblock (PIERCE) at 37°C for 1 h. Then, a solution of BSA (fraction V) was added and incubation was carried out at 37°C for 4 h to form cross-links. A solution of the compound in DMSO (10 μL) at a final concentration of 1 mM was added to the wells with glycated protein and to the wells with BSA. The plate was incubated at 37°C for 16 h. Then, 80 μL/well of antibodies against BSA (1 : 500) were added to the wells, and the plate was incubated at 37°C for 50 min. Next, 80 μL/well of horseradish peroxidase-labeled goat anti-rabbit IgG (1 : 1000) were added to the wells. The wells were incubated at 37°C for 50 min. Subsequently, 3,3',5,5'-tetramethylbenzidine (100 μL/well) was added. The plate was incubated at room temperature in the darkness for 20 min. To stop the reaction, a 2M solution of H_2SO_4 was used. Within 10 min after the reaction, the optical absorption was recorded at 450. Alagebrium (Sigma-Aldrich) was used as a control substance. The results were processed in Microsoft Excel (Microsoft, United States) with the calculation of basic statistical indicators of descriptive statistics.

Quantitatively, the activity of the compounds in the tests for evaluation of the antiplatelet and antiglycation actions, as well as actions to cleave the cross-links of proteins, was evaluated by the value of Δ%, reflecting the ratio of the results obtained for the sample with the substance and the results of the control group. The value was determined by the formula:

$\Delta\% = (1 - data_{test}/Mdata_{control}) \times 100\%$; where the $data_{test}$ is the absolute value of the trait registered in the test (the level of fluorescence, optical absorption or light scattering) in each individual sample with the substance, the $Mdata_{control}$ is the average level of the same trait in the control samples without substance. For a visual representation of the $\Delta\%$ values, we used the definition of indicators $M \pm m$, corresponding to the arithmetic mean (M) and the standard error of the arithmetic mean (m).

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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