IRENA VAŠKEVIČIENĖ

SYNTHESIS AND INVESTIGATION OF *N*-SUBSTITUTED *β*-ALANINES AND AZOLES WITH FUNCTIONALIZED AROMATIC MOIETIES

SUMMARY OF DOCTORAL DISSERTATION

NATURAL SCIENCES, CHEMISTRY (N 003)

Kaunas
2021

KAUNAS UNIVERSITY OF TECHNOLOGY

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KAUNO TECHNOLOGIJOS UNIVERSITETAS

IRENA VAŠKEVIČIENĖ

N-PAKEISTŲ *β*-ALANINŲ BEI AZOLO DARINIŲ, TURINČIŲ FUNKCIONALIZUOTUS AROMATINIUS PAKAITUS, SINTEZĖ IR TYRIMAS

Daktaro disertacijos santrauka
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1. INTRODUCTION

Human health has improved significantly with the discovery and development of effective drugs for microbial infections. However, due to the misuse of anti-infective agents, the microbial resistance to multiple drugs has increased significantly as well. In order to solve this problem, it is necessary to develop new ways to prevent such phenomenon; therefore, it is necessary to discover new classes of drugs that have the original structure and mechanisms of action.

The heterocyclic compounds are extremely important in organic chemistry as they make up more than half of all known organic compounds. The thiazole heterocycle is a component of many natural products and synthetic compounds with a broad spectrum of biological activity (Doregiraee et al., 2015). The thiazole ring fragment is found in biologically active and dynamic molecules, such as sulfathiazole (antimicrobial drug), ritonavir (antiviral drug), abafungin (antifungal drug), bleomycin, and thiazofurin (anticancer preparations). In medical chemistry, the important derivatives are sulfonamides that as well contain a thiazole moiety. These compounds are known as pharmaceutical agents featuring various biological activities, such as antibacterial (Argyropoulou et al., 2009), anticancer (Naaz et al., 2018), and are used as carbonic anhydrase inhibitors (Kilicaslan et al., 2016). The reverse reaction of carbon dioxide hydration is fundamental to many physiological processes. It is catalyzed by carbonic anhydrase (CA) (Kazokaitė, 2015). These enzymes are widespread in both prokaryotes and eukaryotes. In the human body, 12 active CA isoforms are detected. The disorders of gene expression in some CA isoforms have been associated with various diseases, such as glaucoma and tumor developments (Kazokaitė, 2015). Benzenesulfonamide derivatives are the most widely studied as CA inhibitors, despite the synthesis of many different CA sulfonamide inhibitors (Čapkauskaitė, 2008). However, pharmaceutical preparations that have been synthesized on their base have several drawbacks (Čapkauskaitė, 2008). One of the major drawbacks is that the inhibition of carbonic anhydrases throughout the body and the use of sulfonamide inhibitors result in a variety of unexpected side effects, mostly due to their non-specific effect on all CA isoforms, and many of them are toxic (Čapkauskaitė, 2008). Therefore, the development of specific or organo-selective new sulfonamide inhibitors remains to be a very relevant and important challenge. Other important heterocyclic compounds are 2-pyrrolidinone derivatives, which have significant biological and pharmacological effects. The medication belonging to this group is piracetam (2-oxo-1-pyrrolidine acetamide) that has been developed for patients with seizures, Alzheimer's and Senile dementia, concussion, and other neurological diseases. Another product is doxapram (1-ethyl-4-(2-morpholin-4-ylethyl)-3,3-diphenylpyrrolidin-2-one), and it is used for patients with

respiratory failure. The pyrrolidinone moiety is considered the major group of pharmacophores that is involved in the complex of chemical structures, such as ceruletide, which is a ten-amino acid oligopeptide that stimulates muscle and increases digestive secretion. Lastly, gonadorelin (GnRH) is responsible for the release of follicle-stimulating luteinizing hormone (Kellici, 2017).

Recently, a special attention has been paid to *β*-amino acids as a potential precursor of bioorganic, medicinal, and natural products (Juaristi et al., 2005). For example, taxol, which is extracted from short-leaved yew, is used as an anticancer drug. Bleomycin, isolated from *Streptomyces bacteria*, inhibits the growth of dividing cancer cells, and destruxin A is a cyclic peptide that disrupts calcium balance in cells. Dipeptide-carnosine is found in muscles, liver, brain, and other organs and demonstrates antioxidant properties. The *β*-alanine fragment is included in the composition of vitamin B_5 and, therefore, is a precursor of coenzyme A (CoA) and acyl-CoA protein, which carries carbon within the cell (Voet et al, 2006). *N*-substituted *β*-alanines have been extensively studied for their biological activity. They possess properties such as agricultural plant growth stimulators, lysophosphatidic acid (EDG-2 receptor) antagonists; they are used as antimalarial drugs as well (Voet et al, 2006).

The aim of the research: the synthesis of new variously functionalized 3-(-*N*-(3- and 4-sulfamoylphenyl)amino)propanoic, 1-substituted 5-oxopyrrolidine-3-carboxylic acids, their derivatives, and the study of structure, chemical and biological properties of the obtained compounds.

The objectives of the research:

1. To synthesize 3-(-*N*-(4-sulfamoylphenyl)amino)propanoic acid, study the structure of products, formed during its halogenation reactions, and synthesize hydrazone-type compounds by modifying the carboxy group.

2. To synthesize aminothiazole ring-containing derivatives by using the reactivity of the thiocarbonyl moiety of thioureido acids and study the chemical properties of the obtained compounds.

3. To synthesize 1-arylsubstituted 5-oxo-3-pyrrolidinecarboxylic acids and perform modification of the aromatic ring and carboxy group.

4. To analyze the influence of the structural modification of synthesized compounds on the biological activity.

The scientific novelty and practical value of the work

The process for the halogenation of 3-(-*N*-(4-sulfamoylphenyl)amino)propanoic acid has been proposed. In the halogenation method of propanoic acid when using HBr and HCl in the oxidizing environment, in which two hydrogen atoms in the aromatic ring are replaced by halogen atoms, and using *N*-bromosuccinimide, one hydrogen atom in the aromatic ring is replaced by bromine atom. Hydrazones of 3-(-*N*-(4-sulfamoylphenyl)amino)propanoic, 3-(-*N*-(3-sulfamoylphenyl)amino)propanoic, 1-arylsubstituted 5-oxopyrrolidine-3-carboxylic acid hydrazides that have been

obtained by their reaction with monocarbonyl compounds in DMSO-d_6 solutions due to the restricted rotation around the CO-NH bond exist as mixtures of two isomeric *Z*- and *E*- forms. Due to the C=N group isomerism, the formation of *Z*-position isomers in hydrazones is the most possible. The derivatives of a pyrrole and pyrazole rings were synthesized by using dicarbonyl compounds in these reactions, and thiosemicarbazones were obtained in the reaction with phenylthiocyanate, which cyclized to triazole derivatives in an alkaline medium. Some of the synthesized *N*-substituted, *N,N*-disubstituted *β*-alanines, 1-substituted 5-oxo-3-carboxypyrrolidinones and their derivatives have been shown to have better CA inhibitory properties than the parent *β*-alanine compounds. The obtained results show that the influence of the benzenesulfonamide moiety on the inhibitory activity of carbonic anhydrases is greater than that of the heterocyclic/aromatic moiety. The development of CA inhibitors is important because carbonic anhydrase activity is associated with certain diseases, such as nervous system diseases, cancer, glaucoma, and others. The development of new sulfonamide drugs that bind more strongly to carbonic anhydrases and have fewer side effects remains an important challenge. Based on the antibacterial studies of synthesized 1-substituted 2-pyrrolidinones, containing a benzimidazole moiety, new compounds with strong bactericidal activity have been identified. The performed research provides an opportunity to plan and expand the methodology of target synthesis of biologically active substances, to expand the variety of reagents for precise organic synthesis.

The main statements of the doctoral dissertation:

1. Under different specific conditions, halogenation of 3-(-*N*-(4-sulfamoylphenyl)amino)propanoic acid allows to replace one or two hydrogen atoms in the aromatic ring with halogen atoms.

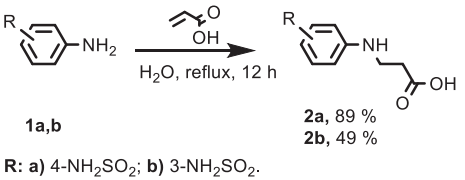
2. *N*-aryl-*N*-thiocarbamoyl-*β*-alanines are convenient intermediates for the synthesis of variously functionalized thiazole heterosystems that have amino acid moiety.

3. The functionalization of the aromatic ring of sulfanilamide-substituted *N*-substituted *β*-amino acids, 1-aryl-5-oxo-3-pyrrolidinecarboxylic acids in the aromatic ring has a positive effect on the activity of carbonic anhydrase inhibitors.

2. RESULTS AND DISCUSSION

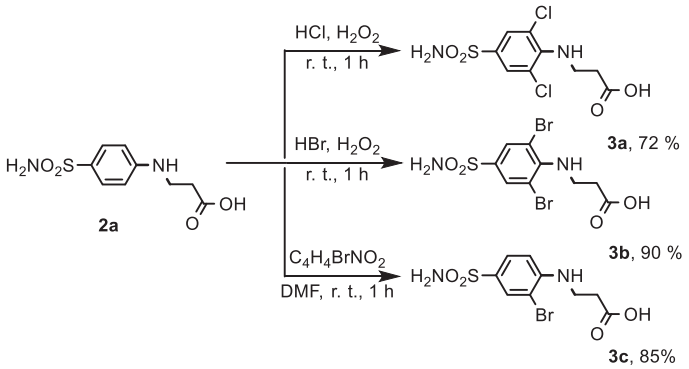
2.1. The synthesis of 3-(*N*-sulfamoylphenyl)-*β*-amino acids

One of the convenient methods for the synthesis of *N*-aryl-*β*-alanines is the interaction of aromatic amines with acrylic acid. 3-(-*N*-(4- and 3-sulfamoylphenyl)-*β*-alanines **2a**, **b** were synthesized by the addition reaction of corresponding amines **1a**, **b** with acrylic acid in water. The reactions were carried out at reflux for 12–24 hours (Scheme 1).



Scheme 1

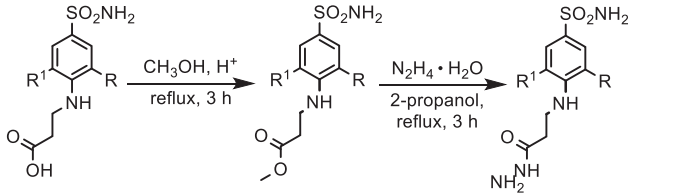
2,6-Dihalosubstituted 3-(-*N*-(4-sulfamoylphenyl)amino)propanoic acids **3a**, **b** were synthesized by reacting *β*-alanine **2a** with HCl or HBr at room temperature and using a catalytic amount of hydrogen peroxide (Scheme 2). During these reactions, two hydrogen atoms in the aromatic ring are replaced by halogen atoms. 3-(-*N*-(2-Bromo-4-sulfamoylphenyl)amino)propanoic acid (**3c**) was synthesized by the treatment of acid **2a** with *N*-bromosuccinimide in dimethylformamide at room temperature.



Scheme 2

2.2. The synthesis of 3-(*N*-sulfamoylphenyl)-*β*-amino acids hydrazides and their chemical transformation

In this work, the esters **4a–d** were obtained by the esterification of acids **2a**, **3a–c** with methanol (Scheme 3). The reactions were performed at the boiling point of the mixtures by using a large excess (8–10 times) of methanol. The interaction of esters **4a–d** with hydrazine monohydrate in 2-propanol at the boiling point of the mixture gave hydrazides **5a–d**, which were used in the further work for the synthesis of hydrazones.

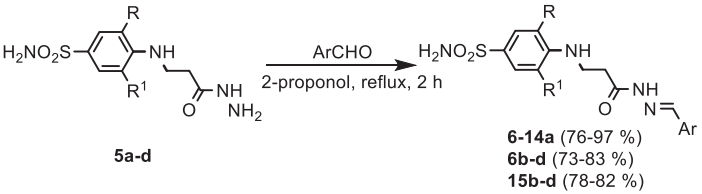


2a, 3a-c **4a-d** (76-84 %) **5a-d** (55-82 %)

2a: R, R^1 = H; **3: a) R, R^1** = Cl; **b) R, R^1** = Br; **c) R** = H, **R^1** = Br; **4, 5: a) R, R^1** = H; **b) R, R^1** = Cl; **c) R, R^1** = Br; **d) R** = H, **R^1** = Br.

Scheme 3

For this purpose, hydrazides **5a–d** were condensed with the corresponding aromatic aldehydes in 2-propanol. Compounds **6–14a**, **6b–d**, **15b–d** (Scheme 4) were obtained in 73–97 % yield.



5a-d

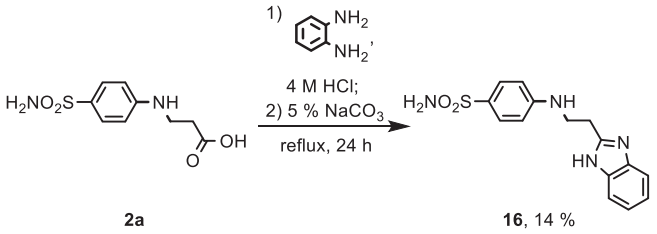
6-14a (76-97 %)
6b-d (73-83 %)
15b-d (78-82 %)

a) R, R^1 = H; **b) R, R^1** = Cl; **c) R, R^1** = Br; **d) R** = H, **R^1** = Br; **6** Ar = C_6H_5;
7 Ar = 4-F-C_6H_4; **8** Ar = 2-Cl-5-NO_2-C_6H_3; **9** Ar = 2-CH_3O-C_6H_4;
10 Ar = 4-CH_3O-C_6H_4; **11** Ar = 2,3-$(CH_3O)_2$-C_6H_3; **12** Ar = 2,4-$(CH_3O)_2$-C_6H_3;
13 Ar = 2,5-$(CH_3O)_2$-C_6H_3; **14** Ar = 3,4,5-$(CH_3O)_3$-C_6H_2; **15** Ar = 4-Cl-C_6H_4.

Scheme 4

The resulting hydrazone-type derivatives contain an HC=N moiety that can lead to geometric isomers. The compounds as well contain amide group that can be characterized by *E/Z* rotational conformational. In ^{1}H NMR spectra in DMSO-d_6 solutions, the proton signals of these fragments are seen in sets of two spectral lines. Thus, in derivatives **6–14a**, **6b–d**, **15b–d**, due to the restricted

rotation about the CO-NH bond, sufficiently stable *E/Z* isomers are formed, the ratio of which is 0.35:0.65 in all the cases. However, the formation of geometric isomers has not been observed.

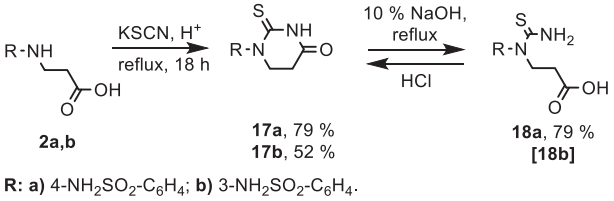


Scheme 5

In this work, 4-(-*N*-(2-(1*H*-benz[*d*]imidazol-2-yl)ethyl)amino)benzenesulfonamide (**16**) was synthesized by the *Phillips* method (Scheme 5). The reaction was performed in boiling 4 M hydrochloric acid, and the target product was obtained in only 16 % yield. The formation of the benzimidazole fragment can be judged from the ^{1}H NMR spectrum of this compound, in which the proton singlet of the N=C-NH group is observed in a low-field area at 12.27 ppm, and an increased number of aromatic proton peaks integrated for 8 protons.

2.3. The synthesis of 3-(4- and 3-sulfamoylphenyl)-*N*-thiocarbamoyl-*β*-alanine

Thioureido acids (**18a**, **b**) were synthesized from aminopropanoic acids (**2a**, **b**) by condensation with potassium thiocyanate in boiling acetic acid for 18 h. The resulting intermediates carbamothioyl amino propanoic acids (**18a**, **b**) are cyclized to more stable compounds **17a**, **b** with concentrated hydrochloric acid (medium pH 1–2) (Scheme 6). 2-Thioxotetrahydropyrimidin-1(2*H*)-yl)benzenesulfonamides (**17a**, **b**) were isolated from the reaction mixtures by dilution with water. Thioureido acids **18a**, **b** were obtained by the decyclization of compounds **17a**, **b** with 10 % NaOH solution. 3-(1-(4-Sulfamoylphenyl)thioureido)propanoic acid (**18a**) was isolated from the reaction mixture by the acidification of the solution with 5 % hydrochloric acid, and 3-(1-(3-sulfamoylphenyl)thioureido)propanoic acid (**18b**) was not isolated from the reaction mixture due to its high water solubility.



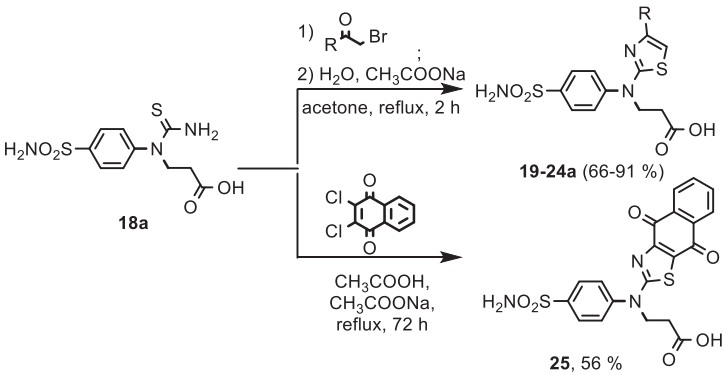
R: a) 4-NH_2SO_2-C_6H_4; **b)** 3-NH_2SO_2-C_6H_4.

Scheme 6

2.4. The synthesis of *N,N*-disubstituted *β*-alanines containing thiazole fragments

The corresponding thiazole derivatives were synthesized from 3-(1-(4-sulfamoylphenyl)thioureido)propanoic acid (**18a**) in work **19–24a** by the *Hantzsch* method, i.e., boiling them with *α*-haloketones in acetone for 2 h.

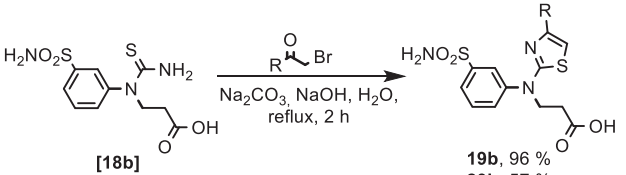
3-(-*N*-(4,9-dioxo-4,9-dihydronaphtho[2,3-d]thiazol-2-yl)(4-sulfamoylphenyl)amino)propanoic acid (**25**) was synthesized by reacting thioureido acid **18a** with 2,3-dichloro-1,4-naphthoquinone in glacial acetic acid at the boiling point.



R: 19a C_6H_5; **20a** 4-F-C_6H_4; **21a** 4-Cl-C_6H_4; **22a** 4-CN-C_6H_4; **23a** 4-NO_2-C_6H_4; **24a** 3,4-Cl_2-C_6H_3.

Scheme 7

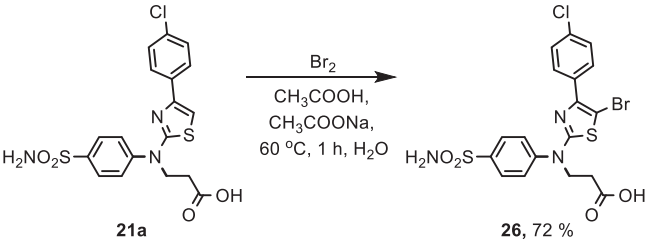
Thiazole derivatives **19b** and **23b** were obtained by adding Na_2CO_3 and NaOH to the reaction mixture of 3-(1-(3-sulfamoylphenyl)thioureido)propanoic acid (**18b**) (Scheme 8). The reaction mixture was boiled for 2 hours, followed by the addition of 2-bromoacetophenones at reflux for 2 hours. The resulting compounds **19b** and **23b** were isolated from the reaction mixture by the acidification to pH 6 with acetic acid.



R: **19b** C_6H_5; **20** 4-F-C_6H_4; **23b** 4-NO_2-C_6H_4

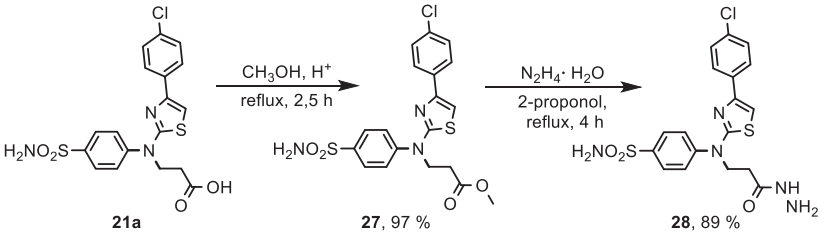
Scheme 8

Aminopropanoic acid **21a** was dissolved in a mixture of sodium acetate and acetic acid to which bromine was slowly added dropwise with stirring. The reaction was carried out at 60 °C for 1 hour, then the reaction mixture was diluted with water to give the 5-bromothiazole derivative **26**.



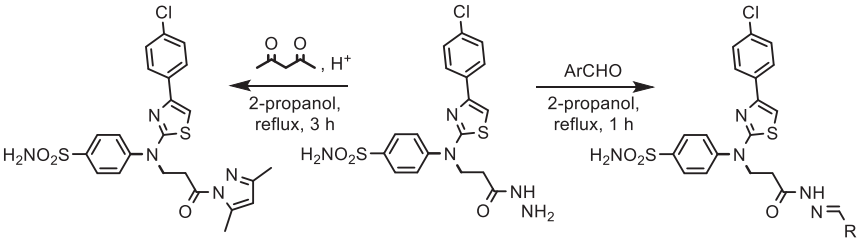
Scheme 9

The esterification of 3-(-*N*-(4-(4-chlorophenyl)thiazol-2-yl)-*N*-(4-sulfamoylphenyl)amino)propanoic acid (**21a**) with methanol catalyzed by sulfuric acid was performed to give methyl 3-(-*N*-(4-(4-chlorophenyl)thiazol-2-yl)-*N*-(4-sulfamoylphenyl)amino) propanoate (**27**), which then was treated with hydrazine monohydrate to obtain 4-(-*N*-(4-(4-chlorophenyl)thiazol-2-yl)-*N*-(3-hydrazinyl-3-(oxopropyl)amino)benzenesulfonamide (**28**) (Scheme 10).



Scheme 10

The condensation of the hydrazide (**28**) with 2,4-pentanedione afforded compound **29**, having a 3,5-dimethylpyrazole ring. This reaction was performed in 2-propanol for 3 h in the presence of a catalytic amount of hydrochloric acid (Scheme 11). In the ^{1}H NMR spectrum of compound **29**, the singlet at 6.14 ppm was observed to the pyrazole CH group proton; two singlets of the CH_3 groups were observed at 2.15 ppm and 2.37 ppm. Hydrazones **30–33** were obtained by condensing acid hydrazide **28** with various aromatic aldehydes in 2-propanol.



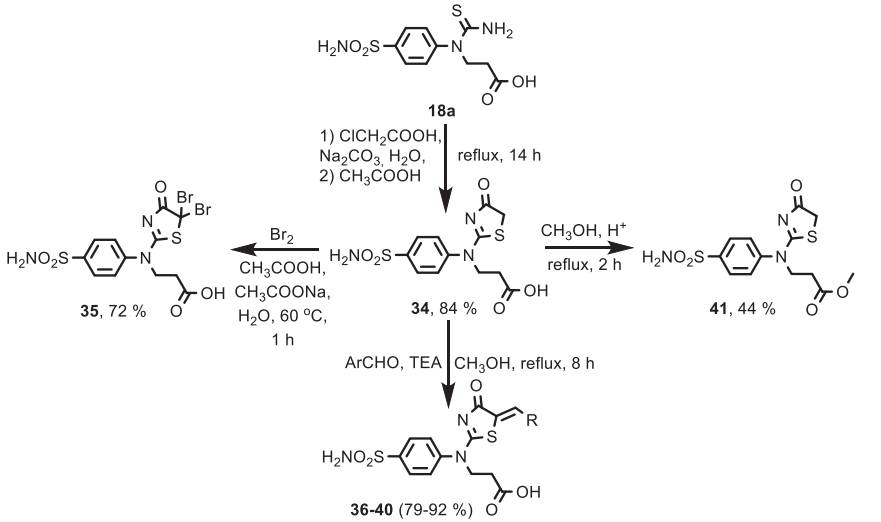
29, 77 % **28** **30-33** (74-86 %)

R: 30 C_6H_5; **31** 4-F-C_6H_4; **32** 4-Cl-C_6H_4; **33** 4-CH_3O-C_6H_4.

Scheme 11

2.5. The synthesis of 3-(-*N*-(4-oxo-4,5-dihydrothiazol-2-yl)(4-sulfamoylphenyl)amino)propanoic acid and its derivatives

In the next step, thiazolone **34** was synthesized by the treatment of thioureido acid **18a** with monochloroacetic acid, which was subsequently variously functionalized (Scheme 12). Compound **34** was brominated with bromine in acetic acid at 60 °C with the presence of sodium acetate in the mixture. In this reaction, compound **35**, having two bromine atoms at the 5-position of the thiazole ring, was synthesized. The formation of compound of this structure can be judged from the ^{1}H NMR spectrum, which lacks the proton singlet of the SCH_2 group of the dihydrothiazole ring at 3.99 ppm, compared to the analogous spectrum of the initial compound **34**.



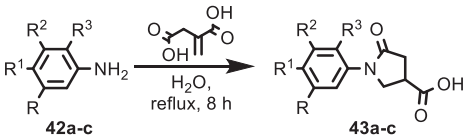
R: 36 C_6H_5; **37** 4-F-C_6H_4; **38** 4-Cl-C_6H_4; **39** 4-CH_3O-C_6H_4; **40** 4-NO_2-C_6H_4.

Scheme 12

The reaction of **34** with the corresponding aldehydes in methanol, using TEA as a catalyst, gave 5-(4-substituted benzylidene)thiazolones **36–40** in 79–92 % yield. In the ^{1}H NMR spectra of these compounds, the singlet at ~7.89 ppm (C=CH-R) and the increase in the peaks of the aromatic fields of the spectra prove the formation of the desired structures. Ester **41** was synthesized according to the conventional procedures with methanol from **34**.

2.6. The synthesis of 1-substituted 5-oxopyrrolidine-3-carboxylic and their derivatives

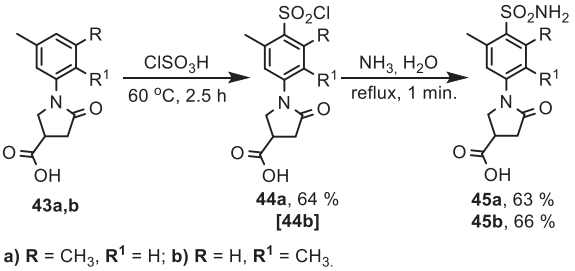
Substituted 5-oxopyrrolidine-3-carboxylic acids **43a-c** were synthesized by alkylation of anilines **42a–c** with itaconic acid (Scheme 13) in water at reflux.



a) R, R^2 = CH_3, **R^1, R^3** = H; **b) R, R^3** = CH_3, **R^1, R^2** = H;
c) R = Cl, **R^1** = OCH_3, **R^2, R^3** = H.

Scheme 13

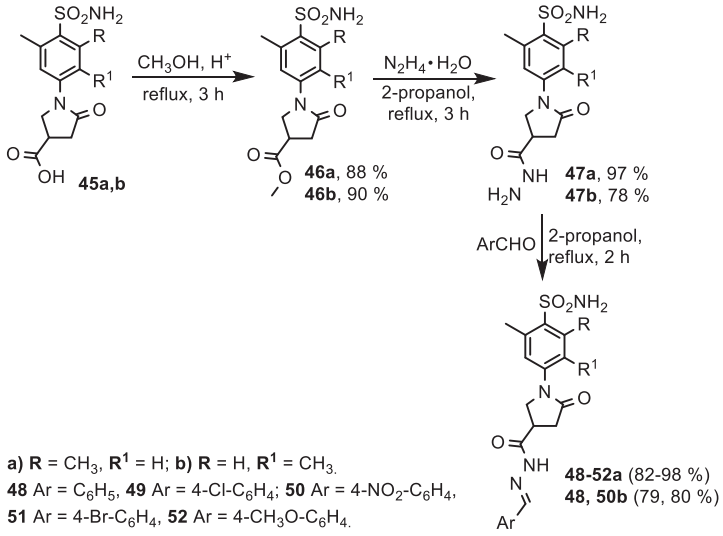
Compounds **43a**, **b** were modified to chlorosulfonyl derivatives **44a**, **b**, which reacted with ammonia to give 4-sulfamoylphenyl-5-oxopyrrolidine-3-carboxylic acids **45a**, **b** (Scheme 14).



Scheme 14

2.7. The synthesis of 5-oxopyrrolidine-3-carboxylic acids hydrazides and their reaction with aromatic aldehydes

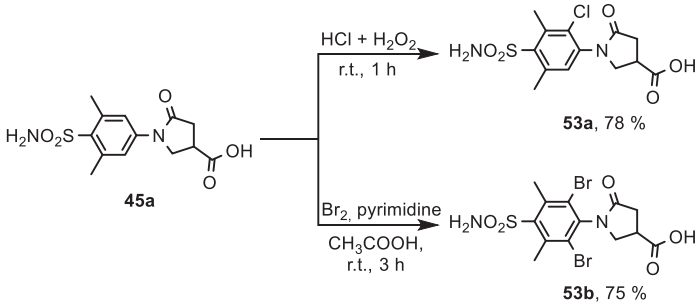
After the esterification of the obtained compounds, followed by the hydrazinolysis reactions of the esters, the acid hydrazides **47a**, **b** were synthesized. The latter were subsequently used in condensation reactions with various aldehydes to give hydrazones **48–52a**, **48b**, and **50b** (Scheme 15).



Scheme 15

2.8. The halogenation reactions of 1-(3,5-dimethyl-4-sulfamoylphenyl)-5-oxopyrrolidine-3-carboxylic acid and its derivatives

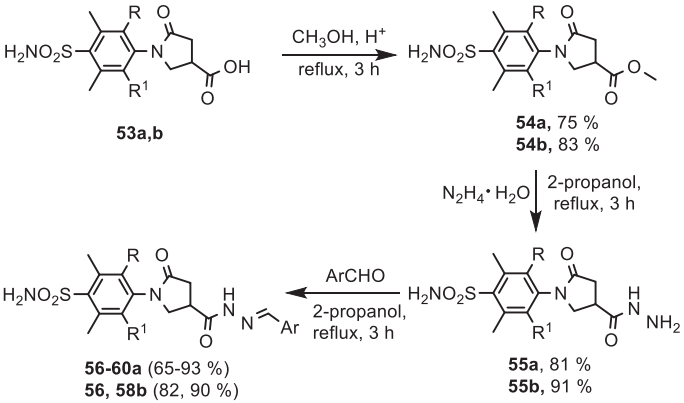
The chlorination was performed by using hydrochloric acid in the presence of hydrogen peroxide in the reaction mixture (Scheme 15). Based on ^{1}H and ^{13}C NMR spectra, the reaction showed the formation of 1-(2-chloro-3,5-dimethyl-4-sulfamoylphenyl)-5-oxopyrrolidine-3-carboxylic acid (**53a**). The chlorination took place in only one position of the benzene ring. The bromination of bromine in acetic acid and a mixture in the presence of pyridine, in contrast to the first case, gave the dibromo derivative 1-(2,6-dibromo-3,5-dimethyl-4-sulfamoylphenyl)-5-oxopyrrolidine-3-carboxylic acid **53b** (Scheme 16), enabling to get monobromo.



Scheme 16

Further, the acids **53a**, **b** that were obtained in the study were transformed into esters **54a**, **b** and the latter into acid hydrazides **55a**, **b** (Scheme 17). The esterification was carried out with methanol in the presence of a catalytic amount of sulfuric acid. The reaction of the esters with hydrazine monohydrate was performed in 2-propanol at reflux. Hydrazones **56–60a**, **56b,** and **58b** were obtained in 65–93% yield during the interaction of hydrazides **55a**, **b** with aromatic aldehydes.

The NMR spectra recorded in DMSO-d_6 solutions show that the resulting hydrazones **48–52a**, **48b**, **50b**, **56–60a**, **56b**, and **58b** exist as a mixture of *E/Z* rotamers.

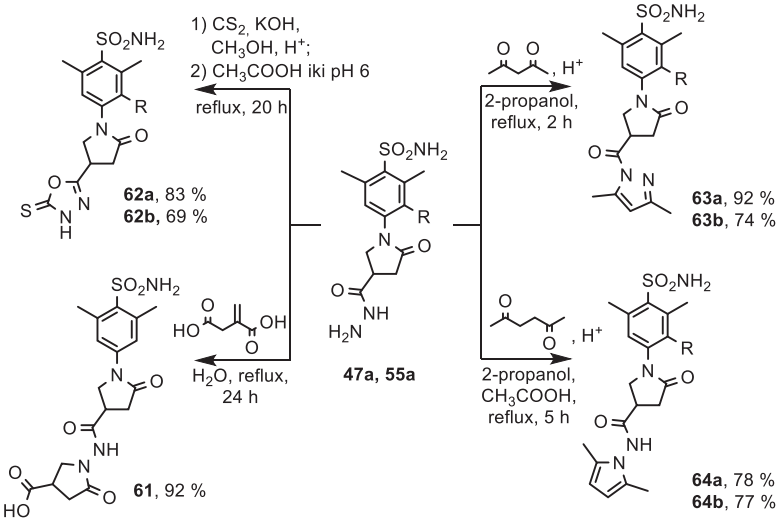


a) **R** = Cl, **R**^{1} = H; **b)** **R, R**^{1} = Br.
56 Ar = C_6H_5, **57** Ar = 4-Cl-C_6H_4; **58** Ar = 4-NO_2-C_6H_4, **59** Ar = 4-Br-C_6H_4,
60 Ar = 4-CH_3O-C_6H_4.

Scheme 17

2.9. The condensation reactions of hydrazides with aliphatic diketones

1-(1-(3,5-Dimethyl-4-sulfamoylphenyl)-5-oxopyrrolidine-3-carboxamide)-5-oxopyrrolidine-3-carboxylic acid (**61**), having two 2-pyrrolidinone moieties in the structure, was synthesized from hydrazide **47a** and itaconic acid in water at reflux for 24 h (Scheme 17). In this work, 1,3,4-oxadiazole-2-thiones **62i**, **f** were prepared by refluxing **47a**, **55a** with carbon disulfide and potassium hydroxide in methanol, without isolation of the potassium dithionate, and treatment of the obtained solution with the acetic acid. The resonances at 178 ppm (C=S) and 163 ppm (C=N) in the ^{13}C NMR spectra confirmed the formation of the oxadiazole ring.



47a R = H; **55a R** = Cl; **62, 63, 64: a R** = H, **b R** = Cl.

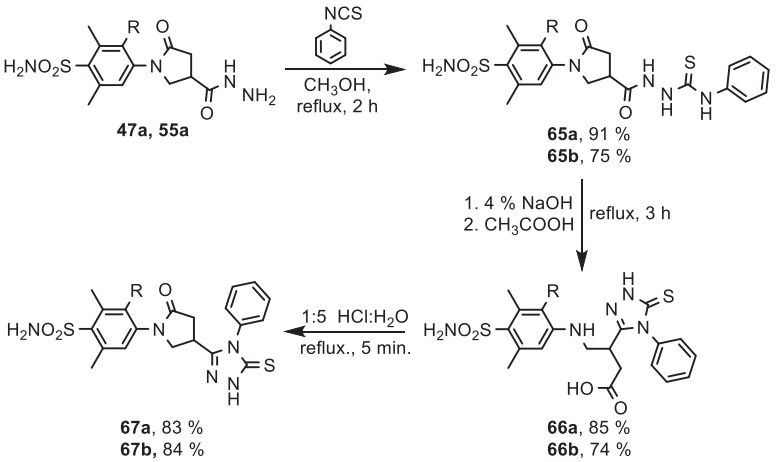
Scheme 18

Dimethylpyrazole derivatives **63a**, **b** were obtained from hydrazides **47a**, **55a** by treatment with 2,4-pentanedione in the presence of a catalytic amount of hydrochloric acid (Scheme 18). In the ^{1}H NMR spectra of compounds **63a**, **b,** two singlets at ~2.2 ppm and ~2.5 ppm were assigned to the protons of the two CH_3 groups, and the singlet at 6.24 ppm exhibited the presence of CH group of the pyrazole hetereocycle. The reaction of hydrazides **47a**, **55a** with 2,5-hexanedione in the presence of acetic acid gave pyrrole derivatives **64a**, **b**. The ^{1}H NMR spectra of **64a**, **b** show characteristic intense proton signals of the two CH_3 (at ~2.0 ppm) and CH (at 5.65 ppm) groups of the pentane pyrrole heterocycle.

2.10. The synthesis of thiosemicarbazides and their cyclization reactions

N-phenylhydrazine-1-carbothioamides **65a**, **b** were obtained by reacting hydrazides **47a**, **55a** with phenylisothiocyanate in methanol at reflux (Scheme 19). The products have already been crystallized during the reaction. The resulting thiosemicarbazides **65a**, **b** were easily cyclized with 4 % aqueous sodium hydroxide solution to 1,2,4-triazole-5-thiones **66a**, **b**. The formation of cyclic derivatives during the reaction can be inferred from the shift of the C=S group carbon resonance peak towards the stronger fields (^{13}C NMR), compared to the analogous spectra of the parent compounds **65** and the appearance of resonance lines at 153.57 (**a**) and 153.72 (**b**) ppm of newly formed N=CN fragment to the triazole ring. However, the decyclization of the pyrrolidinone

ring occurred during the reaction with sodium hydroxide. The salts of *N*-substituted *γ*-amino acids were transferred to free γ-amino acid by acidifying their solutions with acetic acid. The after-action of these acids with HCl for several minutes at reflux led to the target compounds **67a**, **b**.

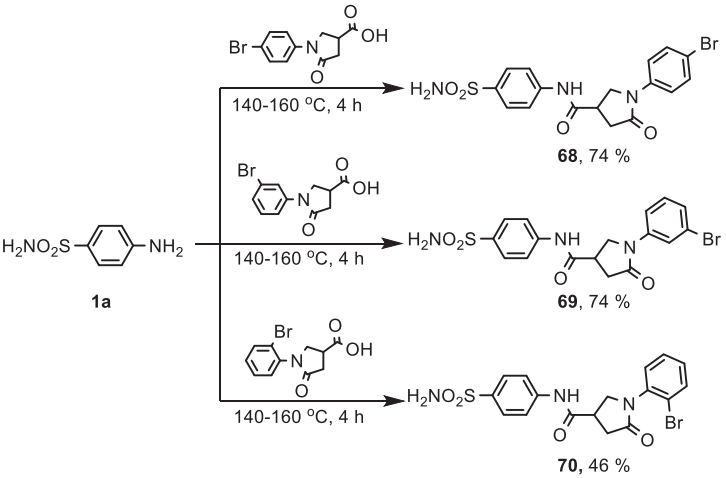


47a R = H; **55a R** = Cl; **65, 66, 67**: **a R** = H; **b R** = Cl;

Scheme 19

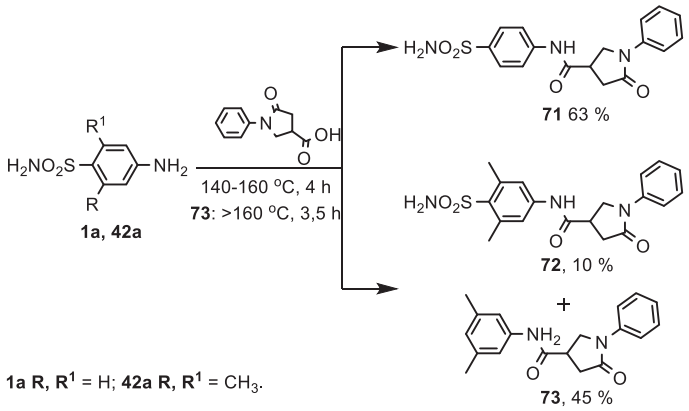
2.11. The condensation reactions of 4-aminobenzenesulfonamide with phenyl-5-oxopyrrolidine-3-carboxylic acids

Compounds **68–70** and **71** were obtained by melting 4-aminobenzenesulfonamide (**1a**) and the corresponding *N*-bromophenyl-5-oxopyrrolidine-3-carboxylic acids at 140–160 °C (Scheme 19), while melting 1-phenyl-5-oxopyrrolidine-3-carboxylic acid with 4-amino-2,6-dimethylbenzenesulfonamide **42a** at 140 °C afforded a mixture of *N*-(3,5-dimethyl-4-sulfamoylphenyl)-5-oxo-1-phenylpyrrolidine-3-carboxamide (**72**) and a derivative without an aminosulfonyl group **73** (Scheme 20).



Scheme 20

These compounds were isolated by column chromatography. The reaction above 160 ºC gave only one derivative, i.e., *N*-(3,5-dimethylphenyl)-5-oxo-1-phenylpyrrolidine-3-carboxamide (**73**).

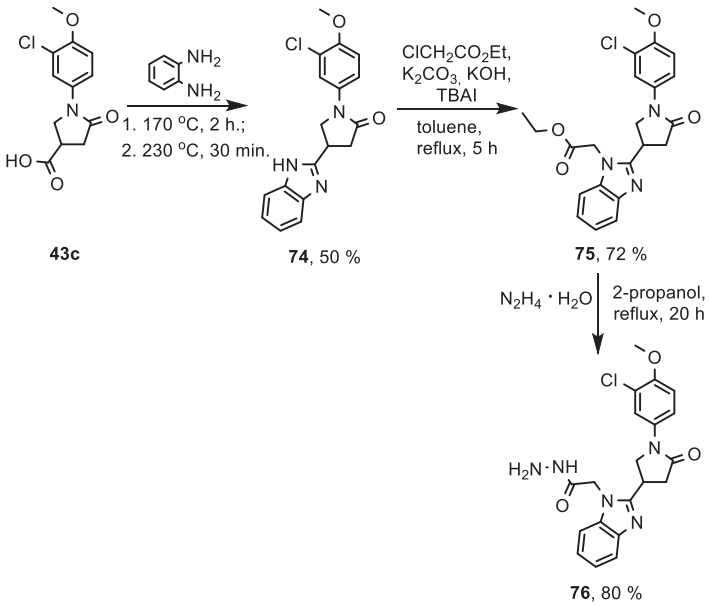


Scheme 21

2.12. The synthesis of 3-(1*H*-benzo[*d*]imidazol-2-yl)-1-(3-chloro-4-methoxyphenyl)-5-oxopyrrolidines and their derivatives

In this work, 1-(3-chloro-4-methoxyphenyl)-5-oxopyrrolidine-3-carboxylic acid (**43c**) was synthesized according to a known procedure by boiling the corresponding aromatic amine with itaconic acid in water. This compound was used for the synthesis of 2-substituted benzimidazole **74** by melting it with 1,2-diaminobenzene (Scheme 22).

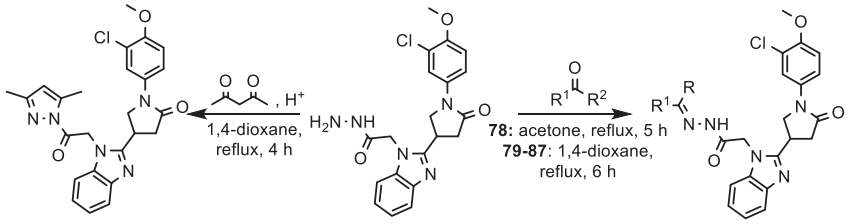
The 1,2-disubstituted benzimidazole derivative **75** was obtained by alkylation of the benzimidazole derivative **74** with ethyl chloroacetate in toluene in the presence of potassium carbonate, potassium hydroxide, and a catalytic amount of tetrabutylammonium iodide.



43c **74**, 50 % **75**, 72 % **76**, 80 %

Scheme 22

The reflux of the resulting ester **75h** with hydrazine monohydrate in 2-propanol for 20 h gave 2-(2-(1-(3-chloro-4-methoxyphenyl)-5-oxopyrrolidin-3-yl)-1*H*-benzimidazol-1-yl)acetohydrazide (**76h**), which then was used for the preparation of hydrazones **78–87**. The reaction of acid hydrazide **76h** with 2,4-pentanedione in 1,4-dioxane at reflux in the presence of a catalytic amount of hydrochloric acid afforded *N*-substituted pyrazole derivative **77** (Scheme 23).

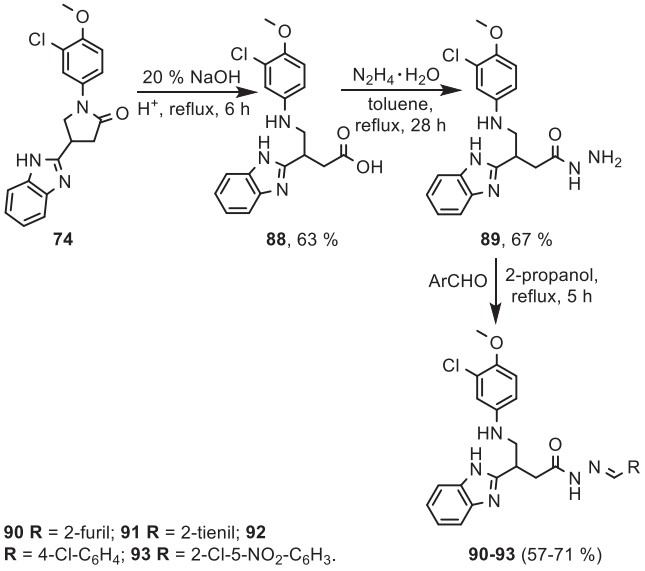


77, 85 % **76** **78-87** (60-98 %)

78 **R** = CH_3, **R^1** = CH_3; **79** **R** = H, **R^1** = 4-Br-C_6H_4; **80** **R** = H, **R^1** = 4-Cl-C_6H_4; **81** **R** = H, **R^1** = 2-Cl-5-NO_2-C_6H_3; **82** **R** = H, **R^1** = 3-piridil; **83** **R** = H, **R^1** = 2-furil; **84** **R** = H, **R^1** = 5-nitro-2-furil; **85** **R** = H, **R^1** = 2-tienil; **86** **R** = H, **R^1** = 5-nitro-2-tienil; **87** **R** = H, **R^1** = 5-brom-2-tienil.

Scheme 23

In this work, the pyrrolidinone ring of **74** was decyclized by using it in 20 % sodium hydroxide solution. Initially, the corresponding sodium salt of *γ*-amino acid was formed, which then was acidified with acetic acid to separate free 3-(1*H*-benzimidazol-2-yl)-4-(3-chloro-4-methoxyphenylamino)butanoic acid (**88**) (Scheme 24).



74 **88**, 63 % **89**, 67 %

90 **R** = 2-furil; **91** **R** = 2-tienil; **92** **R** = 4-Cl-C_6H_4; **93** **R** = 2-Cl-5-NO_2-C_6H_3. **90-93** (57-71 %)

Scheme 24

The structural changes of compound **88** were determined by comparing the NMR spectra of this compound with those of the **74** compound having a pyrrolidinone ring. In the spectra of amino acid **88**, the signals at 5.7 ppm

(NHPh, ^{1}H NMR) and 173.4 ppm (COOH, ^{13}C NMR) indicate the presence of decyclized structure in the molecule. The ^{13}C NMR spectrum shows that the difference in the peak displacements of the carbon atoms of the open-chain CH and CH_2CO groups is very small, i.e., only 0.6 ppm, while in the cyclic compound, this difference is even 6.8 ppm. Further, the hydrazones of *γ*-amino acid **90–93** were synthesized in the work. For this purpose, the acid **88** was refluxed with hydrazine monohydrate in toluene to give the carbohydrazide **89**, which was used for the condensation with various aromatic aldehydes. The analysis of the ^{1}H NMR spectra of **90–93** showed that in DMSO-d_6 solutions, due to the restricted rotation around the CO-NH bond, the hydrazones exist as a mixture of *E/Z* rotamers, where the Z isomer predominates.

3. RESULTS OF BIOLOGICAL TESTS

3.1. The investigation of the interaction of synthesized *N*-substituted and *N*, *N*-disubstituted β-alanine derivatives with CA

In search of compounds that were highly related and selective inhibitors of human carbonic anhydrases, *N*-substituted and *N,N*-substituted *β*-amino acids and their derivatives, containing a benzenesulfonamide moiety, were synthesized. The research was performed under the guidance of Prof. D. Matulis at the Institute of Biotechnology of Vilnius University.

The inhibitory activity of all compounds was measured by the fluorescence thermal shift method (FTSA). The inhibitory activity of compounds is expressed as a dissociation constant (K_d), which is the inverse proportion to the binding constant (K_b). The binding constants of fourteen synthesized compounds with all human catalytically active CA isoforms were measured by using the thermal shift analysis method (Appendix 1). The binding constants of other compounds were measured by selecting CA I, II, VII, XII, and XIII isoforms (Appendix 2).

p-Halogen-substituted compounds are better inhibitors than the original β-alanine **2a**. The introduction of electron acceptor groups (Cl and Br) in the compounds in the *m*-position of the benzene ring improved the binding to CA. Appendix 1 shows that the replacement of chlorine atoms (compound **6b**) with bromine atoms (compound **6c**) reduced the binding strength with many CAs by 2–5-fold, except for CA VB, IX, XII, and XIII. It binds to CA XII **6c** over 100 times more strongly than **6b**. The observation of the pair of ligands **15c** and **15b** shows that the compound **15c** with bromine substituents binds to CA VB 12.6-fold and CA IX 5.8-fold more strongly than compound **15b**, while the affinity for CA I isoforms decreased 100-fold. In most cases, the compounds with two bromine substituents (6c and **15c**) had higher affinity compared to the compounds with one bromine substituent (**6d** and **15d**). However, a significant difference in the affinity was observed between compounds **15c** and **15d**, when the affinity of CA I, IV, VA, VI, XII was reduced 5–10-fold by the introduction of the second bromine atom at the m-position, and CA XIV and CA II by 100-fold. However, it has been observed that the binding strength to CA IX and CA XIII increased 3-fold and that of CA VB increased 64-fold. The compounds **6b**, **6c**, and **15c** bound most strongly to CA VB (K_d 2.3–10 nM) and were highly selective for CA VB isoforms (selectivity ratio greater than 10-fold compared to the other CAs). This high affinity of the CA VB can be explained by additional contacts that can be formed by chlorine or bromine atoms upon the attachment to the CA VB active site.

The presence of a phenyl group in compound **6b** increased the binding strength for all CA isoforms compared to HCBSA, especially CA VB, and the binding affinity for this isoform was increased 33-fold.

The position of the sulphonamide group in the benzene ring greatly affects the binding strength of the CA isoforms. m-Substituted benzenesulfonamides (compounds **2b**, **17b**, **19b**, and **23b**) were weak CA inhibitors with K_d = 1–100 μM CA I, II, III, and XII. *m*-Substituted compound **19b** inhibits CA I and CA VII more than 300-fold less than *p*-substituted compound **19a**, which has an identical substituent, with CA I K_d values of 3700 and 33 nM, CA VII values of 50,000 and 167 nM, respectively (Appendix 1). For all remaining CA isoforms, the K_d of compound **19b** more than doubles.

4-(-*N*-(3-(2-Benzylidenhydrazinyl)-3-oxopropyl)amino)-3,5-dibromobenzenesulfonamide (**6c**) and 3,5-dibromo-4-(-*N*-(3-(2-(4-chlorobenzylidene)hydrazinyl))-3-oxopropyl)amino)benzenesulfonamide (**15c**) showed strong binding and more than 10-fold greater selectivity for the mitochondrial CA VB isoform compared to the other 11 catalytically active human CAs.

3.2. The binding strength of 1-substituted 5-oxo-3-carboxypyrrolidines to CA

The dissociation constants (K_d) of structurally related benzenesulfonamides, having a 5-oxopyrrolidine moiety in the *p*-position for 12 catalytically active human CA isoforms, were determined by FTSA (Appendix 3).

The methyl groups in the *o*- and *m*-positions have an influence on the CA binding. The *o*-substituted derivative **46a** binds to CA I 3 times more strongly than compound **A**. Meanwhile, the binding capacity of compound **46a** to CA II, IV, VI, VII, IX was 3 to 10 times lower than that of the compound without methyl substituents. The comparison of *o*-/*o*- and *o*-/*m*-methyl substituted compounds (**46b** and **46a**, respectively) showed that these ligands had very similar binding to CA II, III, VI, IX. Nevertheless, *o*-/*m*-methylbenzenesuldonamide **46b** binds to CA VB and VII significantly stronger than with *o*-/*o*-methylbenzensuldonamide **46a**. In summary, the presence of methyl groups in the *o*-/*m*-position strongly increased the attachment to CA VB and VII compared to the compound **46a** and **A**.

The influence of chlorine atom in *m*-position should be discussed. The chlorine atom is an electron-with drawing substituent; thus, the pK_a values of all chlorine-substituted sulfonamide groups are lower than those of non-chlorinated compounds. Therefore, based on this observation, it was obvious that the replacement of hydrogen atom with a chlorine atom increases the bond strength at least several times

The introduction of the two methyl groups in the *o-/o*-positions reduced the affinity with all 12 CAs, but the actual bond strength was restored by the attachment of the chlorine atom. Appendix 3 shows that the introduction of a chlorine substituent (compound **54a**) increases the binding strength of CA II (K_d = 29 nM), CA VB (K_d = 25 nM), CA VII (K_d = 13 nM), and CA XIII (K_d = 14 nM), compared to compound **46a**. This indicates that the interaction of chlorine-containing compounds with CA increases up to several-fold, most likely due to the formation of a halogen bond with the amino acids of the protein.

The influence of bromine atoms in the *m*-position is important as well. The binding of benzenesulfonamides, having two methyl groups in the *o-/o*-positions, bromine atoms in the *m-/m*-positions, and a pyrrolidinone moiety in the *p*-position to the CA, was measured. The compounds did not stabilize CA isoforms as measured by the FTSA method, and therefore, did not interact (or weakly interact) with any of the CA isoforms (Appendix 3). The solubility of the compounds in this series at pH 7 was relatively low and could interfere with the binding measurements.

The influence of the pyrrolidinone moiety in the *p*-position should be discussed. In order to investigate the effect of the pyrrolidinone ring, benzenesulfonamides were grouped according to moieties: methyl groups in the *o-/o*-position, **45–52a**, **61**, **62–67a**; methyl group *o-/o-* and chlorine atom in *m*-positions, **53–60a**, **62–67a**, methyl group in *o-/m*-position, **45–48b**, **50b**.

The compounds belonging to the same group bound very similarly to a particular CA, the affinity only slightly increasing with increasing hydrophobicity of the para substituent. The binding of compounds **68–71** and **72** to CA was studied as well, but they bound in a similar manner to the compounds containing the pyrrolidinone moiety.

The study reached several important conclusions. Two methyl groups at the *m-/o-* and *o-/o*-positions in the benzenesulfonamide ring reduced or did not affect the binding affinity for all CAs in all the studied cases, e.g., 20 times with CA I, II, IV, VI, IX, XII, XIII, XIV. The introduction of the chlorine atom into the *m*-position of 2,6-dimethylbenzenesulfonamide derivatives did not affect the attachment to CA I, but increased the affinity for other CAs, especially CA VII and CA XIII (up to 500-fold increase in the observed affinity).

3.3. The investigation of antibacterial activity

The antibacterial activity of compounds **74–76h, 77–87**, and **88–93** was determined at LUHS under the guidance of Prof. J. Šiugždaitė. The effect of various concentrations of the synthesized compounds against gram-positive spore-forming rods *Bacillus cereus*, gram-positive rods *Staphylococcus aureus*, gram-negative rods *Escherichia coli*, and *Pseudomonas aeruginosa* was studied by using the agar diffusion method (500, 250, 125, 62.5, 31.25, 8, 3.9, 1.95, 0.97, 0.485, 0.24, and 0.12 μg / ml).

The lowest concentration of a bactericidal substance in which no growth is observed is considered the minimum bactericidal concentration (MBC), and the lowest concentration of a compound that inhibits visible bacterial growth is considered the minimum inhibitory concentration (MIC). Oxytetracycline was used as a control in the antibacterial activity studies of the synthesized compounds. The values of the minimum inhibitory concentrations (MIC, μg/mL) of the compounds are given in Table 1.

Table 1. Antibacterial activity *in vitro* (MIC μg/mL) of the synthesized compounds

Compounds	*B. cereus*	*E. coli*	*S. aureus*	*P. aeruginosa*
74	+	31.25	7.8	0.485
75	+	+	+	7.8
76	+	7.8	125	125
77	+	+	+	+
78	+	62.5	15.6	125
79	+	0.12	+	+
80	+	+	+	7.8
81	+	0.485	+	+
82	+	+	+	+
83	+	0.24	+	+
84	+	0.485	+	1.95
85	+	+	+	+
86	+	+	+	+
87	+	+	+	+
88	500	+	125	125
89	500	125	125	125
90	500	62.5	+	125
91	500	+	125	500
92	+	+	+	+
93	+	+	+	+
Oxytetracycline, 62.5				

+ – growth of microorganisms

The data in Table 1 show that the test compounds **74h–76h** and **77–93** had almost no effect on the *B. cereus* bacterial strain, only compounds **88–91** with carboxyalkyl substituents or γ-amino acid derivatives in the benzimidazole ring showed a weak inhibitory effect (MIC 500 μg/mL) against *B. cereus*. The compounds **77**, **82**, **85–87**, **92**, and **93** exhibited no antibacterial activity against the test microorganisms.

The derivatives **79** and **83** showed high antibacterial activity against gram-negative rods *E. coli* at MIC value of 0.12 μg/mL and 0.24 μg/mL in vitro. The compounds **81** and **84** showed a great antibacterial activity against *E. coli* at the

value of 0.485 μg/mL *in vitro*. The MIC value of 0.485 μg/mL was received against *P. aeruginosa* for compound **74h** as well. The MIC value of 7.8 μg/mL was obtained against *S. aureus* for the compound **74h** and against *P. aeruginosa* for the compounds **75h** and **80**.

The studies have determined the minimum bactericidal concentrations of the synthesized compounds **74h–76h** and **77–93** as well. The results of the studies are presented in Table 2.

Table 2. Antibacterial activity *in vitro* of the synthesized (MBC, μg/mL) compounds

Compounds	*B. cereus*	*E. coli*	*S. aureus*	*P. aeruginosa*
74	125	62.5	7.8	1.95
75	+	31.25	31.25	7.8
76	+	7.80	250	125
77	+	3.9	250	15.6
78	+	62.5	15.6	250
79	+	0.24	15.6	31.25
80	+	31.25	250	15.6
81	+	0.485	500	62.5
82	+	31.25	500	62.5
83	+	0.24	250	125
84	+	0.485	250	1.95
85	+	125	3.90	31.25
86	+	125	7.80	15.6
87	+	125	62.5	125
88	500	15.6	125	125
89	500	125	125	125
90	500	62.5	125	125
91	500	62.5	125	125
92	+	250	62.5	15.6
93	+	250	62.5	7.8
Oxytetracycline, 62.5				

+ – growth of microorganisms

B. cereus bacteria were sensitive only to **74h**, **88–91** and at high concentrations of these compounds. The hydrazones **79**, **83**, **81**, **84** and hydrazide **76h** were the most active against *E. coli* at the concentrations of 0.24, 0.485 and 7.8 μg/mL, respectively. Pyrolidinones **74h** and **84** (1.95 μg/mL) and pyrazoles **75h** and **93** (7.8 μg/mL) showed the strongest activity against *P. aeruginosa*, while compounds **85** (3.9 μg/mL), **74h**, and **86** (7.8 μg/mL) showed the most effect on *S. aureus*. γ-amino acid derivatives **88–93** were found to have moderate effects on *E. coli*, *S. aureus*, and *P. aeruginosa*.

4. RESULTS AND CONCLUSIONS

1. 3-(-*N*-(4-Sulfamoylphenyl)amino)propanoic acid was resynthesized, and its reactions with halogenating agents were investigated; hydrazides of these acids were obtained. The following conclusions have been determined:

- the reaction with *N*-bromosuccinimide gives 3-(-*N*-(2-bromo-4-sulfamoylphenyl)amino)propanoic acid, while the reaction with hydrogen chloride and bromide in the presence of an oxidizing medium gives 3-((2,6-dichloro-4-sulfamoylphenyl)amino)propanoic and 3-(-*N*-(2,6-dibromo-4-sulfamoylphenyl)amino)propanoic acids;
- in the reaction of acid hydrazides with aromatic aldehydes, hydrazones-type compounds are formed, and in the DMSO-d_6 solutions, they exist as *E/Z* izomers mixture due to the restricted rotation around the CO-NH bond, which the *Z* isomer predominates.

2. 3-(1-(4-Sulfamoylphenyl)thioureido)propanoic acid was synthesized, its reactions with *α*-halocarbonyl compounds were studied, and it has been determined that when the monochloroacetic acid was used, it formed a hydrogenated thiazole derivative, while reaction with α-haloketones obtained the heteroaromatic ring-containing compounds. The 3-(-*N*-(4-(4-chlorophenyl)thiazol-2-yl)-*N*-(4-sulfamoylphenyl)amino)propanoic acid carboxy group changes were made, and 4-(-*N*-(4-(4-chlorophenyl))thiazol-2-yl)-*N*-(3-hydrazinyl-3-oxopropyl)amino)benzenesulfonamide, which was condensed with aromatic aldehydes to gain the corresponding hydrazones, was synthesized; meanwhile, the reaction with 2,4-pentanedione formed a 3,5-dimethylpyrazole ring-containing compound.

3. A series of functionalized aromatic ring-containing 1-aryl-5-oxo-3-pyrrolidinecarboxylic acids, their hydrazides were synthesized, and the reactions of hydrazides with mono- and dicarbonyl compounds, phenylisothiocyanate, carbon disulfide were investigated. Thus, the following conclusions have been made:

- the reaction of 1-aryl-5-oxo-3-pyrrolidinecarboxylic acid hydrazides with aromatic aldehydes forms hydrazones, which the in DMSO-d_6 solutions exist as a mixture of the *E/Z* isomers due to the restricted rotation around CO-NH bond;
- the reaction of hydrazides with diketones 2,4-pentanedione and 2,5-hexanedione affords cyclic pyrazole and pyrrole derivatives;
- the hydrazides are synthesized by the treatment of phenylisothiocyanate with thiosemicarbazides, which cyclize to triazole derivatives in an alkaline derivatives;
- the reaction of acid hydrazides with carbon disulphide forms dithiocarbazides, which are cyclized to oxodiazole derivatives by the action of acid.

4. The influence of some synthesized compounds on CA inhibitory activity and antibacterial properties of some compounds was determined, and the following conclusions have been found:

- the best CA inhibitor properties had sulfanilamide-substituted compounds 4-(-*N*-(3-(2-benzylidenhydrazinyl)-3-oxopropyl)amino)-3,5-dibromobenzenesulfonamide (**6c**) and 3,5-dibromo-4-(-*N*-(3-(2-(4-chlorobenzylidene)hydrazinyl))(3-oxopropyl)amino)benzenesulfonamide (**15c**);
- the introduction of the chlorine atom into the *m*-position of 2,6-dimethylbenzenesulfonamide derivatives did not affect the attachment to CA I but increased the affinity for other CAs, especially CA VII and CA XIII (up to 500-fold increase in binding strength);
- dimethyl groups in *meta/ortho*- or *ortho/ortho*- positions on the benzenesulfonamide ring in all the investigated cases reduced or did not affect the binding affinity to all CAs;
- the best antibacterial properties showed *N′*-(4-bromobenzylidene)-2-(2-(1-(3-chloro-4-methoxyphenyl)-5-oxopyrrolidin-3-yl)-1*H*-benz[*d*]imidazol-1-yl)acetohydrazide (**79**). The testing of compounds by the serial dilution method showed that compound **79** had a minimum inhibitory concentration (MIC) of 0.24 μg/ml against *Escherichia coli*, 15.6 μg/ml against *Staphylococcus aureus* and 31.25 μg/ml against *Pseudomonas aeruginosa*, when the control value was 62.5 μg/ml.

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APPENDIXES

Appendix 1. Compound dissociation constants for all 12 recombinant human carbonic anhydrase (CA) catalytically active isoforms CA I, CA II, CA III, CA IV, CA VA, CA VB, CA VI, CA VII, CA IX, CA XII, CA XIII, and CA XIV as determined by FTSA at pH 7.0 (37 ºC)

Comp.	Dissociation constants K_d (nM)					
	CA VI	CA VII	CA IX	CA XII	CA XIII	CA XIV
5c	1000	2000	$\geq 1\times10^5$	4000	2200	330
5d	2000	2000	$\geq 1\times10^5$	1111	5000	100
5e	12500	1000	$\geq 1\times10^5$	4000	20000	1700
6c	125	500	$\geq 1\times10^5$	3300	330	10
6d	670	670	$\geq 1\times10^5$	2500	330	2.3
6e	2000	200	$\geq 1\times10^5$	1800	4000	170
7a	14000	2200	$>1\times10^5$	10000	17000	5700
15c	200	500	$\geq 1\times10^5$	25000	2000	63
15d	20000	20000	$\geq 1\times10^5$	12500	50000	5.0
15e	2000	200	$\geq 1\times10^5$	2000	5600	320
19a	33	140	17000	2000	40000	6700
19b	3700	3400	10000	12500	36000	33000
26	125	40	$\geq 1\times10^5$	4000	67000	2200
34	3300	2500	$\geq 1\times10^5$	5300	$\geq 1\times10^5$	$\geq 1\times10^5$
HCBSA[a]	$\geq 1\times10^5$	6700	$\geq 1\times10^5$	14000	$\geq 1\times10^5$	$\geq 1\times10^5$
BSA	7100	1790	$\geq 1\times10^5$	17000	6700	33000
Comp.	Dissociation constants K_d (nM)					
	CA VI	CA VII	CA IX	CA XII	CA XIII	CA XIV
5c	6700	1700	500	6700	400	500
5d	2500	1000	200	5000	333	500
5e	12500	5000	670	6700	5000	360
6c	2000	500	125	2000	50	250
6d	1700	250	50	10000	20	200
6e	2500	1000	180	5000	500	170
7a	17000	20000	670	33000	50000	2500
15c	5000	5000	250	$\geq 1\times10^5$	140	670
15d	20000	20000	4.3	$\geq 1\times10^5$	200	2500
15e	2500	1000	140	10000	670	250
19a	10000	170	25	670	290	125
19b	17000	50000	670	29000	2000	1250
26	10000	67	33	140	40	100
34	29000	3300	670	2500	8300	1700
HCBSA[a]	$\geq 1\times10^5$	$\geq 1\times10^5$	5000	67000	$\geq 1\times10^5$	5000
BSA	14000	6670	1500	12500	10000	9100

[a] HCBSA-(4-{[(2-hydrazinecarbonyl)ethyl]amino}benzene-1-sulfonamide), compound 8 in the manuscript (Rutkauskas et al., 2014).

Appendix 2. Compound dissociation constants for recombinant human carbonic anhydrase (CA) catalytically active isoforms CA I, CA II, CA XII, and CA XIII determined by the fluorescent thermal shift assay (FTSA) at pH 7.0 (37 ºC)

Comp.	Dissociation constants K_d (nM)			
	CA I	CA II	CA XII	CA XIII
2a	100000	6700	15000	33000
2b	100000	10000	17000	100000
3c	1000	1800	2100	400
3d	1250	3300	1250	110
3e	20000	3300	4000	5600
4c	56	200	2500	50
4d	220	290	3300	50
4e	1000	100	2500	1000
16	36000	3300	8300	20000
17b	59000	4200	4000	12500
18a	10000	5000	10000	33000
20a	50	290	1000	125
21a	25	100	560	200
22a	40	170	1000	500
23a	50	200	1100	290
23b	3300	8300	59000	1250
24a	48	200	560	220
25	1700	7700	10000	1250
27	140	180	50000	2000
28	190	560	100000	2500
29	10	33	4300	100
30	67	140	100000	290
31	50	250	100000	1000
32	33	460	100000	1700
33	25	500	100000	1250
35	2500	5900	1100	10000
36	1700	150	3000	400
37	8300	400	5300	500
38	17000	710	10000	1250
39	8300	400	8300	830
40	7100	220	6700	500
41	10000	500	10000	1250

Appendix 3. The observed dissociation constants (nM) for compound interaction with the recombinant human carbonic anhydrase (CA) catalytically active isoforms determined by the fluorescent thermal shift assay (FTSA) at pH 7.0 and 37 °C. The standard deviation of the FTSA measurements is ± 1.6-fold in K_d. The limit of K_d determination is ≥200000 nM

Comp.	Dissociation constant (K_d), nM					
	CA I	CA II	CA III	CA IV	CA VA	CA VB
43f	≥200000	≥200000	≥200000	≥200000	ND	≥200000
44f	≥200000	≥200000	≥200000	≥200000	ND	≥200000
45f	20000	12000	≥200000	5900	8300	3300
45g	38000	6400	160000	19000	ND	1100
46f	1500	2000	≥200000	6700	ND	≥200000
46g	11000	1600	≥200000	10000	ND	180
47f	17000	11000	≥200000	9100	200000	1400
47g	58000	8100	≥200000	14000	ND	660
48f	2200	2200	≥200000	5000	2000	710
48g	7100	1300	≥200000	7600	ND	28
49f	1700	2000	≥200000	18000	ND	670
50f	1000	1700	≥200000	4700	ND	400
50g	6200	2100	≥200000	17000	ND	28
51f	2500	2900	≥200000	20000	ND	630
52f	2000	2000	≥200000	2200	ND	670
53i	4300	220	110000	360	ND	530
53j	≥200000	≥200000	≥200000	≥200000	ND	≥200000
54i	670	29	≥200000	1000	ND	25
54j	≥200000	≥200000	≥200000	≥200000	ND	≥200000
55i	2500	140	≥200000	500	ND	140
55j	≥200000	≥200000	≥200000	≥2000000	ND	≥200000
56i	560	56	≥200000	770	ND	20
56j	≥200000	≥20000	≥200000	≥200000	ND	≥200000
57i	670	100	≥200000	510	ND	3.8
58i	330	50	≥200000	480	ND	18
58j	≥200000	≥200000	ND	ND	ND	ND
59i	560	50	≥200000	340	ND	7.7
60i	710	56	≥200000	180	ND	3.2
61	13000	7100	≥200000	9100	ND	7700
62f	10000	7700	≥200000	6900	ND	2000
62i	830	100	≥200000	NA	ND	250
63f	1500	910	≥200000	6000	ND	500
63i	250	25	≥200000	ND	ND	13
64f	2900	2000	≥200000	2000	ND	500
64i	1100	20	ND	ND	ND	100
65f	910	1100	≥200000	6900	ND	770
65i	360	32	ND	ND	ND	200
66f	100000	100000	ND	≥200000	ND	≥200000
66i	40000	17000	ND	10000	ND	10000
67f	2500	2000	≥200000	7000	ND	770
67i	440	33	≥200000	NA	ND	40
68	3300	640	≥200000	5600	ND	3800

69	310	190	≥200000	810	ND	740
70	210	87	≥200000	400	ND	190
71	260	88	≥200000	850	ND	550
72	1600	NA	≥200000	6800	ND	ND
73	≥200000	≥200000	≥200000	≥200000	ND	≥200000
Comp.	Dissociation constant (K_d), nM					
	CA VI	CA VII	CA IX	CA XII	CA XIII	CA XIV
43f	≥200000	≥200000	≥200000	≥200000	≥200000	ND
44f	≥200000	≥200000	≥200000	≥200000	≥200000	ND
45f	25000	20000	5000	7700	8300	3200
45g	22000	27000	2600	8100	670	3900
46f	50000	3400	3000	NA	2000	ND
46g	29000	31	1600	12000	580	840
47f	50000	50000	13000	50000	100000	5000
47g	24000	4400	9000	21000	2800	4000
48f	25000	30000	2000	29000	22000	2000
48g	9600	1900	1400	9300	420	470
49f	14000	≥200000	3300	≥200000	≥200000	ND
50f	10000	33000	2000	22000	17000	ND
50g	9900	1300	1100	14000	320	ND
51f	18000	37000	2500	33000	33000	ND
52f	14000	18000	2000	18000	21000	ND
53i	6300	56	290	1300	220	ND
53j	≥200000	≥200000	≥200000	≥200000	≥200000	ND
54i	4800	13	100	NA	14	ND
54j	≥200000	≥200000	≥200000	≥200000	≥200000	ND
55i	4800	100	330	NA	200	40
55j	≥200000	≥200000	≥200000	≥200000	≥200000	ND
56i	1400	33	100	770	33	12
56j	≥200000	≥200000	≥200000	≥200000	≥200000	ND
57i	650	35	91	2000	200	ND
58i	810	43	100	1400	56	ND
58j	ND	ND	ND	≥200000	≥200000	ND
59i	920	63	200	1400	67	ND
60i	590	16	77	NA	50	ND
61	83000	33000	4000	22000	67000	ND
62f	50000	20000	3300	≥200000	≥200000	ND
62i	3300	130	67	NA	100	ND
63f	28000	3300	2000	8300	3700	ND
63i	3300	20	140	1000	ND	ND
64f	29000	33000	2000	67000	8300	ND
64i	3300	25	100	2000	10	ND
65f	≥200000	6500	1400	5000	1000	ND
65i	2200	100	100	360	14	ND
66f	≥200000	≥200000	ND	50000	33000	ND
66i	10000	7700	1000	2500	1400	ND
67f	41000	5400	1400	10000	2000	ND
67i	3100	8.7	89	500	17	ND

68	17000	1500	430	39000	3100	ND
69	2400	170	56	10000	1100	ND
70	3700	51	38	3500	200	ND
71	3600	110	53	5600	660	ND
72	45000	8300	ND	≥200000	3900	ND
73	≥200000	ND	≥200000	≥200000	≥200000	ND

ND – not determined, NA – not available.

LIST OF SCIENTIFIC PUBLICATIONS ON THE THEME OF THE DISSERTATION

Articles in the journals included in the list of Institute of Scientific Information (ISN)

1. Vaškevičienė, Irena; Paketurytė, Vaida; Pajanok, Nikita; Žukauskas, Šarūnas; Sapijanskaitė, Birutė; Kantminienė, Kristina; Mickevičius, Vytautas; Zubrienė, Asta; Matulis, Daumantas. Pyrrolidinone-bearing methylated and halogenated benzenesulfonamides as inhibitors of carbonic anhydrases // Bioorganic and Medicinal Chemistry. London: Elsevier. ISSN 0968-0896. eISSN 1464-3391. 2019, Vol. 27, iss. 2, p. 322-337.
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REZIUMĖ

Žmonių sveikata žymiai pagerėjo, kai buvo atrasti ir sukurti veiksmingi vaistai nuo mikrobų sukeliamų infekcijų. Tačiau dėl netinkamo antiinfekcinių agentų naudojimo mikrobų atsparumas sudėtiniams vaistams ženkliai išaugo. Susidūrus su šia problema, būtina sukurti naujus būdus, kaip išvengti šio reiškinio. Taigi reikia atrasti naujas vaistų klases, turinčias originalią struktūrą ir veikimo mechanizmus.

Heterocikliniai junginiai yra nepaprastai svarbūs organinėje chemijoje – jie sudaro daugiau nei pusę visų žinomų organinių junginių. Tiazolo heterociklas yra daugelio natūralių produktų ir sintetinių junginių, pasižyminčių plačiu biologiniu aktyvumu, komponentas (Doregiraee, at al., 2015) Biologiškai aktyviose ir dinamiškose molekulėse, tokiose kaip sulfatiazolis (antimikrobinis vaistas), ritanaviras (antivirusinis vaistas), abafunginas (priešgrybelinis vaistas), bleomicinas ir tiazofurinas, yra tiazolo fragmentas. Medicininėje chemijoje reikšmingi dariniai yra sulfonamidai, turintys tiazolo fragmentą. Šie junginiai yra gerai žinomi kaip farmaciniai agentai, pasižymintys įvairiu biologiniu aktyvumu, tokiu kaip antibakteriniu (Argyropoulou et al., 2009) priešvėžiniu (Naaz, et al., 2018) ir naudojami kaip karboanhidrazių inhibitoriai (Kilicaslan, et al., 2016). Grįžtama anglies dioksido hidratacijos reakcija yra fundamentinė daugelyje fiziologinių procesų. Ją katalizuoja karboanhidrazės (CA) (Kazokaitė, 2014). Šie fermentai plačiai paplitę tiek prokariotuose, tiek eukariotuose. Žmogaus organizme aptinkama 12 aktyvių CA izofomų. Kai kurių CA izoformų genų raiškos sutrikimai siejami su įvairiomis ligomis, pavyzdžiui, glaukoma ar navikų vystymusi (Kazokaitė, 2014). Benzensulfonamido dariniai yra plačiausiai tiriami kaip CA inhibitoriai. Susintetinta daug įvairių CA sulfonamidinių slopiklių, tačiau jie turi nemažai trūkumų (Čapkauskaitė, 2008). Vienas iš svarbiausių trūkumų yra tai, kad karboanhidrazės slopinamos visame organizme ir, naudojant sulfonamidinius slopiklius, pasireiškia įvairūs nelaukti pašaliniai poveikiai, dažniausiai dėl to, kad jie veikia nespecifiškai visas CA izoformas, bei daugelis pasižymi toksiškumu (Čapkauskaitė, 2008). Todėl specifinių arba atskiriems organams selektyvių naujų sulfonamidinių slopiklių sukūrimas lieka labai aktualus ir svarbus uždavinys. Dar vieni svarbūs heterocikliniai junginiai yra 2-pirolidinono dariniai, kurie pasižymi reikšmingu biologiniu ir farmakologiniu poveikiu. Vienas iš vaistinių preparatų – piracetamas (2-okso-1-pirolidino acetamidas) skirtas pacientams, sergantiems ligomis, kai pasireiškia traukuliai, Alzheimerio ir senatvine demencija, smegenų sukrėtimu ir kitomis neurologinėmis ligomis. O doksapramas (1-etil-4-(2morfolin-4-iletil)-3,3-difenilpirolidin-2-onas) naudojamas esant kvėpavimo nepakankamumui. Pirolidinono fragmentas, laikomas pagrindine farmakoforų grupe, įeina į sudėtingas chemines struktūras, kaip ceruletidas, kuris yra dešimties aminorūgščių oligopeptidas stimuliuojantis lygiuosius raumenis ir aktyvinantis

virškinimo sulčių sekreciją. O gonadorelinas (GnRH) yra atsakingas už folikulus stimuliuojančio liuteinizuojančio hormono išsiskyrimą (Kellici, et al., 2017).

Pastaruoju metu nepaprastas dėmesys buvo skiriamas β-aminorūgštims, kaip potencialioms pradinėms medžiagoms bioorganinių, vaistinių ir natūralių produktų sintezei (Juaristi, et al., 2005). β-alanino fragmentą turintys junginiai pasižymi skirtingu biologiniu aktyvumu.

Pavyzdžiui, taksolis, kuris išskiriamas iš trumpaspyglio kukmedžio, naudojamas kaip priešvėžinis preparatas. Iš Streptomyces bakterijų išskirtas bleomicinas slopina besidalijančių vėžio ląstelių augimą. O destruksinas A yra ciklinis peptidas, sutrikdantis kalcio pusiausvyrą ląstelėse. Dipeptidas – karnozinas randamas raumenų, kepenų, smegenų ir kituose organuose. Jis pasižymi antioksidacinėmis savybėmis. β-alanino fragmentas įeina į sudėtį vitamino B5 ir todėl yra kofermento A (CoA) ir acil-KoA baltymo, kuris perneša anglį ląstelėje, pirmtakas (Voet, et al., 2006). N-pakeistieji β-alaninai buvo plačiai ištirti dėl jų biologinio aktyvumo, pavyzdžiui, kaip žemės ūkio augalų augimo stimuliatoriai, kaip lizofosfatidinės rūgšties (EDG-2 receptorių) antagonistai, kurie yra naudingi kaip vaistas, ir jų antimaliarinio aktyvumo (Voet, et al., 2006).

Darbo tikslas – susintetinti naujas įvairiai funkcionalizuotas 3-(-*N*-(3- ir 4-sulfamoilfenil)amino)propano, 1-pakeistas 5-oksopirolidin-3-karboksirūgštis, jų darinius, nustatyti gautų junginių struktūrą, chemines savybes ir išanalizuoti biologinį aktyvumą.

Tikslui pasiekti buvo iškelti šie **uždaviniai**:

1. Susintetinti 3-(-*N*-(4-sulfamoilfenil)amino)propano rūgštį, ištirti jos halogeninimo reakcijų metu susidarančių produktų struktūrą, bei modifikuojant karboksigrupę susintetinti hidrazonų tipo junginius.
2. Pasinaudojus tioureido rūgščių tiokarbonilinio fragmento reaktingumu susintetinti aminotiazolo ciklą turinčius darinius, ištirti gautų junginių chemines savybes.
3. Susintetinti 1-arilpakeistą 5-okso-3-pirolidinkarboksirūgštį, atlikti aromatinio žiedo, karboksigrupės kitimus.
4. Išanalizuoti, kokią įtaką biologiniam aktyvumui daro susintetintų junginių struktūros kitimas.

Darbo mokslinis naujumas ir praktinė reikšmė

Pasiūlytas 3-(-*N*-(4-sulfamoilfenil)amino)propano rūgšties halogeninimo būdas, naudojant HBr ir HCl esant oksidacinei aplinkai, kai du vandenilio atomai aromatiniame žiede yra keičiami halogeno atomais, o naudojant N-bromsukcinimidą yra pakeičiamas vienas vandenilio atomas Br atomu

aromatiniame žiede. 3-(-*N*-(4-sulfamoilfenil)amino)propano, 3-(-*N*-(3-sulfamoilfenil)amino)propano, 1-arilpakeistiems 5-oksopirolidin-3-karboksilrūgščių hidrazidams reaguojant su monokarboniliniais junginiais gauti hidrazonai, kurie DMSO-d6 tirpaluose dėl suvaržyto sukimosi apie CO-NH ryšį egzistuoja E/Z posūkio izomerų mišinių pavidalu. Dėl C=N grupės izomerijos hidrazonuose labiausiai tikėtinas Z padėties izomerų susidarymas. Šiose reakcijose panaudojus dikarbonilinius junginius susintetinti pirolo ir pirazolo ciklus turintys dariniai, o reaguojant su feniltiocianatu gauti tiosemikarbazidai, kurie šarminėje terpėje ciklizuojasi į triazolo darinius. Išanalizuota, kad dalis susintetintų N-pakeistų, N,N-dipakeistų β-alaninų, 1-pakeistų 5-okso-3-karboksipirolidinonų ir jų darinių pasižymi geresnėmis CA slopinančiomis savybėmis nei pradiniai β-alanino junginiai. Gauti rezultatai įrodo, kad benzensulfonamidinės dalies įtaka karboanhidrazių slopinančiam aktyvumui yra didesnė nei heterociklinės/aromatinės dalies. CA slopiklių kūrimas svarbus tuo, kad karboanhidrazių veikla siejama su tam tikromis ligomis, pavyzdžiui, nervų sistemos ligomis, vėžiu, glaukoma ir kt. Svarbiu uždaviniu išlieka naujų sulfonamidinių vaistų sukūrimas, kurie stipriau jungtųsi prie karboanhidrazių ir pasižymėtų mažesniu šalutiniu poveikiu. Remiantis susintetintų 1-pakeistų 2-pirolidinonų, turinčių benzimidazolo fragmentą, antibakterinių tyrimų duomenimis, nustatyti nauji junginiai, pasižymintys ryškiu baktericidiniu aktyvumu. Atlikti darbai sudaro galimybę planuoti ir praplėsti biologiškai veiklių medžiagų tikslinės sintezės metodologiją, išplėsti tiksliosios organinės sintezės reagentų įvairovę.

Ginamieji teiginiai:

1. Halogeninant 3-(-*N*-(4-sulfamoilfenil)amino)propano rūgštį skirtingomis sąlygomis, yra įmanoma vieną arba du vandenilio atomus aromatiniame žiede pakeisti halogenatomais.
2. *N*-aril-*N*-tiokarbamoil-*β*-alaninai yra patogūs tarpiniai junginiai įvairiai funkcionalizuotoms tiazolo heterosistemoms, turinčioms aminorūgšties fragmentą, sintetinti.
3. Sulfanilamidinį pakaitą aromatiniame žiede turinčių *N*-pakeistų *β*-aminorūgščių, 1-arilpakeistų 5-okso-3-pirolidinkarboksirūgščių aromatinio žiedo funkcionalizavimas, turi teigiamą poveikį jungimosi stiprumui prie karboanhidrazių.

REZULTATAI IR IŠVADOS

1. Resintezuota 3-(-*N*-(4-sulfamoilfenil)amino)propano rūgštis, ištirtos jos reakcijos su halogeninimo agentais bei gauti šių rūgščių hidrazidai ir nustatyta, kad:

- reakcijoje su *N*-bromsukcinimidu susidaro 3-(-*N*-(2-brom-4-sulfamoilfenil)amino)propano rūgštis, tuo tarpu reakcijoje su vandenilio chloridu ir bromidu, esant oksidacinei terpei, gaunamos atitinkamai 3-(-*N*-(2,6-dichlor-4-sulfamoilfenil)amino)propano ir 3-(-*N*-(2,6-dibrom-4-sulfamoilfenil)amino)propano rūgštys;
- reaguojant gautiems karboksirūgščių hidrazidams su aromatiniais aldehidais susidaro hidrazonai, kurie DMSO-d_6 tirpaluose dėl fragmento CO-NH buvimo molekulėse ir suvaržyto sukimosi apie CO-NH ryšį, egzistuoja *E/Z* izomerų mišinių, kuriuose vyrauja Z izomeras, pavidalu.

2. Susintetinta 3-(1-(4-sulfamoilfenil)tioureido)propano rūgštis, ištirtos jos reakcijos su α-halogenkarboniliniais junginiais ir nustatyta, kad reakcijoje panaudojus monochloracto rūgštį susidaro hidrintos struktūros tiazolo darinys, tuo tarpu reakcijoje su *α*-halogenketonais gaunami heteroaromatinį žiedą turintys junginiai. Atlikta 3-(-*N*-(4-(4-chlorfenil)tiazol-2-il)-*N*-(4-sulfamoilfenil)amino)propano rūgšties karboksigrupės kitimai ir susintetintas 4-(-*N*-(4-(4-chlorfenil)tiazol-2-il)-*N*-(3-hidrazinil-3-oksopropil)amino)benzensulfonamidas, kurį kondensuojant su aromatiniais aldehidais gauti atitinkami hidrazonai, tuo tarpu reakcijoje su 2,4-pentandionu susidaro 3,5-dimetilpirazolo ciklą turintis junginys.

3. Susintetinta visa eilė funkcionalizuotą aromatinį žiedą turinčių 1-arilpakeistų 5-okso-3-pirolidinkarboksirūgčių, jų hidrazidų, ištirtos hidrazidų reakcijos su mono- ir dikarboniliniais junginiais, fenilizotiocianatu, anglies disulfidu ir nustatyta, kad:
   - 1-arilpakeistų 5-okso-3-pirolidinkarboksirūgčių hidrazidams reaguojant su aromatiniais aldehidais susidaro hidrazonai, kurie DMSO-d_6 tirpaluose dėl suvaržyto sukimosi apie CO-NH ryšį, egzistuoja E/Z izomerų mišinių pavidalu;
   - hidrazidams reaguojant su diketonais – 2,4-pentandionu ir 2,5-heksandionu – susidaro ciklinės stuktūros pirazolo ir pirolo dariniai;
   - hidrazidus veikiant fenilizotiocianatu susintetinti tiosemikarbazidai, kurie šarminėje terpėje ciklizuojasi į triazolo darinius;
   - rūgščių hidrazidams reaguojant su anglies disulfidu susidaro ditiokarbazatai, kurie dėl rūgšties poveikio ciklizuojasi į oksodiazolo darinius.

4. Išanalizuota dalies susintetintų junginių įtaka CA slopinančiam aktyvumui ir kai kurių junginių antibakterinės savybės ir nustatyta, kad:
   - iš susintetintų sulfanilamidinį pakaitą aromatiniame žiede turinčių junginių geriausiomis CA slopiklio savybėmis pasižymėjo 4-(-*N*-(3-(2-benzilidenhidrazinil)-3-oksopropil)amino)-3,5-dibrombenzensulfonamidas (**6c**) ir 3,5-dibrom-4-(-*N*-(3-(2-(4-chlorbenziliden)hidrazinil)-3-oksopropil)amino)benzensulfonamidas (**15c**);

- chloro atomo įvedimas į 2,6-dimetilbenzensulfonamido darinių *m*-padėtį neturėjo įtakos prisijungimui prie CA I, tačiau padidino gimiringumą likusioms CA, ypač CA VII ir CA XIII (iki 500 kartų padidėjo jungimosi geba);
- dvi metilgrupės *m-/o-* ir *o-/o*-padėtyse benzensulfonamido žiede visais tirtais atvejais sumažino arba neturėjo įtakos jungimosi gimiringumui su visomis CA;
- *N′-(*4-brombenziliden)-2-(2-(1-(3-chlor-4-metoksifenil)-5-oksopirolidin -3-il)-1*H*-benz[d]imidazol-1-il)acetohidrazidas (**79**) pasižymi geriausiomis antibakterinėmis savybėmis. Tiriant junginius praskiedimo metodu nustatyta, kad junginio **79** minimali inhibicijos koncentracijos vertė (MIC) prieš *Escherichia coli* buvo 0,24 μg/ml, prieš *Staphylococcus aureus* – 15,6 μg/ml, prieš *Pseudomonas aeruginosa* - 31,25 μg/ml, kai kontrolinė vertė yra 62,5 μg/ml.

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