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SEARCH FOR BİOLOGİCALLY ACTİVE SUBSTANCES USİNG THE EXAMPLE OF 2.4-DIOXO- AND 4-IMINO-2-OXO-3-PHENYL-5-R-6-R`-THIENO[2.3-D]PYRIMIDINES, ROSPECTS FOR THEİR USE İN PHARMACY AND MEDİCİNE

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Key words: *ureide derivatives; carboxylic acids; alkylation; acylation; diuretic action*

Introduction

The research and synthesis of new compounds for the purpose of creating effective medicines is defined as an urgent task of modern pharmaceutical science. For this purpose, special attention was paid to the study of thieno[2.3-d]pyrimidine derivatives. Thieno[2.3-d]pyrimidines have recently become widespread among annelated derivatives of thiophene and pyrimidine. Firstly, this is due to the variety of methods for obtaining the appropriate precursors, for example, the well-known and widespread Thorpe-Ziegler and Gevald reactions, which allow the synthesis of a functionalized thiophene cycle, in particular 2- and 3-aminothiophene derivatives. Secondly, many derivatives of ringed thiophenes and pyrimidines have a wide range of biological effects (immunomodulatory, anti-inflammatory, antiviral, neurotropic, analgesic, antiallergic, antitumor, antimicrobial, antibacterial, growth-regulating). Of particular interest were the reactions of substituted thieno[2.3-d]pyrimidines, which open the way to various heterocyclic systems – products and semi-products of fine organic synthesis and other substances with valuable properties. Considering this, the urgent task was the development of methods for the synthesis of new substituted thieno[2.3-d]pyrimidines and the study of their physicochemical, biological properties, as well as the pharmacological screening of the synthesized compounds.

The purpose of this paper was to synthesize new biologically active substances – derivatives of 2.4-dioxo- and 4-imino-2-oxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines; their study and further pharmacological research. For this, the following goals were set.

On the basis of ethyl 4-R-5-R`-2-amino-thiophene-3-carboxylates and nitriles of 4-R-5-R`-2-aminothiophene-3-carboxylic acids, by interacting with phenylisocyanate, to synthesize the corresponding ureide derivatives, which can be obtained by cyclization sodium salts and acid forms of 2.4-dioxo- and 4-imino-2-oxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines.

To investigate the possibility of interaction of synthesized substances with Grignard reagents and their ability to undergo Dimrot rearrangement. To investigate the possibility and directions of alkylation, acylation and cyanoethylation of 2.4-dioxo- and 4-imino-2-oxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines and the properties of its products. To investigate the structure of the obtained compounds using instrumental analysis methods (ultraviolet-visible (UV), infrared (IR), NMR1H spectroscopy, elemental analysis). To carry out a detailed analysis of the obtained substances (PASS (Prediction of Activity Spectra for Substances) program) to identify potential biologically active substances.

To carry out a pharmacological study of the synthesized compounds, taking into account the results of predicting the biological action. To study the peculiarities of the relationship between chemical structure and biological activity. To identify promising compounds for further in-depth biological research.

The research object is the synthesis of biologically active compounds in a number of derivatives of 2.4-dioxo- and 4-imino-2-oxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines and their synthesis intermediates (ethyl 4-R-5-R`-2-aminothiophene-3-carboxylates, nitriles of 4-R-5-R`-2-aminothiophene-3-carboxylic

acids, their ureide derivatives).

The subject of research includes methods of creation, physical, chemical and biological properties of ethyl 4-R-5-R`-2-aminothiophene-3-carboxylates, nitriles of 4-R-5-R`-2-aminothiophene-3-carboxylic acids, their ureide derivatives, 2.4-dioxo- and 4-imino-2-oxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines, as well as products of their alkylation, acylation, cyanoethylation and other chemical transfermations, methods of studying the biological activity of new compounds.

The study of thieno[2.3-d]pyrimidines was partially carried out by a group of scientists under the leadership of Professor C.M. Khrypak et al. [1] and G.V. Rizak et al. [2]. Researchers S.B. Salib et al. [3] proved that thieno[2.3-d]pyrimidines, which had a thiosemicarbazide framework, were excellent candidates for further chemical transformations in order to obtain highly selective anticancer drugs. French scientists P. Lagardere et al. [4] studied in detail the properties of three isomers – thieno[2.3-d]pyrimidines, thieno[3.2-d]pyrimidinnes, and thieno[3.4-d]pyrimidines. It was found that drugs derived from the first compound had mainly antituberculosis and antifungal effects, while the derivatives of the second compound had antiviral effect. It was established that derivatives of thieno[3.4-d]pyrimidines manifested antibacterial and antifungal effects. Scientists from the University of Toledo recently discovered a new thienopyrimidine analog that mediates immunogenic death in breast cancer cells, which gave promising results in the treatment of this disease [5].

As a result of the paper of the author of this research, preparative methods were proposed for creating ureide derivatives of 2-aminothiophenes, 2.4-dioxo- and 4-imino-2-oxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines, as well as their alkyl, acyl and cyanoethyl derivatives. The results of studying the reactivity of ethyl 4-R-5-R`-2-aminothiophene-3-carboxylates, nitriles of 4-R-5-R`-2-aminothiophene-3-carboxylic acids, their ureide derivatives, 2.4-dioxo- and 4-imino-2-oxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines, the products of their chemical transformations and the methods of their preparation are important for conducting a targeted search for biologically active substances in the mentioned series of compounds. For further in-depth pharmacological studies, 2-acetyloxy-4-oxo-3-phenyl-5,6-dimethyl-thieno[2.3-d]pyrimidine is proposed, which exhibits high diuretic and anti-inflammatory activity, low toxicity and is characterized by a relatively affordable synthesis method. A draft quality control method has been developed for this compound.

Materials and methods

Physical and chemical methods were used for studying the properties of ethyl 4-R-5-R`-2-aminothiophene-3-carboxylates, nitriles of 4-R-5-R`-2-aminothiophene-3-carboxylic acids, their ureide derivatives, 2.4-dioxo- and 4-imino-2-oxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines, their alkyl, acyl and cyanoethyl derivatives and products of their transformations. Synthesis and study of physicochemical properties was carried out at the Department of Organic Chemistry of the National University of Pharmacy (Kharkiv).

The diuretic activity of the synthesized substances was investigated on white rats weighing 200-250 g at the Department of Physiology of the National University of Pharmacy according to the method of E.B. Berkhin [6]. Five animals were used in each series. Solutions of compounds stabilized by Tween 80 were injected into the stomach of rats in an amount corresponding to the semi-effective dose of hypothiazide – 40 mg/kg. At the same time, control experiments were conducted. Subsequently, the animals were given a water load per os, with a calculation of 3 ml per 100 g of mass and placed in individual cages for urine collection. Urine volumes were measured every hour for four hours. The urinary activity of the kidneys was calculated as a percentage and the ability of the substance to stimulate diuresis was determined. Calculations were made according to formula 1:

$DA = (V_1 / V_2) \cdot 100\%$

where: DA is diuretic activity expressed as a percentage; V1 is urine volume (experimental group), ml; V2 is urine volume (control group), ml.

The study of the antiexudative activity of substances synthesized by the author was carried out at the Department of Physiology of the National University of Pharmacy using the carrageenan edema method on white mice weighing 18-22 g. To reproduce acute aseptic inflammation, a 1% carrageenan solution was used, which was injected under the plantar

aponeurosis in the amount of 0.05 ml after 1 hour after administration of the test substance per os. After 3 hours, the animals were removed from the experiment, the hind feet were amputated at the level of the hip joints. The control group of rats received an equivalent amount of solvent. Diclofenac sodium at a dose of 3.8 mg/kg was chosen as the comparison drug. The activity of the studied substances is determined by their ability to reduce swelling compared to the control and is expressed as a percentage. Edema growth was assessed by increasing the volume of the inflamed foot, which was measured every hour for five hours with a mechanical oncometer using the method of A. Zakharzhevskyy. The size of the edema was calculated as the difference between the volumes of the intact and affected limbs. The antiexudative effect of the synthesized compounds was determined by the degree of reduction of tissue swelling in experimental animals compared to control animals. The percentage of edema suppression is calculated according to formula 2:

$AA = (\Delta Ve - \Delta Vc / \Delta Vc) \cdot 100\%$

where: AA is the anti-inflammatory activity in %; ΔVe and ΔVc are the difference between the inflamed and non-inflamed limbs in the experiment group and in the control group.

The antimicrobial activity of the substances synthesized by the author and the products of their chemical transformations was conducted in the laboratory of the Mechnikov Institute of Microbiology and Immunology in accordance with the recommendations of the State Pharmacopoeia of Ukraine [7; 8], type 1. The antimicrobial activity was studied in vitro using a standard set of reference test strains of gram-positive and gram-negative bacteria.

The antifungal effect was studied on a reference culture of a yeast-like fungus of the Candida genus. Cultures with microorganisms were kept in a thermostat at a temperature of 37°C for 24 hours. Results were calculated from the absence of growth in the last tube corresponding to the minimum inhibitory concentration (MIC). To determine the minimum bactericidal concentration (MBC), 0.1 ml of the last two or three test tubes of the series were placed in a Petri dish with solid nutrient media. After 18-24 hours of incubation at a temperature of 37°C, a minimum concentration was noted that did not allow growth on agar and corresponded to the MBC value. The microbial load for museum strains was 5x10 CFU/ml. Dimethyl sulfoxide was used as a solvent in the research; solutions of the studied substances were adjusted to a concentration of 1 mg/ml. Microscopic examination was carried out under a "Biolan" microscope at x100, x200, x400 magnification.

The synthesis of ureide derivatives 3.1a-d was performed by investigating the interaction of 2-amino-3-carbethoxy(cyano)thiophenes 2.1 with phenylisocyanate. A classic Gevald reaction was used to obtain the aminothiophenes 2.1. Instead, the initial thiophenes 2.1a-d were isolated from the reaction medium. To synthesize ureide derivatives 3.1a-c containing an ester group in position 3, a non-polar solvent, benzene, was used, and the optimal synthesis time was found to be 3 hours [9]. The process of synthesizing the compounds involved the use of physical and chemical methods for studying the properties of various substances. As a result of the synthesis, both the primary compounds and their transformed products were obtained. To separate the non-transformed parts of the primary substances from the reaction products or the process of purification of the main product, various techniques were employed such as filtration, recrystallization, and column chromatography. These techniques allowed for the isolation of pure compounds that were then subjected to further analysis of their physicochemical and biological properties. The use of these purification methods was crucial to obtaining accurate results during the study of the compounds' diuretic, antiexudative, and antimicrobial activity.

Based on the obtained data, was obtained a Patent of utility model No. 72647 "2-alk(acyl)oxy-4-oxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines, which exhibit diuretic activity" [10] and a Patent for the invention No. 104197 "2-alk(acyl)oxy-4-oxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines, which exhibit diuretic activity" [11].

Results

Synthesis of ureide derivatives of 2-amino-3-carbethoxy(cyano)thiophenes and study of their cyclization. Condensed pyrimidines occupy an important place among biologically active heterocycles of natural and synthetic origin. It is known that thienopyrimidine derivatives have a wide

spectrum of biological activity, therefore the synthesis of new condensed systems based on thiophene and pyrimidine was of interest both from the viewpoints of chemistry and the study of their pharmacological properties [12; 13].

In order to synthesize the original ureides 3.1, the author investigated the interaction of 2-amino-3-carbethoxy(cyano)thiophenes 2.1 with phenylisocyanate (Figure 1-2) (aminothiophenes 2.1 were obtained by the classic Gevald reaction). It was established that the formation of ureide derivatives 3.1a-d does not occur under the conditions of the described synthesis of similar thioureides (boiling for 2-3 hours in 1.4-dioxane). Initial thiophenes 2.1a-d were isolated from the reaction medium. The author developed a methodology for the synthesis of ureide derivatives 3.1a-c containing an ester group in position 3, which involves the use of a non-polar solvent – benzene. The optimal synthesis time is 3 hours; ıt was found that the optimal solvents for the synthesis of ureide derivatives with a cyano group in position 3 are toluene or a mixture of xylene isomers. It was found that the best time for synthesis is three hours (Figure 1-2).

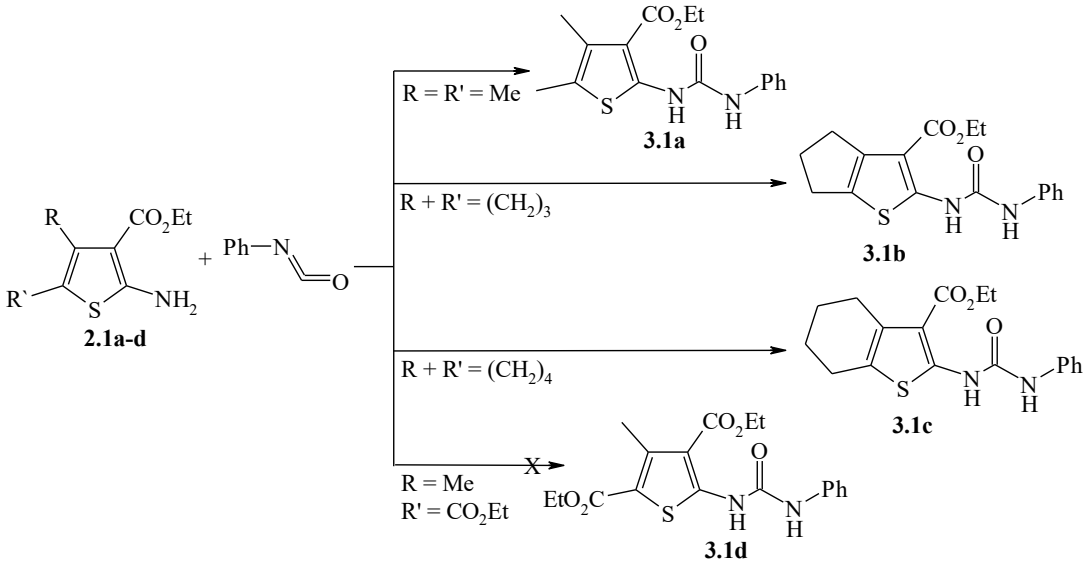

Figure 1. *Interaction of 2-amino-3-carbethoxy(cyano)thiophenes 2.1 with phenylisocyanate*

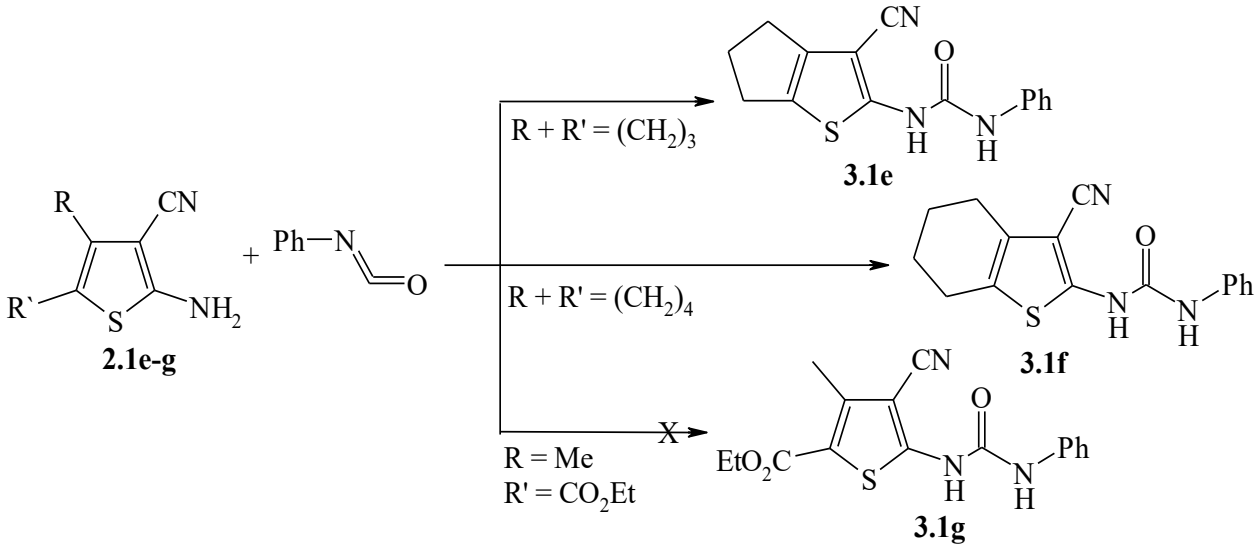

Figure 2. *Interaction of 2-amino-3-carbethoxy(cyano)thiophenes2.1 with phenylisocyanate*

The author also established that the reaction can be carried out at a lower temperature (for example, boiling in benzene), but this required an increase in the synthesis time. Ureides 3.1e,f were obtained with a yield of 75% upon boiling for 3.5 h.

An increase in the duration of the reaction to 4-5 h was accompanied by the processes of

partial tarification of the starting 2-amino-3-carbethoxy and 2-amino-3-cyano-thiophenes 2.1a-g and target products 3.1a-c,e,f, which caused a decrease in the yield of ureide derivatives 3.1a-c,e,f.

In the case of using thiophenes 2.1d and 2.1g (R = Me, R` = CO2Et) for the reaction, the initial compounds were isolated under the reaction conditions, which can probably be explained by a decrease in the nucleophilic properties of the amino group in position 2 of the thiophene nucleus due to the electron-accepting effect of the second ester group in position 5. The 1H NMR spectra were used to characterize the synthesized organic substances. The spectra showed signals corresponding to the different functional groups present in the compounds. For example, the spectra of nitriles of 4-R-5-R-2-aminothiophene-3-carboxylic acids showed signals in the range of 3.3-3.9 ppm corresponding to the cyano group, while the spectra of ethyl 4-R-5-R-2-aminothiophene-3-carboxylates showed signals in the range of 1.1-1.4 ppm corresponding to the ethyl group. The spectra of the ureide derivatives showed signals in the range of 1.6-2.8 ppm corresponding to aliphatic substituents in positions 4 and 5 of the thiophene nucleus, and signals of aromatic protons in the range of 6.9-7.7 ppm and two NH groups within 10.0-11.2 ppm. The IR spectra were also used to analyze the synthesized compounds. For example, the IR spectra of the nitriles showed absorption bands at around 2230-2260 cm-1 corresponding to the cyano group, while the spectra of the ureide derivatives showed absorption bands at around 3300 cm-1 corresponding to NH groups. The analysis of IR spectra allowed to confirm the presence of functional groups in the synthesized compounds and to assess the purity of the obtained substances.

Cyclization of ureide derivatives 3.1a-c was carried out in a 90% aqueous ethanol solution under the action of a two-fold excess of alkali; in this case, sodium salts of 2-oxy-4-oxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines 3.2a-c were obtained, to which 10% aqueous solution of acetic acid was added the corresponding 2.4-dioxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines 3.3a-c were isolated (Figure 3).

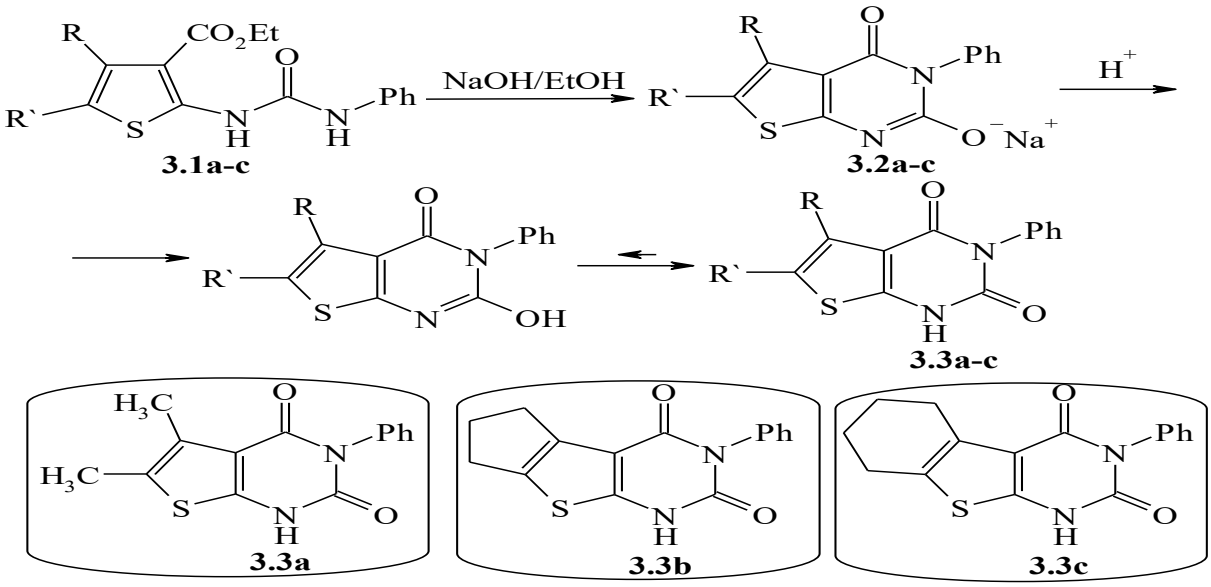

Figure 3. *Cyclization of ureide derivatives*

Sodium salts of 4-imino-2-oxy-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines 3.2e.f and 4-imino-2-oxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines 3.3e.f were obtained under similar conditions (Figure 4). The NMR1H spectra of 2.4-dioxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines 3.3a-c were characterized by the presence of signals corresponding to aliphatic substituents in positions 4 and 5 of the thiophene nucleus, signals of aromatic protons in the range 6.9-7.7 ppm. In contrast to the original ureide derivatives 3.1a-c, the spectra lacked the signals of the NH groups of the ureide fragment, but the signal of the endocyclic NH group appeared in a weaker field (~12 ppm). 1H NMR spectra of 4-imino-2-oxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines 3.3e.f additionally

contained signals of the exocyclic NH group (9.0-9.2 ppm).

The 13C NMR spectra of the synthesized derivatives were used to characterize the carbon atoms in the structures. The spectra of the compounds containing alkyl groups showed signals in the range of 20-60 ppm corresponding to the carbon atoms of the aliphatic substituents. The signals of the carbon atoms in the aromatic ring were observed in the range of 110-160 ppm. The spectra of the compounds containing aromatic groups showed signals in the range of 120-160 ppm corresponding to the carbon atoms in the aromatic ring, and the signals of the carbon atoms in the aliphatic substituents were observed in the range of 20-60 ppm. In addition, the IR spectra were used to confirm the presence of functional groups in the synthesized compounds. The presence of characteristic absorption bands in the IR spectra allowed for identification of the functional groups present in the compounds, including carbonyl, amine, and thioamide groups. Overall, the combination of NMR and IR spectroscopy provided a comprehensive analysis of the synthesized derivatives. The study of alkylation and acylation processes of 2.4-dioxo- and 4-imino-2-oxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines. 2.4-dioxo- and 4-imino-2-oxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines 3.3a-c.e.f are optimal compounds for chemical modification, which made it possible to obtain a wide range of new potential biologically active substances. One of the ways that made it possible to significantly modify the chemical structure was the Grignard reaction. The interest in these studies was also due to the fact that such experiments in a series of thieno[2.3-d]pyrimidines had not been carried out before, and there are few literature data on the interaction of structurally related benzopyrimidines, and the direction of the reaction depended on many factors.

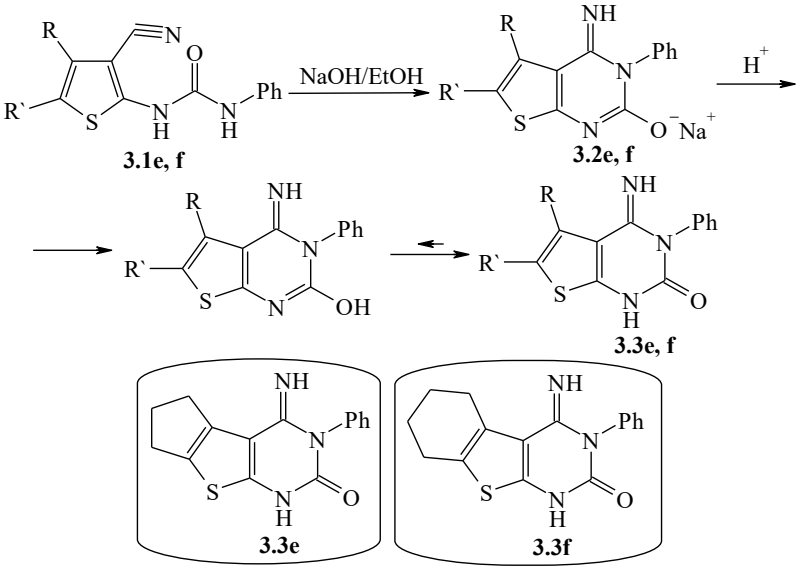

Figure 4. *Sodium salts of 4-imino-2-oxy-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines 3.2e.f and 4-imino-2-oxo-3-phenyl-5- R-6-R`-thieno[2.3-d]pyrimidine 3.3e.f 3.3e.f*

In the studies carried out by the author, it was shown that 2.4-dioxo- and 4-imino-2-oxo-3-phenyl-5-R-6-R`-thieno-[2.3-d]pyrimidine 3.3a-c.e.f did not interact with arylmagnesium halides. In the classical solvent for this reaction, diethyl ether, the original thienopyrimidines 3.3a-c,e,f are insoluble, so the reaction was carried out in tetrahydrofuran. Even the use of the reagent in a significant excess (1:7) did not give a positive result – the original compounds were isolated from the reaction medium.

It is known from the literature that the corresponding analogues – 4-imino-3-phenylbenzo- and 4-imino-2-thio-3-phenylfuro[2.3-d]pyrimidine – at high temperatures, in particular, when boiling in DMF, underwent Dimrot rearrangement: there was a migration of the phenyl substituent at position 3 to the imino group at position 4 of the condensed system. It was established that this structural rearrangement is not characteristic under the given conditions for 4-imino-2-oxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines 3.3e.f – the original

thienopyrimidines were isolated from the reaction medium 3.3e.f (Figure 5).

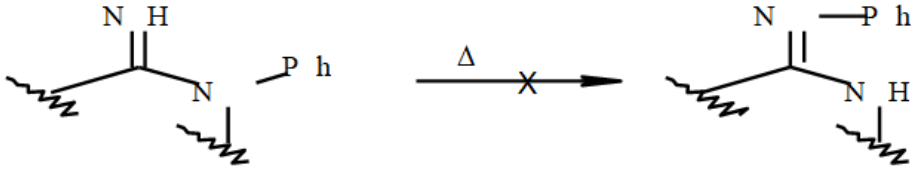

Figure 5. *Effect of the method at high temperatures*

Other methods that were used to synthesize new series of compounds included alkylation and acylation. It was established that the alkylation of sodium salts of 2-oxy-4-oxo(imino)-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines 3.2a-c.e.f with alkyl halides (methyl iodide, ethyl bromide, chloroacetate acid, methyl bromoacetate, allyl bromide, and 3-bromopropanoic acid) occurred at the exocyclic oxygen atom in position 2 of the pyrimidine ring, i.e., similarly to the corresponding thioanalogues, with the formation of 2-alkoxy-4-oxo(imino)-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines 4.1a-o, with the yields of 70-92% (Figure 6).

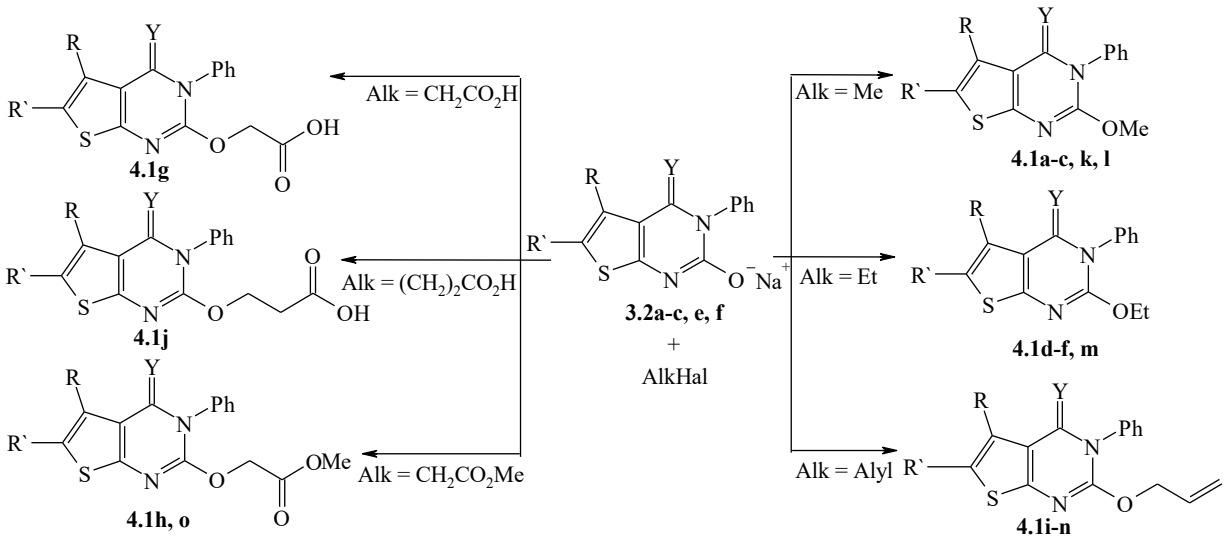

Figure 6. *Alkylation of sodium salts of 2-oxy-4-oxo(imino)-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines 3.2a-c,e,f with alkyl halides*

Note: **4.1a** R = R` = Me, Y = O; **4.1b** R + R` = $(CH_2)_3$, Y = O; **4.1c** R + R` = $(CH_2)_4$, Y = O; **4.1d** R = R` = Me, Y = O; **4.1e** R + R` = $(CH_2)_3$, Y = O; **4.1f** R + R` = $(CH_2)_4$, Y = O; **4.1g** R + R` = $(CH_2)_4$, Y = O; **4.1h** R + R` = $(CH_2)_4$, Y = O; **4.1i** R + R` = $(CH_2)_4$, Y = O; **4.1j** R + R` = $(CH_2)_4$, Y = O; **4.1k** R + R` = $(CH_2)_3$, Y = NH; **4.1l** R + R` = $(CH_2)_4$, Y = NH; **4.1m** R + R` = $(CH_2)_4$, Y = NH; **4.1n** R + R` = $(CH_2)_4$, Y = NH; **4.1o** R + R` = $(CH_2)_4$, Y = NH.

To prove the direction of the reaction for the thienopyrimidine system, the author obtained O- (4.1j) and N-alkylation products (4.5c), which contained the same substituent (carboxyethyl) – by the interaction of salt 3.2c with 3-chloropropanoic acid and hydrolysis of the nitrile 3-(2.4-dioxo-3-phenyl-5.6-tetramethylenethieno[2.3-d]pyrimidin-1-yl) propanoic acid 4.3c, respectively. The physicochemical properties (melting point, NMR1H spectrum data) of products 4.1j and 4.5c differed.

This result of the reaction was explained by a decrease in the nucleophilic properties of the nitrogen atom in position 1 of the pyrimidine cycle due to the influence of the thiophene nucleus. This was evidenced by an unsuccessful attempt to carry out N-alkylation of 2-amino-3-carbethoxy(cyano)thiophenes 2.1a-g with ethyl chloroacetate (boiling in ethanol or DMF in the presence of Na2CO3) – the author isolated the starting thiophenes 2.1a-g.

The 1H NMR spectra of 2-alkoxy-4-oxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines 4.1a-j were characterized by the presence of signals corresponding to aliphatic substituents in positions 4 and 5 of the thiophene core, signals of the alkyl substituent in position 2 of the pyrimidine cycle, signals of aromatic protons in the range of 7.2-7.6 ppm. Unlike the unsubstituted oxygen atom of 2.4-dioxo-3-phenyl-5-R-6-R`-thieno[2.3-d]-pyrimidines 3.3a-c, they lack the signals of the exocyclic NH group of the pyrimidine fragment. The 1H NMR spectra of 2-alkoxy-4-imino-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines 4.1k-o additionally contained signals of the NH group in position 4 of the pyrimidine nucleus in the range of 5.8-7.9 ppm.

As a result of acylation of sodium salts of 2-oxy-4-oxo(imino)-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines 3.2a-c.e.f with halogen anhydrides of carboxylic acids (acetyl chloride, benzoyl chloride) 2-acyloxy-4-oxo(imino)-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidine 4.2a-j was isolated (Figure 7). The 1H NMR spectra of 2-acyloxy-4-oxo-3-phenyl-5-R-6-R`-thieno[2.3-d]-pyrimidines 4.2a-f were characterized by the presence of signals corresponding to aliphatic substituents in positions 4 and 5 of the thiophene nucleus, signals of the acyloxyl substituent in position 2 of the pyrimidine ring, signals of aromatic protons within the range of 7.2-7.6 ppm. Unlike 2.4-dioxo-3-phenyl-5-R-6-R`-thieno[2.3-d]-pyrimidines 3.2a-c, they lack the signals of the exocyclic NH group of the pyrimidine ring. NMR1H spectra of 2-acyloxy-4-imino-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines 4.2g-j additionally contained signals of the NH group in position 4 of the pyrimidine nucleus.

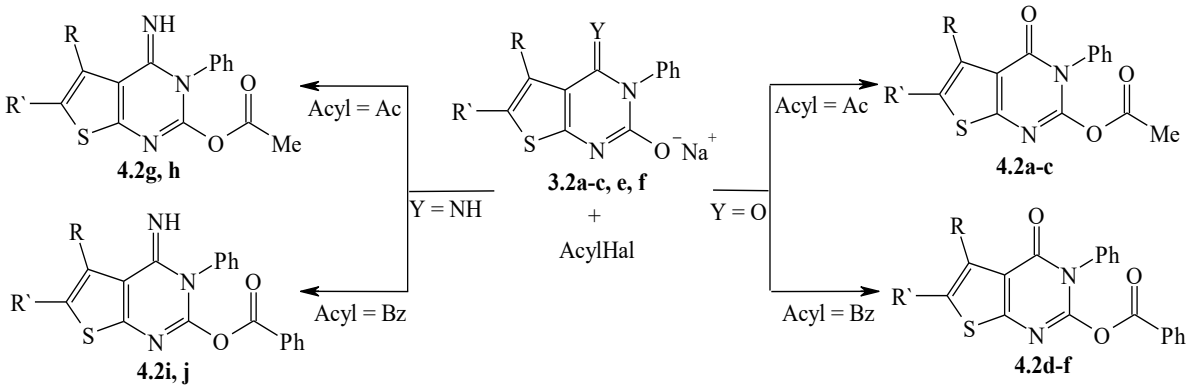

Figure 7. *Acylation of sodium salts of 2-oxy-4-oxo(imino)-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines 3.2a-c.e.f with carboxylic acid halides*

Note: **4.2a** R = R` = Me; **4.2b** R + R` = $(CH_2)_3$; **4.2c** R + R` = $(CH_2)_4$; **4.2d** R = R` = Me; **4.2e** R + R` = $(CH_2)_3$; **4.2f** R + R` = $(CH_2)_4$; **4.2g** R + R` = $(CH_2)_3$; **4.2h** R + R` = $(CH_2)_4$; **4.2i** R + R` = $(CH_2)_3$; **4.2j** R + R` = $(CH_2)_4$.

The author investigated the features of the cyanethylation reaction of 2.4-dioxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidine derivatives. During the interaction of 2.4-dioxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines 3.3a-c with acrylonitrile, 3-(2.4-dioxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidin-1-yl) nitriles were formed of the propanoic acids 4.3a-c. It is known that the introduction of the amidoxime group affected the manifestation of antimicrobial properties. When nitriles 4.3a-c were treated with hydroxylamine, amidoximes of 3-(2.4-dioxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidin-1-yl) propanoic acids 4.4a were obtained. 3-(2.4-dioxo-3-phenyl-5-R-6-R`-thieno-[2.3-d]pyrimidin-1-yl) propanoic acids 4.5a-c were obtained by hydrolysis of nitriles 4.3a-c in an acidic medium (Figure 8). The NMR spectra of 1H nitriles of 3-(2.4-dioxo-3-phenyl-5-R-6-R`-dimethylthieno[2.3-d]pyrimidin-1-yl) propanoic acids 4.3a-c were characterized by the presence of signals corresponding to aliphatic substituents in the positions 4 and 5 of the thiophene core and fragment -(CH2)2-, signals of aromatic protons in the range of 6.9-7.7 ppm. Unlike the corresponding 2.4-dioxo-3-phenyl-5-R-6-R`-dimethyl-thieno[2.3-d]pyrimidines 3.3, they lack signals of the endocyclic NH group of the pyrimidine ring.

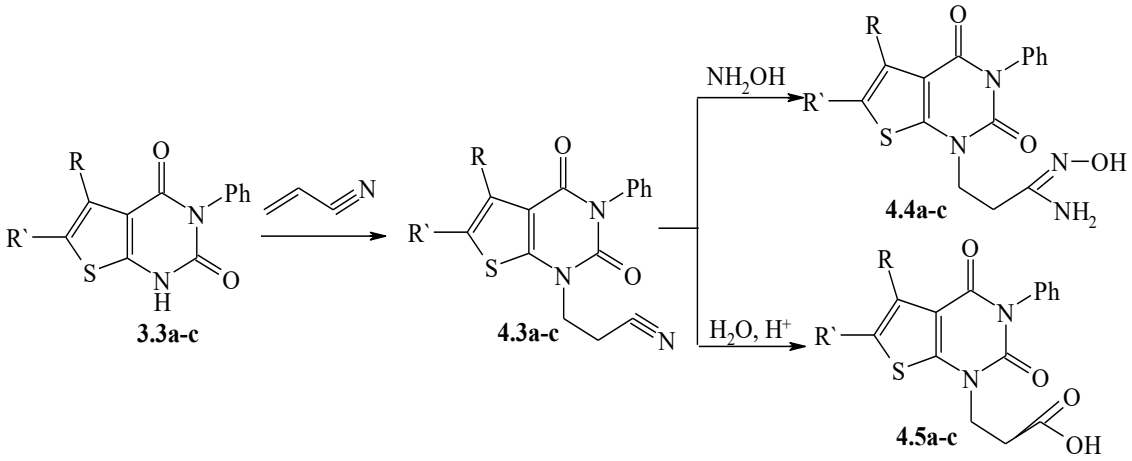

Figure 8. *Cyanethylation of 2.4-dioxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidine derivatives*
Note: **4.3a, 4.4a, 4.5a** R = R` = Me; **4.3b, 4.4b, 4.5b** R + R` = $(CH_2)_3$; **4.3c, 4.4c, 4.5c** R + R` = $(CH_2)_4$.

In the NMR spectra of 1H amidoximes of 3-(2.4-dioxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidin-1-yl) propanoic acids 4.4a-c additional amide (at 5.4 -5.5 m.h.) and OH group (~9 ppm) signals appeared, while in the spectra of acids 4.5a-s, there are signals of the OH group (~12 ppm).

Biological studies of created substances. For a preliminary logical-structural evaluation of the possible biological action of the synthesized compounds, the author used the Prediction of Activity Spectra for Substances (PASS) program, which was used to evaluate the pharmacological effects, mechanisms of action, and specific toxicity of the compound. To conduct PASS forecast, thiophenes 2.1a-e, ureide derivatives 3.1a-e, thieno[2.3-d]pyrimidine 3.3a-e, their alkyl 4.1a-o and acyl 4.2a-j derivatives, nitriles 3-(2, 4-dioxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidin-1-yl) propanoic acids 4.3a-c [14; 15] were synthesized.

A library of 60 compounds was processed by the PASS program to guide planning, synthesis, as well as further biological studies. The calculations were performed with the tolerance Pa>0.7 and Pi<0.005, which made it possible to carry out a more thorough selection and reduce the risk of encountering a known medicinal product. Computer methods of research of the created substances were used and it was shown that these compounds possessed various types of biological activity, the main of which being excretory and anti-inflammatory.

Taking into account the data of computer forecasting and literature search, it was appropriate to test the synthesized compounds for the manifestation of diuretic, antiexudative and antimicrobial activities. Diuretics are used for many diseases that are accompanied by fluid retention in the body: chronic circulatory failure, nephrotic syndrome, liver cirrhosis, as well as hypertension, glaucoma, and others, but many of them have pronounced side effects [16-18]. That is why the identification and creation of new effective means of excretory action was an extremely important relevant problem.

The study of the diuretic effect of the synthesized compounds was carried out at the Department of Physiology of the National University of Pharmacy using the E.B. Berkhin [6] method. The obtained data were analyzed (Figure 9), and it was noted that the ureide derivatives of thiophene 3.1 did not show diuretic activity. Moreover, an antidiuretic effect was noted for compounds 3.1c and 3.1d (the difference compared to the control amounts to -41.78% and -17.41%, respectively). At the same time, the connection between chemical structure and biological activity was not observed; probably, the entire molecule, rather than individual pharmacophores (structural fragments), was responsible for the antidiuretic effect.

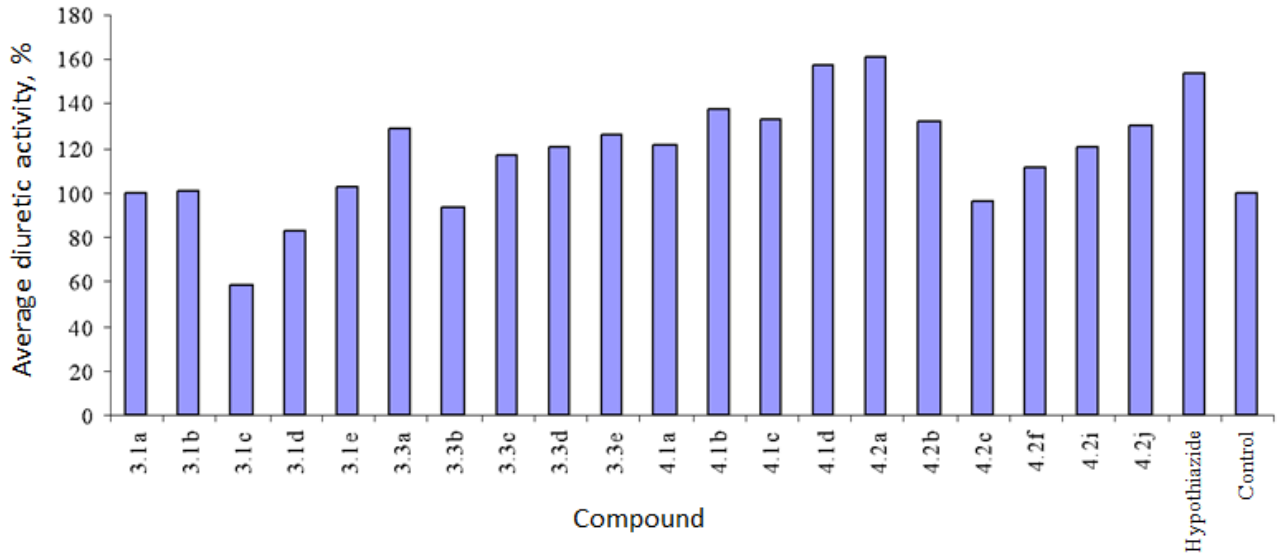

Figure 9. *Average diuretic activity of some synthesized compounds*

When switching to thieno[2.3-d]pyrimidines 3.3, an increase in the level of diuretic activity (with the exception of compound 3.3b) was observed at the level of 11.25%-25.93%. At the same time, imino derivatives 3.3e and 3.3f showed a higher level of activity compared to oxo analogues. Alkylation contributed to the manifestation of diuretic activity. All alkyl derivatives 4.1 showed diuretic activity at the level of more than 28%. The most active was the ethyl derivative 4.1d (57%). Regarding acylated derivatives 4.2, the analysis showed a discrepancy in results: from absence to activity at a level slightly higher than the reference drug.

Therefore, condensed thienopyrimidines were a more promising group for the search for diuretins. In general, compounds 4.1d and 4.2a (+57.21% and +61.48%, respectively) showed diuretic activity at the level of the reference drug, while the latter compound exceeded the specific activity of hypothiazide by 7.48%. The rest of the compounds showed weak diuretic activity at the level of 25.9%-37.5%.

Diuretic and anti-inflammatory activities showed a synergistic effect, therefore substances that showed a high (4.1d, 4.2a) and moderate (3.3e, 4.1b, 4.1c, 4.2b, 4.2f and 4.2j) level of diuretic activity were also investigated for detection of anti-inflammatory action.

The study of the antiexudative effect of the synthesized compounds was carried out at the Department of Physiology of the National Academy of Sciences on the model of carrageenan edema. The results of the study showed that a pronounced reduction in edema was observed in mice that received substances 4.1d, 4.2a. Compounds 4.1b, 4.1c, 4.2b had a moderate anti-exudative effect, while substances 3.3e, 4.2f, 4.2j did not show a pronounced anti-inflammatory effect in comparison with the control group of animals and the reference drug (voltaren). A comparative characterization with the reference drug (diclofenac sodium) was carried out and it was concluded that the most pronounced anti-inflammatory activity was revealed by compound 4.2a, which is competitive with voltaren in terms of anti-inflammatory activity (51.45% and 72.48%, respectively).

Significant experience of chemotherapeutic introduction into clinical use of first-generation antibiotics has convincingly proved that, along with undisputed effectiveness, the uncontrolled and unreasonable use of these drugs was usually accompanied by a progressive increase in side effects [19]. At the same time, the development of resistance to antibiotics caused special concern. The analysis of aspects of antibiotic resistance convincingly proves that, in general, this problem is related to both the antimetabolite mechanism of action of these drugs and significant differences in the absolute levels of bacteriostatic and bactericidal abilities. In this regard, one of the promising directions for the improvement and development of antibiotic therapy in the 21st century was the consistent substitute introduction of drugs with pronounced antiseptic properties into clinical medicine [20; 21]. Their advantages included complex effects in the inherent mechanisms of action on the microbial cell, independence of activity levels from indicators of resistance to pathogens' drugs, predominant or selective microbicidal effect on the microbial cell. This is what the prospects of preventing and overcoming antibiotic resistance were associated with [22; 23].

The antimicrobial activity of the synthesized compounds was carried out in the laboratory of antimicrobial agents of the Mechnikov Institute of Microbiology and Immunology in accordance with the recommendations of the State Pharmacopoeia of Ukraine [7; 8] using a standard set of reference test strains of gram-positive and gram-negative bacteria (S. aureus ATCC 29213, E. soli ATCC 25922, R. aeruginosa ATCC 27853, B. subtilis ATCC 6633). The antifungal effect was studied on the reference culture of S. albicans ATSS 885-653. Microbiological studies of 12 original derivatives were carried out: ethyl 3-R-4-R`-2-aminothiophene-3-carboxylates 2.1a-d, nitriles of 3-R-4-R`-2-aminothiophene-3-carboxylic acids 2.1e-f, their ureides derivatives 3.1, 2.4-dioxo- and 4-imino-2-oxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines 3.3, their alkyl 4.1, acyl 4.2 and cyanethyl derivatives 4.3 and products their chemical transformations by the method of serial dilutions, which made it possible to give a quantitative assessment of the antimicrobial action of the studied compounds. In the course of research (Table 1), it was established that the inhibitory concentration of compounds relative to gram-positive microorganisms (S.aureus ATCC 29213, B. subtilis ATCC 6633) ranged from 25 to 100 μg/ml, while the bactericidal concentration amounted to 50-200 μg/ml.

Table 1. *Results of the study of the antimicrobial activity of some synthesized compounds, μg/ml*

	Antibacterial and fungicidal activity relative to strains, μg/ml									
	S. aureus ATCC 25923		**E. coli ATCC 25922**		**P.aeruginosa ATCC 27853**		**B. sulbtilis ATC 6633**		**C. albicans ATC 885-653**	
Comp.	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
2.1a	25	50	50	100	50	100	50	100	50	100
2.1b	25	50	50	100	50	200	100	200	25	50
2.1e	25	50	50	100	100	100	50	100	50	100
3.1a	50	100	50	100	50	100	50	100	50	100
3.1c	200	200	50	100	100	100	100	200	100	100
3.1d	100	200	50	100	200	200	100	200	50	100
3.1e	100	200	50	100	50	200	100	200	50	100
3.3a	50	50	50	100	100	100	50	200	50	100
3.3c	50	50	100	100	100	200	100	100	200	200
4.2a	200	200	100	200	100	100	100	200	100	200
4.2j	50	100	50	100	50	100	100	200	100	100
4.3a	50	100	50	100	50	100	100	200	50	50
C	100	200	100	200	100	200	100	200	100	200

Among the 12 studied compounds, compounds 2.1a, 2.1b, 2.1e – 2-amino-3-carbethoxy(cyano)thiophenes, which showed inhibitory activity against S.aureus ATCC 29213 at a concentration of 25 μg/ml, should be particularly noted. The bactericidal effect of these substances was moderate and amounted to 50 μg/ml. Relative to B. subtilis ATCC 6633, the compounds showed a moderate inhibitory effect at the same concentration.

Antimicrobial activity against gram-negative microorganisms (E. soli ATCC 25922, P. aeruginosa ATCC 27853) was studied, and it was noted that most of the substances showed a moderate inhibitory effect against E. soli ATCC 25922 at a concentration of 50 μg/ml. Relative to P. aeruginosa ATCC 27853, only half of the substances showed a moderate inhibitory effect at the same concentration. Other compounds did not show high antimicrobial activity against gram-negative pathogens.

Relative to S. albicans ATCC 885-653, a significant number of studied compounds showed a fungistatic effect at concentrations of 50 μg/ml. Only substance 2.1b showed a significant antifungal effect at the minimum fungistatic concentration of 25 μg/ml. Other compounds from this group have demonstrated moderate fungistatic activity.

As follows from the above, as a result of research, it was found that the most active compounds 2.1, which contained functional groups (amino-, carbethoxy- or cyano-), are directly connected to the thiophene nucleus. Moreover, the amino group had a decisive influence on the detection of antimicrobial activity. When it was converted to ureide (compounds 3.1), the ir cated activity decreased, although the carbet

yl and cyano groups remained unmodified. The same pattern was demonstrated in the case of condensed thienopyrimidines 4.1, 4.2 and 4.3 – the absence of the mentioned functional groups (present in the original compounds 2.1) led to almost complete leveling of the antimicrobial activity to the control level. The physical properties of the obtained derivatives of 2.4-dioxo- and 4-imino-2-oxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines are presented in Table 2.

Table 2. *Physical properties of synthesized derivatives*

Derivative	**Aggregate state**	**Melting temperature (°C)**	**Boiling temperature (°C)**	**Density (g/cm³)**	**Refractive index**
2.4-dioxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines	Solid	180-182	460-462	1.25	1.626
Sodium salt of 2-oxy-4-oxo(imino)-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines	Solid	220-222	615-617	1.37	1.673
Ureide derivatives of 2-amino-3-carbethoxy(cyano)thiophenes	Solid	140-142	350-352	1.18	1.582
2-amino-3-cyanothiophenes	Solid	118-120	305-307	1.12	1.554
Ethyl 4-R-5-R`-2-aminothiophene-3-carboxylates	Liquid	-35	168-170	1.05	1.498

Thus, although compound 4.2a with the highest level of diuretic, anti-inflammatory activity and low toxicity did not show antimicrobial activity, however, given the relatively accessible method of synthesis, it is recommended for in-depth pharmacological studies. A project of quality control methods was developed for this compound, and an application for an invention and an application for a utility model were submitted.

Discussion

A huge amount of research has been devoted to the detailed study of the structure and function of pyrimidines. As is known, pyrimidines are structural analogues of natural compounds of the purine series, such as guanine and adenine. It has been studied that they have an extremely wide spectrum of biological activity. The author of this study developed methods for the synthesis of 2.4-dioxo- and 4-imino-2-oxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidine derivatives, analyzed the structure of the invented substances and carried out their pharmacological analysis. It was found that the investigated thienopyrimidines did not undergo Dimrot rearrangement and did not interact with Grignard reagents.

It was established that alkylation and acylation of sodium salts of 2-oxy-4-oxo(imino)-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines by alkyl and acyl halides occurs at the exocyclic oxygen atom in position 2 with the formation of 2-alk(acyl)oxy-4-oxo(imino)-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines.

Virtual screening of synthesized substances, their alkyl, acyl and cyanoethyl derivatives and products of their chemical transformations was carried out. According to the obtained results, biological studies of the created compounds were carried out in order to detect their antimicrobial, excretory, anti-inflammatory activities and acute toxicity. A microbiological study of the synthesized substances was carried out in relation to Staphylococcus aureus, Proteus vulgaris, intestinal, Pseudomonas aeruginosa, and hay bacillus. It was found that the compounds containing functional groups (amino-, carbethoxy- or cyano-) directly connected to the thiophene nucleus were the most active. Considering the high diuretic and anti-inflammatory activity, low toxicity and relatively accessible method of synthesis of the compound 2-acetyloxy-4-oxo-3-phenyl-5.6-dimethylthieno[2.3-d]pyrimidine, it was recommended for in-depth pharmacological studies. A project of quality control methods was developed for the specified compound, an application for an invention and an application for a utility model were submitted.

Today, against the backdrop of an increase in the number and frequency of detection of oncological diseases, the search for highly effective and at the same time affordable drugs is conducted daily. Considering the wide spectrum of activity of pyrimidines, scientists S.B. Salib et al. [3] decided to carry out

a deeper study of these compounds and their derivatives. Scientists synthesized thienopyrimidine derivatives containing a thiosemicarbazidase component. Based on studies, it was found that these substances demonstrated greater antitumor activity against prostate and colon cancer compared to controls. It was established that the inclusion of Br in position 4 of the phenyl ring in the thiosemicarbazide fragment significantly increased the anticarcinogenic effect. Based on this, it was concluded that thieno[2.3-d]pyrimidines, which contained a thiosemicarbazide framework, could be excellent candidates for further chemical transformations in order to obtain more potent and highly selective antitumor agents.

Scientists at the University of Toledo have been searching for drugs to fight triple-negative breast cancer, which is the most lethal and rapidly progressive subtype with early, rapid metastasis. Cancer cells of this subspecies do not have receptors for estrogen, progesterone and human epidermal growth factor 2, which made its treatment much more difficult and significantly reduced the patients' chances of recovery. As a result of long-term research, a new analogue of thienopyrimidines – TPH104 – was synthesized, which induced immunogenic cell death in the triple-negative breast cancer cell line by increasing the stimulating ability of dendritic cells. TPH104 significantly increased adenosine triphosphoric acid levels in the supernatant and mobilized intracellular calreticulin to the plasma membrane in MDA-MB-231 cells, induced rapid membrane permeabilization, and had characteristics of non-apoptotic cancer cell death in culture. TPH104-induced release of adenosine triphosphoric acid increased the surface translocation of CRT and activated the endogenous TNF-a pathway by imparting immunogenic characteristics to dying MDA-MB-231 cells, the content of which, in turn, stimulated dendritic cell maturation and enhanced the action of the inflammatory gene [5].

French scientists P. Lagardere et al. [4] studied in more detail the spectrum of activity of thienopyrimidines and their analogues. Antifungal activity against Candida albicans and Aspergillus niger was investigated by determining the diameter of the zone of growth inhibition. All test substances showed antifungal activity similar to nystatin. Thieno[2.3-d]pyrimidine derivatives were synthesized as anthelmintic agents against Trichinella spiralis. Substitution of the alkyl chain in position 2 of the thienopyrimidine ring with a benzimidazole fragment was essential for increasing the anthelmintic activity. The created compounds showed activity five times higher than that of the comparison drug albendazole. These studies were based on the introduction of various substituents on the pyrimidine and thiophene rings, which allowed quite easily access to a wide range of modulations. Antibacterial, antifungal, and antituberculosis agents were found to be mostly thieno[2.3-d]pyrimidine derivatives, while compounds with antiviral activity were represented by thieno[3.2-d]pyrimidines.

Such significant interest in the study of pyrimidines and their derivatives is primarily explained by the significant spectrum of action of these compounds, their low toxicity, as well as the ability to accelerate cell growth and reproduction. Pyrimidines have been found to have a significant effect on the activity of enzyme systems responsible for the formation or degradation of nucleic acids. It was found that the abovementioned substances have the ability to indirectly affect protein metabolism, with a direct effect on fat and carbohydrate metabolism.

Modern studies have repeatedly proven the ability of pyrimidine derivatives to influence the immune function of the body, stimulating the production of antibodies and normalizing their level, increasing the effectiveness of immunization [24]. A positive effect on hematopoietic function, stimulation of restoration of damaged or dead cell structures was also noted. Antioxidant, anti-inflammatory, antitoxic, antitumor, radioprotective, anabolic, anticatabolic, bactericidal activity and psychotropic, cardiotropic, hepatoprotective effects have been repeatedly proven. It is known that pyrimidine derivatives have the ability to prevent a decrease in the phagocytic activity of leukocytes, which occurs due to the effect of antibacterial agents, cause stimulation of interferon synthesis, increase the level of immunization, the level of normal antibodies. The mechanism of their action as catalysts of the process of immunity formation is related to their inclusion in the exchange of proteins and nucleic acids, which causes a multifaceted effect on immunogenesis and recovery processes in the body.

Pyrimidine derivatives have a significant therapeutic application in the treatment of resistant and multiresistant forms of tuberculo

sis, many oncological diseases, treatment of the human immunodeficiency virus, many diseases caused by helminths, as well as protozoa [25; 26]. For example, the addition of methyl uracil to the complex therapy of dysentery contributed to the normalization of indicators of natural resistance. A wide range of pharmacological properties of drugs that contained a pyrimidine nucleus in their structure indicate the feasibility of further targeted synthesis of new biologically active substances. Thus, the analysis of the formation of resistance to antimicrobial drugs is quite relevant, especially in relation to the pathogens of zoonotic infections.

The main task of many studies was to identify the antitumor effect of pyrimidine derivatives. One of the most important and promising discoveries in this field in recent years has been drugs from the group of tyrosine kinase inhibitors. This enzyme causes the transfer of a phosphate group from adenosine triphosphate acid to a protein in the cell, thus, tyrosine kinases accelerate the phosphorylation of tyrosine residues in the protein, which causes a change in its functioning. Therefore, it can be concluded that the research of pyrimidines, their analogues and derivatives is a rather relevant and promising direction in the modern production of necessary medicines.

Conclusions

As a result of the research, a theoretical generalization and solution of the scientific problem was presented, which lied in the development of preparative methods for the synthesis of 2.4-dioxo- and 4-imino-2-oxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines, research into the structure of the invented substances and their pharmacological analysis.

1. Preparative methods were created for the synthesis of ureide derivatives of 2-amino-3-carbethoxy(cyano)thiophenes, sodium salts of 2-oxy-4-oxo(imino)-3-phenyl-5-R-6-R`-thieno[2.3- d]pyrimidines, 2.4-dioxo- and 2-oxo-4-imino-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines.

2. It was found that the studied thienopyrimidines do not undergo Dimrot rearrangement and do not interact with Grignard reagents, unlike furo- and benzopyrimidines.

3. It was established that alkylation and acylation of sodium salts of 2-oxy-4-oxo(imino)-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines by alkyl and acyl halides occurs at the exocyclic oxygen atom in position 2 the formation of 2-alk(acyl)oxy-4-oxo(imino)-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines.

4. During the interaction of 2.4-dioxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines with acrylonitrile, N-alkylation occurs at the nitrogen atom in position 1 and nitriles 3-(2, 4-dioxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidin-1-yl) propanoic acids, the hydrolysis of which in an acidic medium gave 3-(2.4-dioxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidin-1-yl) propanoic acids, and by interaction with hydroxylamine – corresponding amidoximes.

5. Virtual screening was performed of synthesized ethyl 4-R-5-R`-2-aminothiophene-3-carboxylates, nitriles of 4-R-5-R`-2-aminothiophene-3-carboxylic acids, their ureide derivatives, 2.4-dioxo- and 4-imino-2-oxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines, their alkyl, acyl and cyanoethyl derivatives and products of their chemical transformations. According to the obtained results, biological studies of the created substances were carried out to reveal their antimicrobial, excretory, anti-inflammatory activities and acute toxicity.

6. As a result of the research, regularities of the connection between chemical structure and biological activity were revealed in a number of synthesized compounds. The test compounds that showed a high level of diuretic activity also showed an anti-inflammatory effect. When switching from noncyclic thiophene derivatives to cyclic thieno[2.3-d]pyrimidines, an increase in the level of release activity was observed. Alkylation and acylation of thieno[2.3-d]pyrimidines contributes to the manifestation of diuretic and anti-inflammatory effects.

7. A microbiological study of some synthesized compounds was carried out against Staphylococcus aureus ATCC 26923, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Bacillus subtilis ATCC 6633 and Proteus vulgaris ATCC 4636. The most active were the compounds containing functional groups (amino-, carbethoxy- or cyano-), directly connected to the thiophene nucleus.

Thus, taking into account the high diuretic and anti-inflammatory activity, low toxicity and relatively accessible method of synthesis of the compound 2-acetyloxy-4-oxo-3-phenyl-5.6-dimethylthieno[2.3-d]pyrimidine, it is recommended for in-depth pharmacological studies. A QCM project was developed for the

specified compound. Based on the obtained data, was obtained a Patent of utility model No. 72647 "2-alk(acyl)oxy-4-oxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines, which exhibit diuretic activity" and a Patent for the invention No. 104197 "2-alk(acyl)oxy-4-oxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines, which exhibit diuretic activity". The limitation of the study is that there are no figures depicting the area of the lysis zones, which reflect the microbiological analysis conducted on the synthesized derivatives.

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ПОИСК БИОЛОГИЧЕСКИ АКТИВНЫХ ВЕЩЕСТВ НА ПРИМЕРЕ 2,4-ДИОКСО- И 4-ИМИНО-2-ОКСО-3-ФЕНИЛ-5-R-6-R`-ТИЕНО[2,3-D]ПИРИМИДИНОВ, ПЕРСПЕКТИВЫ ИХ ИСПОЛЬЗОВАНИЯ В ФАРМАЦИИ И МЕДИЦИНЕ

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Ключевые слова: *производные уреидов, карбоновые кислоты, алкилирование, ацилирование, мочегонное действие*

Целью данной работы явилась разработка новых биологически активных веществ – производных 2,4-диоксо- и 4-имино-2-оксо-3-фенил-5-R-6-R`-тиено[2,3-d]пиримидинов; исследование строения изобретенных веществ и проведение их фармакологического анализа. Такие методы исследования с использованием различных химических реакций

были использованы при синтезе этиловых 4-R-5-R`-2-аминотиофен-3-карбоксилатов, нитрилов 4-R-5-R`-2-аминотиофен-3-карбоновых кислот, их уреидных производных, 2.4-диоксо- и 4-имино-2-оксо-3-фенил-5-R-6-R'-тиено[2,3-d]пиримидинов, их алкильных, ацильных и цианоэтилпроизводных и продуктов их химических превращений. В результате исследований изучены особенности взаимодействия 2-амино-3-карбэтокси(циано)тиофенов с фенилизоцианатом и процессы циклизации полученных уреидных производных в соответствующие 2,4-диоксо- и 2-оксо-4-имино-3-фенил-5-R-6-R'-тиено[2,3-d]пиримидины. Изучены особенности алкилирования и ацилирования синтезированных тиено[2,3-d]пиримидинов. При взаимодействии тиено[2,3-d]пиримидиненов с акрилонитрилом образуются нитрилы 3-(2,4-диоксо-3-фенил-5-R-6-R`-тиено[2,3-d]пиримидин-1-ил)пропановых кислот, при гидролизе которых в кислой среде были получены соответствующие пропановые кислоты, а под действием гидроксиламина – соответствующие амидоксимы. На основании данных компьютерного скрининга (программа PASS) изучено секреторное, противовоспалительное и антимикробное действие синтезированных соединений и установлены некоторые закономерности между химическим строением и биологической активностью.

2,4-DİOKSO- VƏ 4-İMİN-2-OKSO-3-FENİL-5-R-6-R`-TİEN[2,3-D]PİRİMİDİNLƏRİN MİSALINDA BİOLOJİ FƏAL MADDƏLƏRİN AXTARISI, ƏCZAÇILIQ VƏ TİBBDƏ İSTİFADƏ PERSPEKTİVLƏRİ

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Açar sözlər: *ureid törəmələri, karboksilik turşular, alkilləşmə, asilləşmə, sidikqovucu təsir*

Tədqiqatın məqsədi yeni bioloji fəal maddələrin – 2,4-diokso- və 4-imin-2-okso-3-fenil-5-R-6-R`-tien[2,3-d]pirimidinlər törəmələri sintezi; sintez edilmiş maddələrin strukturunun öyrənilməsi və onların farmakoloji analizinin aparılması olmuşdur. Müxtəlif kimyəvi reaksiyalardan istifadə etməklə oxşar tədqiqat üsulları etilləşmiş 4-R-5-R`-2-aminotiofen-3-karboksilatların, 4-R-5-R`-2-aminotiofen-3-karboksil turşularının nitrillərinin, onların ureid törəmələrinin, 2,4-diokso- və 4-imin-2-okso-3-fenil-5-R-6-R'-tieno[2,3-d]pirimidinlərin, onların alkil, asil və sianoetil törəmələrinin və onların kimyəvi çevrilmələrinin məhsullarının sintezində istifadə edilmişdir. Tədqiqat nəticəsində 2-amin-3-karbetoksi(siano)tiofenlərin fenilizosianatla qarşılıqlı təsirinin xüsusiyyətləri və alınmış ureid törəmələrinin sikllləşmə prosesi nəticəsində müvafiq olaraq 2,4-diokso- və 2-okso-4-imin-3-fenil-5-R-6-R'-tien[2,3-d]pirimidinlər çevrilməsi prosesləri müəyyən edilmişdir. Sintez olunmuş tien[2,3-d]pirimidinlərin alkilləşmə və asilləşmə xüsusiyyətləri öyrənilmişdir. Tien[2,3-d]pirimidinlərin akrilonitrillə reaksiyası nəticəsində 3-(2,4-diokso-3-fenil-5-R-6-R'-tien[2,3-d]pirimidin-1-il)propan turşusunun nitrilləri əmələ gəlirlər. Onların turş mühitdə hidrolizi zamanı müvafiq propan turşuları, hidroksilamin təsiri altında müvafiq amidoksimləri əmələ gəlirlər. Kompüter skrininqi (PASS proqramı) əsasında sintez edilmiş birləşmələrin sekretor, iltihabəleyhinə və mikrobəleyhinə təsirləri öyrənilmiş, kimyəvi quruluşu ilə bioloji fəallıq arasında bəzi qanunauyğunluqlar müəyyən edilmişdir.

SEARCH FOR BİOLOGİCALLY ACTİVE SUBSTANCES USİNG THE EXAMPLE OF 2.4-DIOXO- AND 4-IMINO-2-OXO-3-PHENYL-5-R-6-R`-THIENO[2.3-D]PYRIMIDINES, PROSPECTS FOR THEİR USE İN PHARMACY AND MEDİCİNE

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***Keywords**: ureide derivatives; carboxylic acids; alkylation; acylation; diuretic action*

The purpose of this paper was the development of new bioactive substances – derivatives of 2.4-dioxo- and 4-imino-2-oxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines; researching the structure of invented substances and conducting their pharmacological analysis. Such research methods were used as synthesis of ethyl 4-R-5-R`-2-aminothiophene-3-carboxylates, nitriles of 4-R-5-R`-2-aminothiophene-3-carboxylic acids, their ureide derivatives 2.4-dioxo- and 4-imino-2-

oxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines, their alkyl, acyl and cyanoethyl derivatives and products of their chemical transformations using various chemical reactions; application of physicochemical methods of analysis to prove the structure and individuality of synthesized compounds; prediction of biological activity using computer methods, a set of standard methods for studying biological properties; assessment and drawing conclusions based on the obtained results. As a result of the research, the peculiarities of the interaction of 2-amino-3-carbethoxy(cyano)thiophenes with phenylisocyanate were studied and the processes of cyclization of the obtained ureide derivatives into the corresponding 2.4-dioxo- and 2-oxo-4-imino-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidines. The features of alkylation and acylation of the synthesized thieno[2.3-d]pyrimidines were studied. It was established that the mentioned reactions take place at the exocyclic oxygen atom with the formation of 2-alk(acyl)oxy derivatives. During the interaction of thieno[2.3-d]pyrimidinenes with acrylonitrile, nitriles of 3-(2.4-dioxo-3-phenyl-5-R-6-R`-thieno[2.3-d]pyrimidin-1-yl)propanoic acids were formed, the hydrolysis of which the corresponding propanoic acids were obtained in an acidic medium, and the corresponding amidoximes were obtained under the action of hydroxylamine. On the basis of computer screening data (PASS program), the secretory, anti-inflammatory and antimicrobial effects of the synthesized compounds were studied and some patterns were established between the connection of chemical structure and biological activity.

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