Synthesis, Molecular Docking, and Hemorheological Activity of New 4-(Thien-2-yl)-3-aminopyridine-2(1H)-one Derivatives

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Abstract—On the basis of 4-(thien-2-yl)-3-aminopyridine-2(1H)-one, the corresponding chloroacetamide and condensed 1H-pyrido[2,3-b][1,4]oxazine-2(3H)-one were synthesized by the reaction of acylation with chloroacetyl chloride. Thiourea derivatives of 3-aminopyridine-2(1H)-one were obtained by reactions with a number of isothiocyanates. It was shown that the carbamothionylmethacrylamide derivative cyclizes rather easily into substituted 1,3-thiazine. Molecular docking of synthesized derivatives for antithrombotic activity was carried out, which showed that the presence of a thiourea fragment in the pyridone core leads to an increase in affinity for the selected protein. The hemorheological study of the compounds using the in vitro model of the increased blood viscosity syndrome also showed activity at the level of the reference drug pentoxifylline.

Keywords: thiophene derivatives, 3-aminopyridine-2(1H)-one, 1H-pyrido[2,3-b][1,4]oxazin-2(3H)-one, thioureide derivatives, intramolecular heterocyclization, hemorheological activity

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Sulfur-containing heterocycles have long ago proved themselves in pharmaceutical practice as effective drugs [1–3]. Over the past 30–40 years, the pharmaceutical industry has been actively searching for new drugs based on compounds containing in their structure the simplest thiophene fragment among numerous sulfur-containing cycles. It is known that thiophene is the most important of the sulfur-containing heterocycles. Many of its derivatives are not only highly resistant to ring opening, but also highly reactive in electrophilic and nucleophilic substitution reactions, which makes it possible to fairly selectively introduce various functional groups and carry out further chemical transformations on their basis. All these properties create wide research opportunities for the preparation of new practically significant thiophene derivatives, for example, as materials for organic electronics [4–6] or pharmacologically active substances with various types of activity, such as anticancer [7–10], analgesic [11], and anti-inflammatory effects [12].

Condensed and conjugated thiophene derivatives also exhibit antiarthritic, antitumor, and anti-HIV activities and exhibit ophthalmic, antimicrobial, and psychotropic properties [13–18]. In the literature there are many other examples of pharmacological agents containing a thiophene fragment, which exhibit other types of biological activity.

To date, there are already many thiophene-based drugs used to treat tonsillitis, coronary heart disease, diabetes, and inflammatory diseases of the upper respiratory tract. However, one of the promising directions in the pharmaceutical chemistry of thiophene derivatives is the synthesis of biologically active compounds, the action of which is aimed at treating the symptoms of AIDS. At present there are relatively few examples of such compounds among thiophene derivatives (Scheme 1). Thus, thiourea derivative 1 is undergoing the stage of biotesting with respect to the mechanism of inhibition of certain types of transcriptases and as an anti-HIV agent.
Compound 2 was proposed by CSIC as an anti-infective agent for AIDS patients [16].

It is known that combining two or more pharmacophore fragments in a molecule is one of the main approaches to the chemical design of new biologically active substances. Among the main synthetic modifiers, pyridine derivatives, which are part of vital vitamins (B₅ and B₆) playing an important role in the vital activity of the body, are quite often used. They are widely applied not only in medical practice in the form of drugs with a variety of therapeutic effects (antibacterial, anti-tuberculosis, antidepressant, antihistamine, analgesic, psychotropic, nootropic, and others) [1-3], but also in agriculture as effective fungicides, herbicides, and growth-stimulating substances [19, 20].

We found in [21] that N-(3-oxoalkenyl)amides are cyclized into 3-amino-substituted pyridine-2(1H)-ones, which previously were not described in the literature, and that they have high antiradical activity. The presence of an inserted amino acid fragment makes such 3-aminopyridine-2(1H)-ones rather attractive building blocks for the synthesis of, for example, peptidomimetics [22–24]. One of the derivatives of 3-aminopyridine-2(1H)-ones (amrinone 3) is used in clinical medical practice as an inotropic (increases the force of the heart contraction) drug with a vasodilating effect [25].

We also developed and optimized a general one-pot method for the preparation of previously unavailable 6-arylbenzo(thieno)[c][1,7]naphthyridin-4(3H)-ones, which were not described in the literature. The method is based on the Pict–Spengler reaction of 3-amino-4-arylpyridine-2(1H)-ones with aromatic aldehydes in acidic media (polyphosphoric acid, 80% H₃PO₄) [26, 27].

Since 3-aminopyridine-2(1H)-ones are binucleophiles, they can be used for the synthesis of pyridine derivatives condensed by the C²–C³ bond, for example, 1H-pyrido[2,3-b][1,4]oxazines. The methods of their preparation are described by a few examples [28–30] based on various pyridine derivatives. We have also shown on single examples [31, 32] that 3-aminopyridine-2(1H)-ones are fairly smoothly cyclized into the corresponding 1H-pyrido[2,3-b][1,4]oxazine-2(3H)-ones under the action of chloroacetyl chloride.

As the object of modification, we chose 4-(thien-2-yl)-3-aminopyridine-2(1H)-one 4, which was not previously studied in respect to chemical modification. We also carried out the reaction of its acylation with chloroacetyl chloride under different conditions. It has been shown that 3-aminopyridine-2(1H)-one 4, as well as 3-aminopyridine-2(1H)-ones previously described in [31], acylates quite smoothly in methylene chloride in the presence of pyridine at room temperature to form the corresponding chloroacetamide 5 in 85% yield (Scheme 2). The replacement of methylene chloride with DMF and an increase in the temperature of the reaction medium to 80–100°C lead to the cyclization of the intermediate chloroacetamide 5 to 1H-pyrido[2,3-b]-[1,4]oxazin-2(3H)-one 6.

Comparison of the ¹H NMR spectra of compounds 5 and 6 shows that the signal of proton at the C² atom of the annelated pyridine ring shifts downfield from 6.42 to 6.96 ppm.

It is known that thioureide derivatives have valuable pharmacological properties and are used as antiepileptic, antidiabetic, antituberculous, and other therapeutically active substances [1–3, 33–35]. Thus, the introduction of sulfur-containing pharmacophore groups, including heterocyclic groups, into the structure of 3-aminopyridinones can lead to enhancement of the basic properties or to appearance of new biological properties.

In order to obtain new functional thiourea derivatives based on 4-(thien-2-yl)-3-aminopyridine-2(1H)-one
we carried out its modification by reacting with some isothiocyanates, since this method is sufficiently preparative and leads to good yields of thioureas [36–38], though their derivatives based on pyridine-2(1H)-ones are described in the literature only in a few examples [39, 40].

The starting acyl isothiocyanates were synthesized in situ (without isolation) by heating the corresponding acid chlorides (benzoyl chloride, p-bromobenzoyl chloride, and methacryloyl chloride) with potassium thiocyanate in acetone. Further reactions of the obtained solutions of isothiocyanates under mild conditions with 3-aminopyridine-2-(1H)-one 4 led to the formation of target products, substituted thiourea derivatives 7a–7c, in 60–80% yields (Scheme 3).

Resulting carbamothioylamides 7a–7c are white or beige finely crystalline powders sparingly soluble in polar organic solvents. The structure of compounds 7a–7c was confirmed by IR and 1H NMR spectroscopy. Thus, in the spectrum of compound 7a, in addition to the main protons, singlets of protons of NH groups are recorded at 11.18, 11.58, and 11.76 ppm.

In order to study the possible intramolecular heterocyclization of methacryloylthiourea derivative 7a under the action of a base, as described in [40, 41], we boiled it in a solution of 2-propanol with the addition of morpholine. It was shown that synthesized compound 7a upon heating in 2-propanol for 10 h undergoes intramolecular heterocyclization to 1,3-thiazine derivative 8 in 53% yield (Scheme 4). The cyclization of compound 7a to 5,6-dihydro-1,3-thiazin-4-one derivative 8 apparently proceeds as a result of an intramolecular nucleophilic attack of the sulfur atom at the electron-deficient carbon atom in the C=C bond.

The formation of 5,6-dihydro-1,3-thiazin-4-one 8 was confirmed by the absence of C=S group vibrations in the region of 1511 cm\(^{-1}\) in its IR spectrum, which appear in case of starting compound 7a. In the \(^1\)H NMR spectrum, there are neither protons at the =CH\(_2\) double bond, which appear for compound 7a as two singlets at 5.74 and 6.05 ppm, nor a singlet of the thioureide NH proton at 11.76 ppm. In addition, in the \(^1\)H NMR spectrum, the signals of methyl protons of the CH\(_3\) group are split into a doublet at 1.19 ppm, which indicates that they interact with the methine CH proton of the thiazine ring, and signals of the methine and methylene protons of the 1,3-thiazine ring appear in the form of multiplets and doublet of doublets, which also confirm the compound 8 formation.

Thus, we have carried out a one-step synthesis of several thioureide derivatives of 3-aminopyridine-2(1H)-one 4 and carried out intramolecular heterocyclization of...
the methacryloylthiourea derivative to obtain 5,6-dihydro-
4H-1,3-thiazin-4-one 8.

The work [42] presents the synthesis of a series of
N,N'-disubstituted thioureas with aromatic and aliphatic
substituents, which were tested as non-anionic antiplatelet
agents against platelet coagulation under the act
uin of arachidonic acid. Some of the thiourea derivatives
reduced the production of both PGE2 and TXB2 in
human platelets, which points to the direct inhibition
of the cyclooxygenase-1 (COX-1) enzyme. In addition,
the series of compounds obtained by the authors of [42]
demonstrates low mutagenic and genotoxic profiles
according to the Ames test and the SOS chromotest
and also good hemocompatibility with healthy human
erthrocytes.

To assess supposed antithrombotic activity of
synthesized derivatives 4–8, we used the molecular
docking method. The protease domain of coagulation
factor XI(F11) in complex with an active site inhibitor
(PDB identifier: 6TS4) [43] was chosen as the target
protein. Three-dimensional (3D) structures were obtained
from the RCSB Protein Data Bank [44], whereas the
ligand molecules were sketched using ChemBio3D Ultra
14.0. The protein structure was prepared for docking
by removing a water molecule and a native ligand, by
adding polar hydrogen atoms, and converting into the
pdbqt-format with the use of the AutoDock MGL software
package [45]. The docking process was carried out using
AutoDock Vina [46]. For the domains of the coagulation
factor protease (PDB: 6TS4) [43], the grid coordinates
of the active site (X = 32.0765, Y = 5.02065, and
Z = −5; size 17×17×20 Å) were applied. The interaction
of ligands in binding sites was interpreted using Discovery
Studio Visualizer [47].

The docking results showed that for the studied
structures, the free energies of complexes with selected
receptors are higher than the free energy of this protein
complex with the corresponding native ligands (Table 1).
It was also found that the presence of a thiourea fragment
in compounds 7a–7c, and also the presence of a cyclic
1,3-thiazine ring in compound 8, increase their affinity
for the selected receptors compared to other derivatives.

The number of intermolecular hydrogen bonds, the
binding energy of ligand-6TS4 stable complexes, and
the number of nearest amino acid residues were also
determined for synthesized compounds 4–8 (Table 2).
All synthesized derivatives formed complexes with target
proteins. Analysis of interactions of the 6TS4 protein
complex and ligand 7a showed that the ligand molecule
is oriented due to one Pi–Pi T-shaped bond with the
HIS57 amino acid fragment and to Pi-alkyl and alkyl
interactions with amino acid residues ALA97, LYS192,
and ALA195, forming three simple hydrogen bonds with
ASP194, GLY193, and ALA195 residues. In addition,
eight van der Waals interactions with amino acid residues
ASP189, CYS219, GLY216, TRP215, SER214, CYS191,
THR213, and ALA190 were found (Fig. 1).

An analysis of the interactions between the 6TS4
protein complex and the 7c ligand was also carried out,
which showed that the ligand molecule is oriented due
to one Pi–Pi T-shaped bond with the HIS57 amino acid
fragment, Pi-alkyl and alkyl interactions with the amino
acid residues ALA190, LEU39, ALA97, one halogen
bond to TYR59A, and four single hydrogen bonds to
ASP194, CYS191, GLY193, and ALA195 residues.

In addition, eight van der Waals interactions with
amino acid residues CYS58, ARG37D, CYS40, GLU98,
SER214, TRP215, THR213, and LYS192 were found (Fig. 2).

In addition, an analysis of the interactions of the 6TS4 protein complex and ligand 8 was performed, which showed that the ligand molecule is oriented due to one Pi–S interaction with the HIS57 amino acid fragment and Pi-alkyl interactions with the amino acid residues ALA195, CYS219, ALA97, and four simple hydrogen bonds with CYS191, GLY193, ALA195, and ASP194. Eight van der Waals interactions with amino acid residues SER214, TRP215, THR213, ALA190, ARG37D, LEU39, LYS192, and GLU98 were also found (Fig. 3).

Thus, the results of the computer docking indicate that the modification of 3-aminopyridone 4 at the amino group leads to an increase in affinity for the selected protein. At the same time, it is the presence of the thiourea fragment that increases the affinity for the selected receptor protein, which is indicative of their potential antithrombotic activity and is confirmed by the previously cited literature data [42].

The initial high antiradical activity of the previously synthesized derivatives of 3-aminopyridine-2(1H)-ones [21] assumes a fairly wide spectrum of biological activity. The active participation of rheological mechanisms in blood circulation disorders has been proven, rheological occlusion being considered as an initiating factor of plasma coagulation mechanisms leading to thrombosis. The deterioration of the viscoelastic properties of erythrocytes leads to a deterioration in microcirculation, which leads to a decrease in local blood flow and tissue hypoxia [48]. It is known that hemodynamic disturbances are determined not only by the state of platelet-vascular hemostasis, but also by the blood rheological properties [49], therefore, it is of interest to study the possibilities of the pharmacological effect of synthesized compounds on the blood viscosity properties. Thus, the known negative effect of excessive lipid peroxidation on hemorheological properties [50, 51] served as the basis for successful attempts to prevent the negative effect of lipid peroxidation on the rheological functions of erythrocytes with the help of antioxidants [52, 53]. This circumstance, and also the results of positive molecular docking for antithrombotic activity, prompted us to perform screening of the synthesized compounds for rheological activity.

For this purpose, we studied the hemorheological activity of compounds 4–8 under conditions of an in vitro model of the increased blood viscosity syndrome. Among the seven samples studied, three samples (5, 7c, and 8) showed the ability to reduce blood viscosity in the in vitro model of blood hyperviscosity. The results of screening samples 5, 7c, and 8 for hemorheological activity in the in vitro blood hyperviscosity model are shown in Table 3.

Table 3 shows that blood incubation for 60 min at 43.0°C leads to a significant increase in blood viscosity compared to the initial values of blood viscosity at
different spindle speeds from 2 to 60 rpm, which points to the formation of blood hyperviscosity.

Screening of compound 5 shows that the blood viscosity at all spindle speeds increases from 48.6 to 76.3% in the control after incubation compared to the initial viscosity. Tested compound 5 prevents the increase in blood viscosity from 3.1 (at a spindle rotation speed of 2 rpm) to 10.2% (at a rotation speed of 60 rpm) compared to...
to the control values. In the case of compound 7c, the blood viscosity in the control increases after incubation from 48.4 to 74.0% at all spindle speeds compared to the initial value. Compound 7c prevents the increase in blood viscosity from 2.1% (at 60 rpm) to 13.7% (at 2 rpm) as compared to the control values. In the case of compound 8, the blood viscosity in the control after incubation increases at all spindle speeds from 52.8 to 96.9% compared to the initial value. Compound 8 prevents the

| Compound | Receptor | H-Bond | Main amino acid interactions | van der Waals interactions |
|----------|----------|--------|-----------------------------|--------------------------|
| 7a       | ASP194, GLY193, ALA195 | ALA97, HIS57, LYS192, ALA195 | CYS191, THR213, ALA190, ASP189, TRP215, SER214 |
| 7b       | SER214, ALA195 | ALA190, SER214, HIS57, CYS219, CYS191 | TRP215, CYS191 |
| 7c       | GLY193, ASP194, ALA195, CYS191 | LEU59, TYR59A, ALA97, ALA190, ALA195, GLY193, HIS57 | CYS58, ARG37D, GLU98, SER214, TRP215, THR213, CYS40 |
| 8        | GLY193, ASP194, ALA195, CYS191 | ALA97, HIS57, CYS219, GLY193, ALA195 | LEU39, ARG37D, LYS192, THR213, ALA190, TRP215, SER214, GLU98 |

*Fig. 1. (a) 3D and (b) 2D docking models of the complex between compound 7a and the domain of the coagulation factor protease (PDF: 6 TS 4).*
Fig. 2. (a) 3D and (b) 2D docking models of the complex between compound 7B and the domain of the coagulation factor protease (PDB: 6TS4).

Fig. 3. (a) 3D and (b) 2D docking models of the complex between compound 8 and the domain of the coagulation factor protease (PDB: 6TS4).
increase in blood viscosity from 2.8 (at 60 rpm) to 11.0% (at 2 rpm) compared to control values.

Thus, the obtained results indicate that compounds 5, 7c, and 8 are not inferior to the reference drug pentoxifylline in the manifestation of hemorheological effects in the in vitro model of blood hyperviscosity.

**EXPERIMENTAL**

The $^1$H and $^{13}$C NMR spectra were recorded on a Jeol JNM-ECA 400 instrument (400 and 100 MHz, respectively) in DMSO-$d_6$, internal standard TMS. Elemental analysis was performed on a Carlo Erba 1106 CHN instrument. Melting points were determined using a Stuart SMP10 heating stage. The reaction progress and product purity were monitored by TLC on Sorbil plates (2-propanol–benzene–ammonia, 10:5:2 system) and developed with iodine vapor or UV light. The samples were analyzed by the HPLC-MS method on an Agilent 1260 Infinity II chromatograph coupled to an Agilent 6545 LC/Q-TOF high-resolution mass spectrometer with a Dual AJS ESI ionization source operating in the positive detection mode. Chromatographic separation was carried out on ZORBAX RRHD Eclipse Plus C18 columns (2.1×50 mm, particle size 1.8 µm). The column temperature during the analysis was maintained at 30°C. The mobile phase consisted of eluents A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile) at a flow rate of 0.5 mL/min. The gradient program was as follows: 0 min, 0% B; 6 min, 0% B; 15 min, 100% B; 20 min, 100% B; 22 min, 0% B; 25 min, 0% B.

**Table 3.** Effect of compounds 5, 7b, and 8 on blood viscosity at different spindle speeds in the blood hyperviscosity in vitro model

| Characteristic | Blood viscosity at different spindle speeds (rpm), MPa s |
|---------------|--------------------------------------------------------|
|               | 2           | 4           | 6           | 8           | 12          | 20          | 40          | 60          |
| Screening of compound 5 ($n = 3$) | | | | | | | | |
| Initial       | 3.10±0.04   | 3.05±0.04   | 2.52±0.03   | 2.36±0.01   | 2.26±0.01   | 2.19±0.01   | 2.15±0.02   | 2.12±0.01   |
| After incubation in control | 5.16±0.28   | 4.85±0.22   | 4.30±0.10   | 4.16±0.14   | 3.67±0.05   | 3.41±0.02   | 3.21±0.03   | 3.15±0.01   |
| After sample incubation with compound 5 | 5.00±0.27   | 4.66±0.20   | 4.07±0.12   | 3.81±0.09   | 3.40±0.04   | 3.12±0.08   | 2.90±0.15   | 2.83±0.16   |
| After incubation | 3.23±0.04   | 3.09±0.03   | 2.89±0.16   | 2.76±0.16   | 2.45±0.05   | 2.30±0.03   | 2.21±0.03   | 2.18±0.03   |
| After incubation in control | 5.62±0.19   | 4.89±0.13   | 4.29±0.04   | 4.14±0.09   | 4.02±0.10   | 3.47±0.03   | 3.37±0.03   | 3.26±0.02   |
| After sample incubation with compound 7c | 5.15±0.15   | 4.33±0.03   | 4.18±0.04   | 3.97±0.11   | 3.83±0.12   | 3.33±0.05   | 3.23±0.03   | 3.19±0.02   |
| After incubation | 3.27±0.02   | 3.19±0.04   | 3.10±0.04   | 2.56±0.03   | 2.49±0.02   | 2.24±0.01   | 2.16±0.01   | 2.12±0.01   |
| After incubation in control | 6.44±0.22   | 5.38±0.23   | 4.74±0.24   | 4.27±0.06   | 3.86±0.07   | 3.54±0.02   | 3.32±0.05   | 3.24±0.03   |
| After sample incubation with compound 8 | 5.73±0.25   | 5.16±0.26   | 4.45±0.19   | 4.09±0.02   | 3.58±0.02   | 3.39±0.02   | 3.20±0.03   | 3.15±0.02   |
| After incubation | 3.94±0.59   | 4.90±0.43   | 4.10±0.38   | 3.87±0.34   | 3.40±0.29   | 2.69±0.26   | 2.32±0.12   | 2.21±0.12   |
| After incubation in control | 7.53±0.45   | 6.36±0.40   | 5.79±0.44   | 5.19±0.31   | 4.37±0.13   | 3.56±0.15   | 2.76±0.09   | 2.53±0.07   |
| After sample incubation with pentoxifylline | 7.03±0.43   | 5.81±0.30   | 5.00±0.21   | 4.56±0.16   | 4.05±0.10   | 3.24±0.14   | 2.56±0.08   | 2.39±0.07   |
| After incubation | 5.1584      | 5.1009      | 5.0357      | 5.0157      | 5.0105      | 5.0063      | 5.0096      | 5.0188      |
| After incubation in control | 7.0306      | 7.2800      | 7.1205      | 7.0855      | 7.0631      | 7.1353      | 7.0999      | 7.1590      |

$n$ is the number of animals in the group; $p$ is the significance level; $p_1 < 0.05$ are statistically significant differences compared to the initial values; $p_2 < 0.05$ are statistically significant differences compared to the corresponding values in the control samples.
acid solution in deionized water) and B (0.1% formic acid solution in acetonitrile). Chromatographic separation was performed according to the following elution scheme: 0–10 min 95% A, 10–13 min 100% B, and 13–15 min 95% A. The mobile phase flow was maintained throughout the analysis at 400 μL/min. In all experiments, the sample injection volume was 1 μL. The sample was prepared by dissolving the entire sample (in 1000 μL) in methanol (for HPLC). Sample dilution was carried out immediately before analysis. The recorded data were processed with the Agilent MassHunter 10.0 software.

2-Chloro-N-[6-methyl-2-oxo-4-(thien-2-yl)-1,2-dihydropyridine-3-yl]acetamide (5) was obtained in a similar manner as described in [31]. Chloroacetyl chloride (1.0 mL, 1.2 mmol) was added to a solution of 0.206 g (1 mmol) of 3-amino-6-methyl-4-(thien-2-yl)pyridine-2(1H)-one in 5 mL of DMF, 0.276 g (2 mmol) of K₂CO₃, and upon stirring in a similar manner as described in [31]. Chloroacetyl chloride was obtained in a dihydropyridine-3-yl]acetamide (5) was obtained in a similar manner as described in [31].

For C₁₂H₁₂ClN₂O₂S⁺ (NC=O), 3303 (NH). 13C NMR spectrum, δ, ppm: 18.4 (СН3), 42.9 (CH2Cl), 102.8 (C5), 118.8, 127.2 (C3-Th), 129.8 (C4-Th), 129.7 (C5-Th), 137.1, 140.8, 143.2, 160.6, 165.8. Mass spectrum (HRMS), m/z: 334.1076 [M + H]+ (calculated for C₁₂H₁₂ClN₂O₂S⁺: 283.0300).

6-Methyl-8-(thien-2-yl)-1H-pyrido[2,3-b][1,4]-oxazin-2(3H)-one (6). To a solution of 0.206 g (1 mmol) of 3-amino-6-methyl-4-(thien-2-yl)pyridine-2(1H)-one 4 in 5 mL of DMF, 0.276 g (2 mmol) of K₂CO₃ and, upon cooling, 0.12 mL (1.5 mmol) of monochloroacetic acid were added. The reaction mixture was stirred at 80–100°C for 2 h. The resulting precipitate was filtered, washed with water, dried, and recrystallized from dioxane. Yield 0.207 g (62%), white fine crystalline powder, mp 199–201°C. IR spectrum (KBr), ν, cm⁻¹: 1690 (NC=O), 3387 (NH). 1H NMR spectrum, δ, ppm: 2.23 s (3H, CH3), 4.73 s (2H, CH2Cl), 6.42 s (1H, H3-Th), 7.15 br. s (1H, H4-Th), 7.64 br. s (1H, H5-Th), 7.72 br. s (1H, H2-Th), 9.44 s (1H, NHCO′), 11.58 s (1H, 1-NH). 13C NMR spectrum, δc, ppm: 18.4 (CH3), 42.9 (CH2Cl), 102.8 (C5), 118.8, 127.2 (C3-Th), 129.8 (C4-Th), 129.7 (C5-Th), 137.1, 140.8, 143.2, 160.6, 165.8. Mass spectrum (HRMS), m/z: 334.1076 [M + H]+ (calculated for C₁₂H₁₀ClN₂O₂S⁺: 334.0668). Found, %: C 54.46; H 4.91; N 12.29. C₁₅H₁₅N₃O₂S₂. Calculated, %: C 54.03; H 4.53; N 12.60.

N′-[6-Methyl-2-oxo-4-thien-2-yl-1,2-dihydropyridine-3-yl]carbamothioyl]methacrylamide (7a). Yield 0.301 g (83%), light brown fine crystalline powder, mp 236–238°C. IR spectrum (KBr), ν, cm⁻¹: 1632 (C=O), 1504 (C= S), 3145, 3075, 3013 (NH). 1H NMR spectrum, δ, ppm: 2.23 s (3H, CH3), 6.48 s (1H, H2-Th), 7.15 br. s (1H, H4-Th), 7.54 t (2H, H3-Th, J = 6.9 Hz), 7.68 m (3H, H3-Th, H5-Th), 8.01 d (2H, H2-Th, J = 7.3 Hz), 11.67 br. s (3H, NH). 13C NMR spectrum, δc, ppm: 18.5 (CH3), 102.7 (C3-Th), 120.4, 127.2 (C3-Th), 128.5 (C2-Th), 128.7 (C3-Th), 128.9 (C4-Th), 130.2 (C5-Th), 133.2, 137.5, 140.6, 143.5, 159.7, 169.3, 182.2. Mass spectrum (HRMS), m/z: 334.1076 [M + H]+ (calculated for C₁₅H₁₆N₂O₂S⁺: 334.0668). Found, %: C 54.46; H 4.91; N 12.29. C₁₅H₁₅N₃O₂S₂. Calculated, %: C 54.03; H 4.53; N 12.60.

4-Bromo-N′-[6-methio-2-oxo-4-thien-2-yl-1,2-dihydropyridine-3-yl]carbamothioyl]benzamide (7c). Yield 0.394 g (88%), mp 203–205°C. IR spectrum (KBr),
5-Methyl-2-[(6-methyl-2-oxo-4-thien-2-yl-1,2-dihydropyridine-3-ylamino)-5,6-dihydro-4H-1,3-thiazine-4-one (8)]. Morpholine (1.5 mL) was added to a mixture of 0.333 g (1 mmol) of carbanothionylamide 7a in 10 mL of isopropanol. The reaction mixture was refluxed for 10 h. The precipitate formed was filtered off, washed with cold 2-propanol, and recrystallized from a 2-propanol–chloroform (1 : 2) mixture. Yield 0.203 g (53%), mp 279–281°C, pale yellow finely crystalline powder. IR spectrum (KBr), ν, cm⁻¹: 1653, 1628 (C=O), 1510 (C=S), 3391, 3150, 2928 (NH). H¹ NMR spectrum, δ, ppm: 2.23 s (3H, CH₃), 6.48 s (1H, H'), 7.14 d. d (1H, H₃Th), 3J 5.0, 4.1 Hz), 7.67 d. d (1H, H₃Th), 3J 3.9, 4J 1.1 Hz), 7.71 d. d (1H, H₃Th), 3J 5.0, 4J 0.9 Hz), 7.75 d (2H, H₅₄₅₆₇, J 8.7 Hz), 7.94 d (H₂₆₇₈₉₁₀, J 8.7 Hz), 11.67 s (1H, NHCS'), 11.79 s (1H, NHCO'), 11.82 s (1H, 1-NH). ¹³C NMR spectrum, δC, ppm: 18.5 (CH₃), 102.7 (C⁵), 120.4, 127.2 (C³Th), 129.0 (C⁴Th), 130.2 (C⁵Th), 130.8 (C²₆₇₈₉₁₀), 131.1, 131.5 (C³₅₆₇₈₉₁₀), 136.8, 140.6, 143.6, 159.7, 162.2, 167.4, 182.3. Found, %: C 48.44; H 3.50; N 9.61. C₁₈H₁₄BrN₃O₂S₂. Calculated, %: C 48.22; H 3.15; N 9.37.

The hemorheological activity of compounds 4–8 was studied on 15 12-week-old female Wistar rats weighing 220–240 g. After blood sampling from laboratory animals, the initial blood viscosity was determined, then blood samples with the tested substances were incubated at 43.0°C for 60 min and the parameters under study were measured. Blood with tested objects in DMSO was incubated dissolved; the final concentration of compounds 4–8 was 10–5 g/mL of blood. Blood samples with DMSO solvent added in an equivolume amount served as controls. A compound with known hemorheological properties, pentoxifylline [54], at a final concentration of 10–5 g/mL of blood, was used as a reference sample. Blood incubation for 1 h under these conditions was accompanied by the formation of blood hyperviscosity [55]. The initial blood viscosity of each animal was measured once; the blood viscosity after incubation was measured in two samples from each animal, both in control and experimental samples. Statistical processing of the results was carried out using the Excel program. The results obtained are presented as “mean ± standard error of the mean.”

The whole research work with laboratory animals was performed in accordance with generally accepted ethical standards for the treatment of animals, based on standard operating procedures that comply with the rules adopted by the European Convention for the Protection of Vertebrate Animals used for Research and other Scientific Purposes (Strasbourg, 1986). The study protocol of the project “Search for means of pharmacological correction of the syndrome of increased blood viscosity associated with endocirne pathology” was approved on August 07, 2020 by the Local Ethical Commission of the National Center for Biotechnology.

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SYNTHESIS, MOLECULAR DOCKING, AND HEMORHEOLOGICAL ACTIVITY

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CONFLICT OF INTEREST
No conflict of interest was declared by the authors.

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REFERENCES
1. Soldatenkov, A.T., Kolyadina, N.M., and Shendrik, I.V., Osnovy organicheskoy khimii lekarstvennykh veshchestv (Fundamentals of Organic Chemistry of Medicinal Substances), Moscow, Khimiya, 2001.
2. Mashkovskii, M.D., Lekarstva XX veika (Medicines of the 20th Century), Moscow: Novaya Volna, 1998.
3. Mashkovskii, M.D., Lekarstvennyye sredstva (Medicines), Moscow: Novaya Volna, 2007.
4. Kostyuchenko, A.S., Yurpalov, V.L., Kurowska, A., Domagala, W., Pron, A., and Fisyuk, A.S., Beilstein J. Org. Chem., 2014, vol. 10, p. 1596.
   https://doi.org/10.3762/bjoc.10.165
5. Kostyuchenko, A.S., Averkov, A.M., and Fisyuk, A.S., Org. Lett., 2014, vol. 16, no. 7, p. 1833.
   https://doi.org/10.1021/ol500356w
6. Kurowska, A., Kostyuchenko, A.S., Zassowski, P., Skorka, L., Yurpalov, V.L., Fisyuk, A.S., Pron, A., and Domagala, W., J. Phys. Chem., 2014, vol. 118, no. 43, p. 25176.
   https://doi.org/10.1021/jp507838c
7. Ahmed, M.M., Khan, M.A., and Rainsford, K.D., Molecules, 2013, vol. 18, no. 2, p. 1483.
   https://doi.org/10.3390/molecules18021483
8. Bober, L., Kawczak, P., and Baczek, T., Int. J. Mol. Sci., 2012, vol. 13, no. 6, p. 6665.
   https://doi.org/10.3390/ijms13066665
9. Lukevics, E., Arsenyan, P., Shestakova, I., Zharkova, O., Kanepe, I., Mezapuke, R., and Pudova, O., Met.-Based Drugs, 2000, vol. 7, p. 63.
   https://doi.org/10.1155/MBD.2000.63
10. Connor, D.T., Cetenko, W.A., Mullican, M.D., Sorenson, R.J., Unangst, P.C., Weikert, R.J., Adolphson, R.L., Kennedy, J.A., Thueson, D.O., Wright, C.D., and Conroy, M.C., J. Med. Chem., 1992, vol. 35, no. 5, p. 958.
   https://doi.org/10.1021/jm00083a023
11. Wardakhan, W.W., Abdel-Salam, O.M.E., and Elmegeed, G.A., Acta Pharm., 2008, vol. 58, no. 1, p. 1.
   https://doi.org/10.2478/v10007-007-0041-5
12. Mohamed, A.A.R., Shehab, M.A., and El-Shenawy, S.M., Monatsh. Chem., 2009, vol. 140, p. 445.
   https://doi.org/10.1007/s00706-008-0067-5
13. Castacer, J. and Prous, J., J. Drugs Fut., 1992, vol. 17, p. 683.
14. Lohmeyer, M., Castacer, J., and Tomudex, T.M., J. Drugs Fut., 1995, vol. 20, p. 371.
15. Uckun, F.M., Sudbeck, E.A., and Venkatachalam, T.K., Tetrahedron Lett., 2001, vol. 42, no. 38, p. 6629.
   https://doi.org/10.1016/S0040-4039(01)01290-4
16. Arranz, E., Diaz, J.A., Ingate, S.T, Witvrouw, M., Pannecoque, C., Balzarini, J., De Clercq, E., and Vega, S., J. Med. Chem., 1998, vol. 41, no. 21, p. 4109.
   https://doi.org/10.1021/jm9802012
17. Sorbera, L.A., Castacer, R.M., and Castacer, J., J. Drugs Fut., 2000, vol. 25, no. 9, p. 907.
18. Moore, N.A., Hotten, T.M., and Tupper, D.E., J. Drugs Fut., 1994, vol. 19, no. 2, p. 114.
19. Mel'nikov, N.N., Pestitsidy. Khimiya, tekhnologiya i primeneniy (Pesticides. Chemistry, Technology and Application), Moscow: Khimiya, 1987.
20. Shimanskaya, M.V., and Leitis, L.Ya., Chem. Heterocycl. Compd., 1989, vol. 25, no. 5, p. 477.
   https://doi.org/10.1007/BF00482487
21. Kulakov, I.V., Matsukevich, M.V., Shulgau, Z.T., Sergazy, S., Selikhanov, T.M., Puzari, A., and Fisyuk, A.S., Chem. Heterocycl. Compd., 2015, vol. 51, p. 991.
   https://doi.org/10.1007/s10593-016-1809-7
22. Verissimo, E., Berry, N., Gibbons, P., Cristiano, M.L.S., Rosenthal, P.J., Gut, J., Ward, S.A., and O’Neill, P.M., Bioorg. Med. Chem. Lett., 2008, vol. 18, p. 4210.
   https://doi.org/10.1016/j.bmcl.2008.05.068
46. Trott, O. and Olson, A.J., *J. Comput. Chem.*, 2010, vol. 31, p. 455.
https://doi.org/10.1002/jcc.21334

47. *Discovery Studio 2015*: Dassault Systemes BIOVIA, Discovery Studio Modelling Environment, Release 4.5, San Diego: Dassault Systemes.

48. Cecchi, E., Mannini, L., and Abbate, R., *G. Ital. Nefrol.*, 2009, vol. 26, no. 46, p. 20.

49. Roitman, E.V., *Tromboz, Gemostaz i Reologiya*, 2003, no. 3, p. 13.

50. Nemeth, N., Peto, K., Magyar, Z., Klarik, Z., Varga, G., Oltean, M., Mantas, A., Czigany, Z., and Tolba, R.H., *Int. J. Mol. Sci.*, 2021, vol. 22, no. 4, p. 1864.
https://doi.org/10.3390/ijms22041864

51. Caprari, P., Massimi, S., Diana, L., Sorrentino, F., Maffei, L., Materazzi, S., and Risoluti, R., *Front. Mol. Biosci.*, 2019, vol. 6, p. 142.
https://doi.org/10.3389/fmolb.2019.00142

52. Becatti, M., Marcucci, R., Gori, A.M., Mannini, L., Grifoni, E., Alessandrello, Liotta, A., Sodi, A., Tartaro, R., Taddei, N., Rizzo, S., Prisco, D., Abbate, R., and Fiorillo, C., *J. Thrombosis Haemostasis*, 2016, vol. 14, no. 11, p. 2287.
https://doi.org/10.1111/jth.13482

53. Mo, J., Fan, J., Guo, Z., Hunag, C., Yan, B., Wang, F., Wang, D., and Sun, S., *Med. Hypotheses*, 1993, vol. 41, no. 6, p. 516.
https://doi.org/10.1016/0306-9877(93)90107-2

54. McCarty, M.F., O’Keefe, J.H., and DiNicolantonio, J.J., *Open Heart.*, 2016, vol. 3, no. 1, p. e000365.
https://doi.org/10.1136/openhrt-2015-000365.

55. Plotnikov, M.B., Koltunov, A.A., and Aliev, O.I., *Eksperimental’nyay i klinicheskaya farmakologiya* (Experimental and Clinical Pharmacology), 1996, vol. 6, p. 57.