

Synthesis, antimicrobial activity, DFT-calculation, and docking of 4-(1,3,4-thiadiazol-2-yl)-containing polysubstituted pyrroles

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CHRONICLE

Article history:

Received October 4, 2023

Received in revised form

January 10, 2024

Accepted March 23, 2024

Available online

March 23, 2024

Keywords:

5-Chloro-4-formyl-1H-pyrrole-3-carboxylates

4-(1,3,4-Thiadiazol-2-yl)pyrroles

Antimicrobial activity

DFT calculation

Docking

ABSTRACT

A series of new 4-(1,3,4-thiadiazol-2-yl)-containing polysubstituted pyrroles **3 a-k** has been synthesized by a preparative convenient method from ethyl 5-chloro-4-formyl-1H-pyrrole-3-carboxylates **1 a-e**, which were selectively transformed into the corresponding polysubstituted pyrrole-4-carboxylic acids **2 a-e** using sodium hypochlorite as an oxidizer. Further, they were transformed into the target compounds with a high yield using the cyclocondensation with N-mono- or N,N-disubstituted thiosemicarbazides in the boiling phosphorus trichloroxide. As seen from the screening of antimicrobial activity, the synthesized compounds exhibit the inhibiting and bactericide activity against some bacteria and fungi. The highest activity has been established for the compounds **3 a, c, e-h, j** against the strain *Klebsiella pneumoniae* (MIC=31.25 µg/mL). The calculated HOMO energy level proves that the compound **3 c** is the most reactive ligand for the interaction with a protein receptor. The molecular docking data show that the compound **3 h** has the highest affinity to the ThiM *Klebsiella pneumoniae* kinase.

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1. Introduction

Pyrrole is a unique nitrogen-containing heterocyclic system¹⁻³ that is a key part of such vitally important compounds as heme, chlorophyll, bile pigments, vitamin B12, and the alkaloids extracted from some sea algae.^{4,5} Besides, the pyrrole nucleus is a part of many synthetic and natural pharmaceutical compositions such as antitumor medicine Sunitinib⁶, hypolipidemic drug Atorvastatin⁷, non-steroid anti-inflammatory drug Tolmetin⁸, *anti-Alzheimer composition* Aloracetam⁹, and the sea shellfish-originated anti-cancer medications Obatoclax¹⁰, Lamellarin O, and Lamellarin R¹¹ (**Fig. 1**). According to the molecular structure, the above-shown medications are polysubstituted derivatives of pyrrole. That is why close attention is focused on the development of effective synthetic methods for such compounds¹²⁻¹⁴ and the investigation of their biomedical properties. As a result, many highly active anticancer, antimicrobial, antiviral, anti-inflammatory, and other pharmaceutically important compounds have been identified among the functionalized polysubstituted pyrrole derivatives.¹⁵⁻¹⁸ In the context of bioscreening, special attention is given to the molecular structures containing a pyrrole cycle bound with another pharmacophoric heterocyclic nucleus – so-called heterocyclic ensembles. It should be noted that a relatively small number of such compounds is currently reported among tetra- and penta-substituted pyrroles with the furan(thiophene)¹⁹, isoxazole²⁰, imidazole²¹, and isatine²² fragments. It should also be emphasized that the isoxazole- and isatine-modified cycles exhibit a pronounced anti-inflammatory activity²³.

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In this work, we investigated the polyfunctional derivatives of pyrrole with the basic nucleus modified by the 1,3,4-thiadiazole cycle. This choice is based on the presence of that cycle in some diuretics and ophthalmologic medications Acetazolamide and Methazolamide²⁴, antibacterial composition Megazol²⁵, and in semi-synthetic antibiotics Cefazedone²⁶ and Cefazolin²⁷ (Fig. 2).

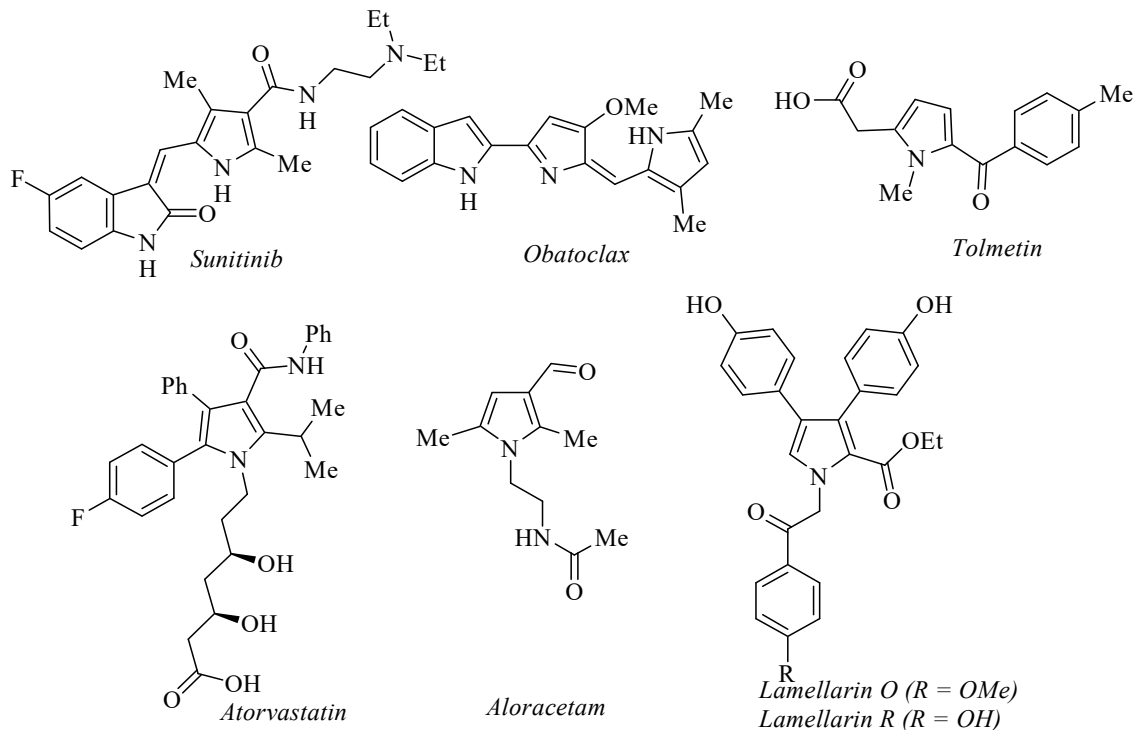


Fig. 1. Some pyrrole-containing drugs

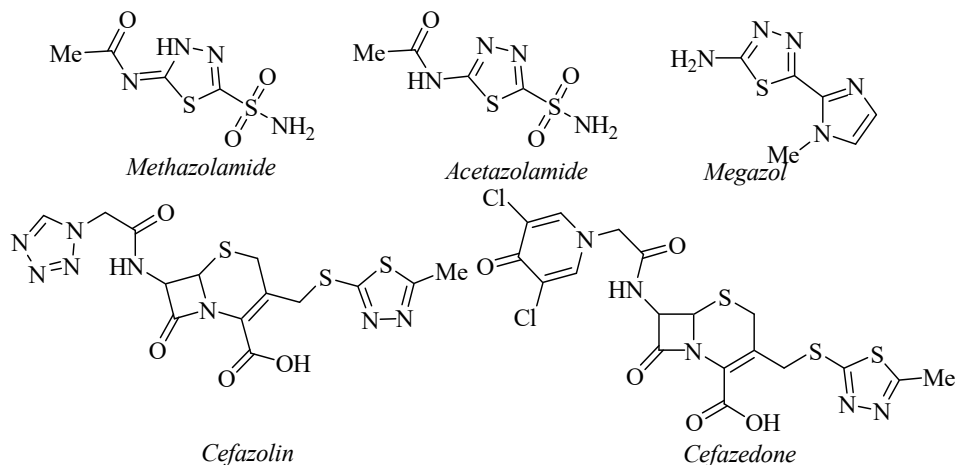


Fig. 2. Some medications with a 1,3,4-thiadiazole nucleus

In general, the derivatives of 1,3,4-thiadiazole exhibit a wide range of bioactivities,²⁸⁻³⁰ including the antimicrobial³¹ and antiparasite^{32, 33} properties. Based on the above, it seems reasonable to develop the synthesis of new hybrid compounds with the functionalized pyrrole and 1,3,4-thiadiazole cycles, investigate their antimicrobial action, and perform the DFT calculation and docking simulation for the most active representatives.

2. Results and Discussion

2.1 Chemistry

Ethyl 5-chloro-4-formyl-1H-pyrrole-3-carboxylates **1 a-e** synthesized in one of our recent works³⁴ were used as base substrates for the targeted introduction of a 1,3,4-thiadiazole fragment. Those compounds were also successfully used for

the obtaining of derivatives of 3-(pyrrol-4-yl)acrylamides, which exhibit a moderate antimicrobial activity³⁵. The introduction of a 1,3,4-thiadiazole nucleus into the 4th position of the pyrrole cycle in compounds **1a-e** assumes two preparative simple stages. In the first stage, the carboxylic acids **2a-e** were obtained by the soft selective oxidizing of an aldehyde group by sodium hypochlorite in a DMSO-H₂O solution with a yield of 88-92 %. The IR spectra of the synthesized acids reveal the medium-intensity absorption bands of the carboxylic (1708-1712 cm⁻¹) and carboxylate (1724-1729 cm⁻¹) C=O groups and the wide absorption bands of the carboxylic OH within the range (2544-2854 cm⁻¹).

It is known that depending on the synthesis conditions, the following derivatives can be obtained in the cyclocondensation of carboxylic acids with substituted thiosemicarbazides: 1,3,4-triazole-2-thiones^{36,37}, 2-amino-1,3,4-oxadiazoles^{38, 39} or 2-amino-1,3,4-thiadiazoles⁴⁰. A synthetic method from the latter cited work was found suitable for the second stage of the synthesis of the target 4-(1,3,4-thiadiazol-2-yl)pyrroles **3 a-k** (Tab. 1). They were obtained with a yield of 71-93 % after a 2-h long heating of equimolar amounts of the acids **2 a-e** with mono- or disubstituted thiosemicarbazides in a threefold excess of phosphorus chloroxide. The absorption bands of the carboxylate C=O fragment (1724-1730 cm⁻¹) and N-H groups (3282-3294 cm⁻¹ for the compounds **3 b-h, j, k**) were identified in the IR spectra of synthesized compounds, while their ¹H NMR spectra showed the proton peaks of all substituents in the pyrrole and 1,3,4-thiadiazole cycles (see Experimental below).

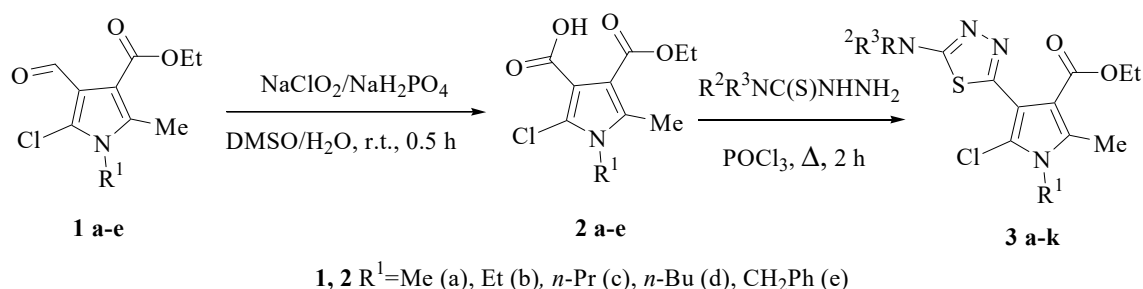
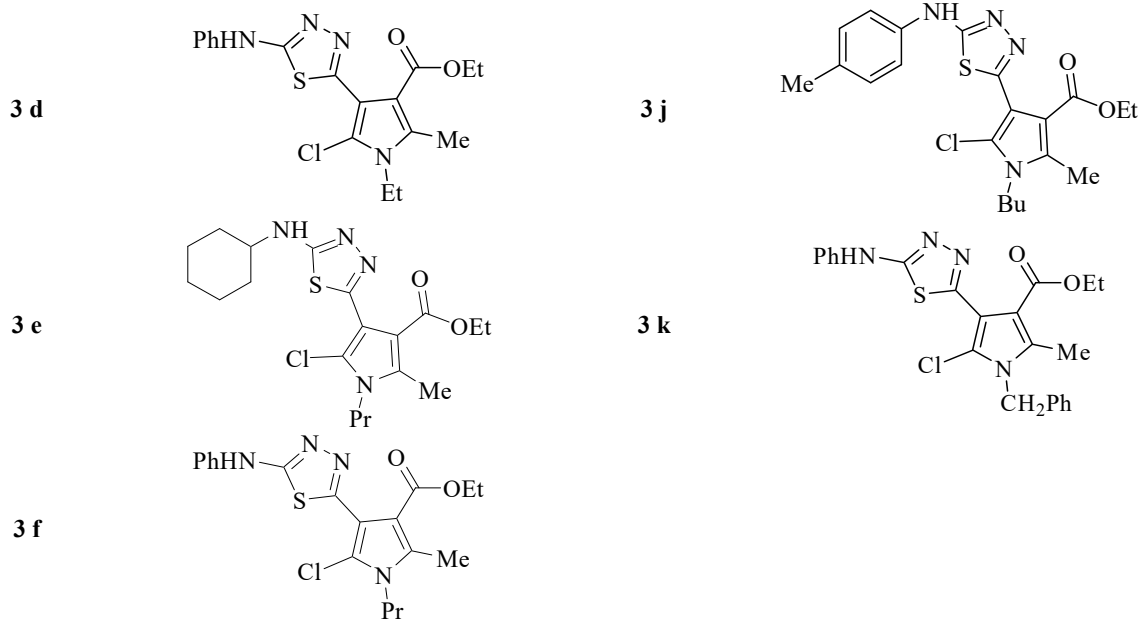


Fig. 3. Synthesis of new derivatives of 4-(1,3,4-thiadiazol-2-yl)pyrroles

Table 1. Structure of synthesized compounds

Compound	Structure	Compound	Structure
3 a		3 g	
3 b		3 h	
3 c		3 i	



2.2 Antimicrobial activity

In 2017, NHO published a list of the highest-priority antibiotic-resistant pathogens. Among others, it includes carbapenem-resistant strains *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli*, a representative of *Enterobacteriaceae*, which can produce the wide-range active β -lactamases⁴¹. That is why our antimicrobial activity search was focused on these strains and some other important bacteria and fungi. An antimicrobial activity of the synthesized compounds **3 a-k** was evaluated by their minimal inhibition concentration (MIC) and minimal bactericide concentration (MBC) against the following gram-positive and gram-negative bacteria *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 1388, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* 4636, *Staphylococcus aureus* ATCC 25923, and fungi *Aspergillus niger* K9 and *Candida albicans* ATCC 885/653. The antibacterial activity data (Tab. 2) prove that all synthesized compounds suppress the proliferation of the abovementioned germs for the concentrations of 31.25-250 $\mu\text{g/mL}$. The lowest MIC found in our research was 31.25 $\mu\text{g/mL}$. The compounds **3 a-k** showed the best results against *Klebsiella pneumoniae* ATCC 13883, for which an inhibition activity was found at 31.25 $\mu\text{g/mL}$ for the seven agents (**3 a, c, e-h, j**). The five agents (**3 c, e-g, j**) were active against *Escherichia coli* ATCC 25922 at the same concentration. Only **3 e** was found active against *Pseudomonas aeruginosa* ATCC 27853 at 31.25 $\mu\text{g/mL}$, while only **3 g** was able to inhibit the proliferation of fungus *Candida albicans* ATCC 885/653. Higher concentrations of the antigerm compounds should be applied to restrain the proliferation of *Proteus vulgaris* 4636, *Staphylococcus aureus* ATCC 25923, and *Aspergillus niger* K9.

Table 2. Antimicrobial activity of new derivatives of 4-(1,3,4-thiadiazol-2-yl)pyrroles **3 a-k**

No	<i>K. pneumoniae</i>		<i>S. aureus</i>		<i>E. coli</i>		<i>P. vulgaris</i>		<i>P. aeruginosa</i>		<i>C. albicans</i>		<i>A. niger</i>	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC	MIC	MFC
3 a	31.25	62.5	62.5	125	62.5	125	62.5	125	62.5	125	62.5	62.5	62.5	125
3 b	62.5	62.5	62.5	125	62.5	62.5	62.5	125	62.5	62.5	62.5	62.5	62.5	125
3 c	31.25	62.5	125	125	31.25	62.5	62.5	62.5	62.5	125	62.5	62.5	62.5	125
3 d	62.5	125	125	250	62.5	125	125	250	62.5	125	62.5	62.5	62.5	125
3 e	31.25	62.5	62.5	125	31.25	62.5	62.5	125	31.25	62.5	62.5	62.5	62.5	125
3 f	31.25	62.5	62.5	125	31.25	62.5	62.5	62.5	62.5	125	62.5	62.5	62.5	125
3 g	31.25	62.5	62.5	62.5	31.25	62.5	62.5	62.5	62.5	125	31.25	62.5	62.5	125
3 h	31.25	62.5	62.5	125	62.5	62.5	125	250	62.5	62.5	62.5	62.5	62.5	125
3 i	62.5	125	125	250	62.5	125	62.5	125	125	125	62.5	62.5	62.5	125
3 j	31.25	62.5	62.5	125	31.25	62.5	62.5	62.5	62.5	125	62.5	62.5	62.5	125
3 k	62.5	62.5	125	125	62.5	62.5	125	125	62.5	62.5	62.5	62.5	62.5	125
DMSO*	+		+		+		+		+		+		+	
C**	15.625	31.25	0.48	0.97	1.95	3.9	7.81	15.625	31.25	31.25	0.97	1.95	0.48	0.48

* proliferation of bacteria detected, ** Decasan (a solution consisting of 0.2 mg/mL of decamethoxin) made by "Yuria-Pharm" was used as an antibacterial control drug, ** Clotrimazole (a solution consisting of 10 mg/mL of clotrimazole) made by PJSC SIC "Borshchahivskiy CPP" was used as an antifungal control drug

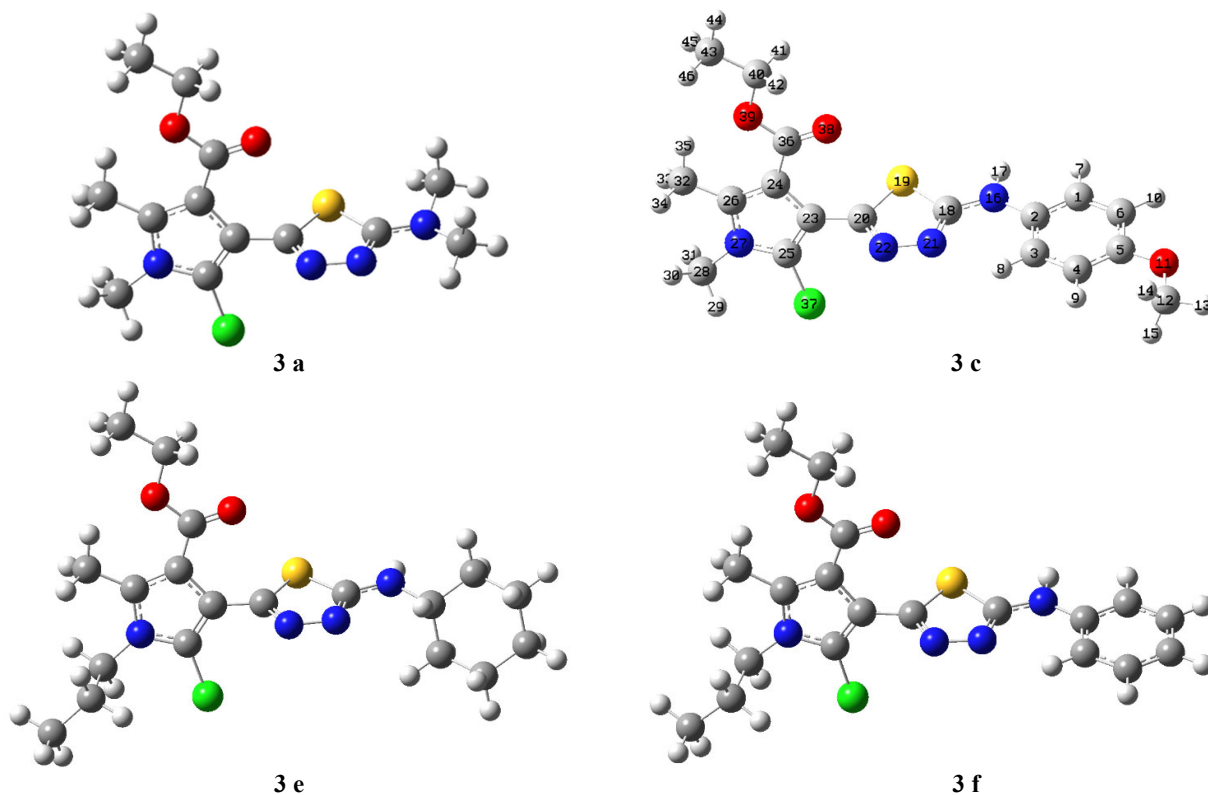
2.3 Structure and reactivity analysis by the method of DFT-calculation

The software Gaussian 09⁴² was employed to optimize the structure of the most active antimicrobial compounds **3 a, c, e-h, j**, and GaussView 5.0.8 was used to visualize optimization results. To optimize the geometric composition of the synthesized antimicrobial agents, the Density Functional Theory was used in the B3LYP approach with the standard set of basic functions 6-311++G(d,p).

The following parameters of reactivity were calculated for **3 a, c, e-h, j**: chemical hardness (η), electrodonating (ω^-) and electroaccepting (ω^+) powers. Ionization potential (IP) and electron affinity (EA) were calculated by the equations:

$$\begin{aligned} \text{IP} &= -E_{\text{HOMO}} \\ \text{EA} &= -E_{\text{LUMO}} \\ \eta, \omega^-, \omega^+ &\text{ – by the equations }^{43,44}: \\ \eta &= 0,5(\text{IP} - \text{EA}) \\ \omega^- &= \frac{\text{IP}^2}{2(\text{IP} - \text{EA})} \\ \omega^+ &= \frac{\text{EA}^2}{2(\text{IP} - \text{EA})} \end{aligned}$$

As seen from the B3LYP/6-311++G(d,p)-based DFT-simulation of molecular structures in vacuum, due to large size of the ester group and chlorine atom located as substituents in the 3rd and 5th positions of the pyrrole ring, an angle between the pyrrole and thiadiazole ring planes is ranging between 65° and 75° (the angle C25-C23-C20-N22 for the compounds **3 a, c, e-h, j** is 73.5°; 68.9°; 71.4°; 74.5°; 75.2°; 65.3°; and 72.4° correspondingly (**Fig. 4**)). The R² aromatic substituents of the compounds **3 c, f, g, j** are in the same plane with 1,3,4-thiadiazole ring, and the angle C18-N15-C2-C3 is close to 0°.



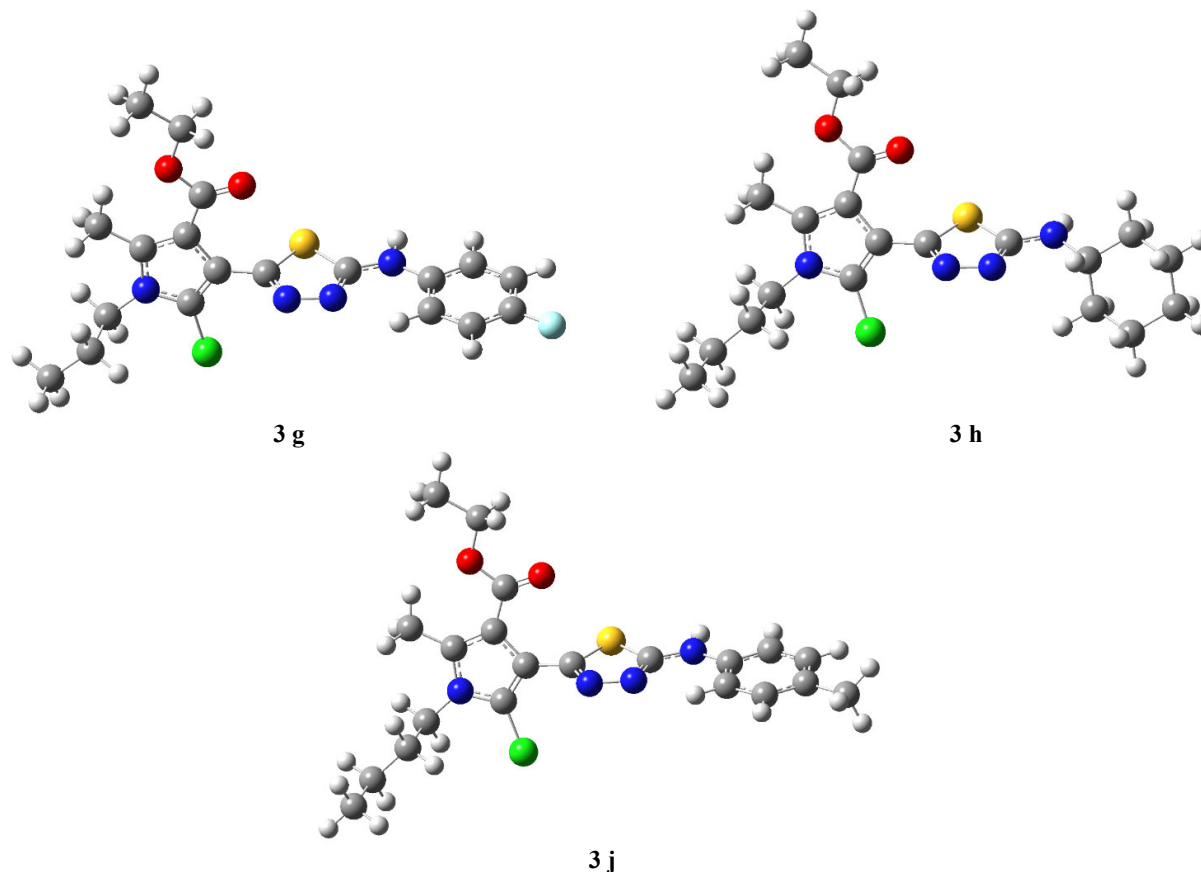


Fig. 4. Optimized structure of the compounds **3 a, c, e-h, j** calculated by the B3LYP/6-311++G(d,p)-based DFT method in vacuum

The energy of frontier orbitals is practically the same (Table 3), but a lower conjugation in the compounds **3 a, e, h** causes some widening in the bandgap width (E_g). Thus, the LUMO and HOMO values for these compounds are the highest and lowest, respectively. The energy of both frontier orbitals gets slightly increased because of more distinct donor properties of the butyl substituent R^1 . In the case of **3 c, f, g, j**, the bandgap width of these compounds with N-aryl substituents in the thiadiazole cycle is narrower, and fluorine, as an acceptor substituent (compound **3 g**) decreases the energy of frontier orbitals, while the donor methoxyl and methyl groups (compounds **3 c, j**) increase this energy, especially the HOMO value. All calculated energy parameters of the compounds **3 a, c, e, f, g, h, j** are given in Table 3. According to the calculated HOMO energies, these compounds can be arranged in the following way: **3 c** > **3 j** > **3 f** > **3 a** = **3 h** > **3 g** > **3 e**, which means that **3 c** is expectedly the most reactive ligand for protein receptors.

Table 3. The calculated energy of frontier MO, bandgap width (E_g), ionization potential (IP), electron affinity (EA), chemical hardness (η), and electrodonating (ω^-) and electroaccepting (ω^+) powers

	3 a	3 c	3 e	3 f	3 g	3 h	3 j
E_{LUMO} , eV	-0,84	-0,98	-0,82	-0,97	-1,03	-0,84	-0,95
E_{HOMO} , eV	-5,73	-5,34	-5,77	-5,69	-5,76	-5,73	-5,54
E_g , eV	4,89	4,36	4,95	4,71	4,73	4,89	4,59
IP, eV	5,73	5,34	5,77	5,69	5,76	5,73	5,54
EA, eV	0,84	0,98	0,82	0,97	1,03	0,84	0,95
η , eV	2,44	2,18	2,47	2,36	2,37	2,45	2,29
ω^- , eV	0,07	0,11	0,07	0,10	0,11	0,07	0,10
ω^+ , eV	3,36	3,27	3,36	3,43	3,50	3,36	3,34

The electrostatic surface potential (ESP) is an important parameter characterizing the active centers of a ligand.^{45, 46} It has been calculated for the molecules of compounds **3 a, c, e-h, j** using optimized structures with the basis B3LYP/6-311++G(d,p) for studying nucleophilic and electrophilic surface spots. Fig. 5 shows two positive (nucleophilic) spots: a smaller but more positive spot on the –NH group and a wider but less positive one – on the alkyl substituents of the pyrrole cycle. Another wide and positive spot can be seen on the N,N-dimethylamino group of compound **3 a**. The positive charge on the amino group of compound **3 g** increases because of the electron-acceptor fluorine atom in the aromatic substituent R^2 , while the electron-donor non-aromatic substituents in **3 a, e, h** cause some decrease in this positive charge. One negatively charged electrophilic center can be found on the nitrogen atoms of thiadiazole, while another less negatively

charged one – near the oxygen atom in the ester group. Another small spot with an even less negative charge can be found on the highly electronegative oxygen and fluorine atoms of the aromatic substituents R^2 in compounds **3 c** and **3 g**. Non-aromatic electron-donor substituents lead to an increase in the negative charge of these spots, while electron-acceptor substituents cause a decrease in this charge.

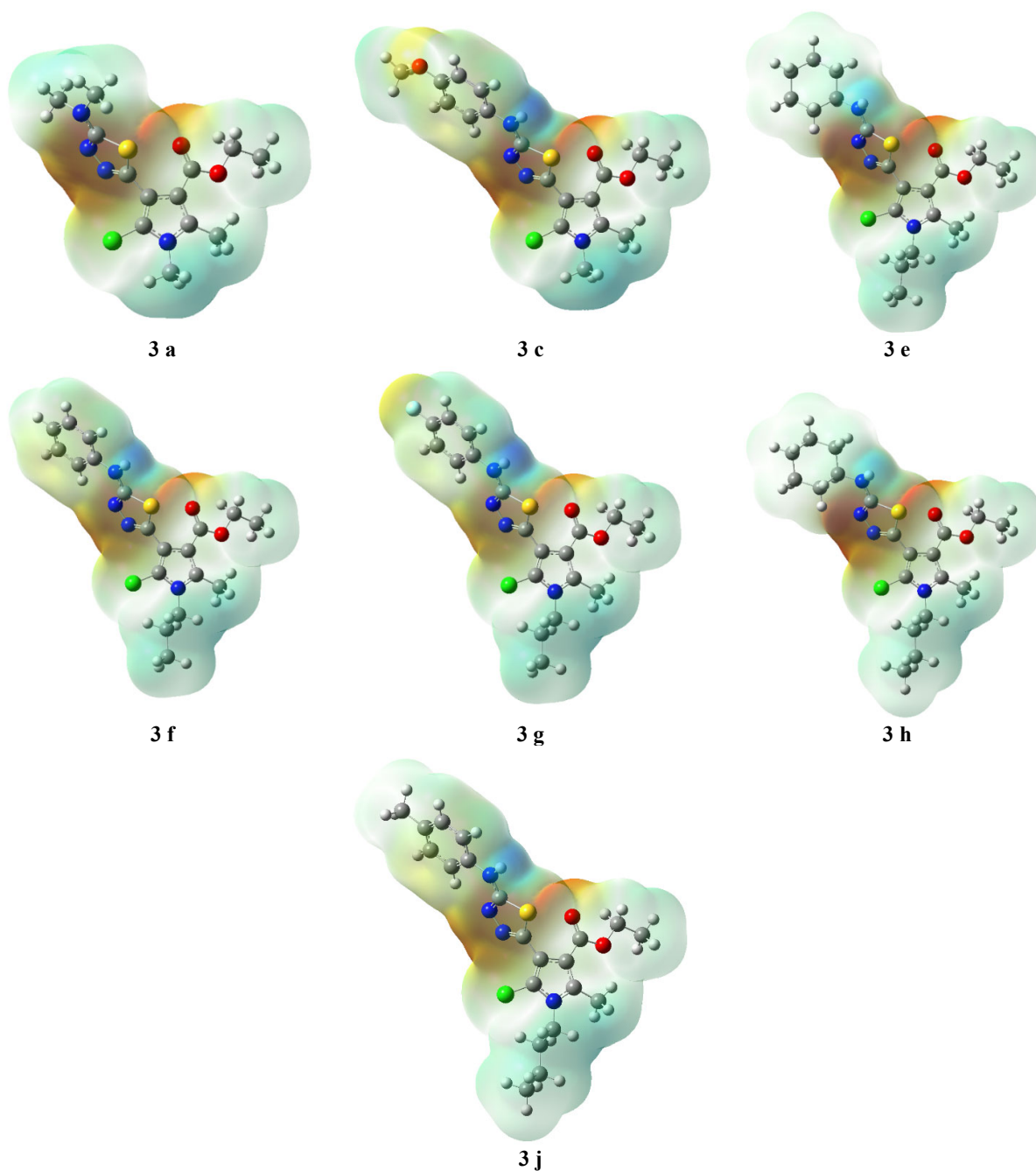


Fig. 5. The calculated ESP for the molecules of compounds **3 a**, **3 c**, **3 e-h**, **3 j** (for the reason of easiness, the color gamma is set for all compounds from red for $-0,06942$ a.u. ($-43,6$ kcal/mol) to blue for $+0,06942$ a.u. ($+43,6$ kcal/mol))

2.4 Molecular docking Study

The molecular docking study was performed using the software Autodoc Vina⁴⁷. The preliminary optimized structures (B3LYP/6-311++G(d,p)) were used in all our calculations. The crystal structure of kinase ThiM *Klebsiella pneumoniae* was downloaded from Protein Data Bank (PDB 6k28), the water molecules were removed, the polar hydrogen atoms and the Gasteiger charges were added to the protein structure. The C chain of the protein was also removed since the investigated

docking site is located between chains A and B. A center of the ligand docking cavity (21.0; 62.4; 37.2) was determined using BIOVIA Discovery Studio Visualizer v21.1. The cavity dimension was 20; 20; 20. The docking was visualized using BIOVIA Discovery Studio Visualizer v21.1.

According to the molecular docking simulation, 9 positions were found with the corresponding ligand-protein affinity for every ligand, and the following affinities were determined for the complexes: -6.1 (**3 a**), -7.3 (**3 c**), -7.1 (**3 e**), -7.4 (**3 f**), -7.5 (**3 g**), -7.6 (**3 h**), and -7.1 (**3 j**) kcal/mol. It means that the compound **3 h** is the most affine to *Klebsiella pneumoniae*, followed by **3 g** and **3 f**, while **3 a** is the least affine to this germ.

The ligand-protein docking schemes are shown for all investigated compounds in **Fig. 6**. It can be seen that compound **3 a** is stabilized inside its docking site by two weak carbon-hydrogen bonds with the residues Val 195 and Gly 71, three π -alkyl bonds between π -electrons of thiadiazole ring and Cys 200, and π -electrons of pyrrole cycle with Val 100 and Ala 101 of the B-chain. There is only one bond between this compound and the A-chain that is established with Ala 48 by the π -alkyl interaction.

The compound **3 c** is stabilized by three hydrogen bonds: through oxygen atoms bonded with Arg 124, Cys 200, and Arg 108 of the B-chain, the alkyl interaction between a methoxy group and Leu 105 and Ala 101 of the B-chain, and with Val 50 of the A-chain. A phenyl ring is bonded with the A-chain through Ala 48 and with the B-chain through Val 100 by the π -alkyl interaction. Also, a thiadiazole ring is bonded with the B-chain through Val 196 and Cys 200 by the π -alkyl interaction.

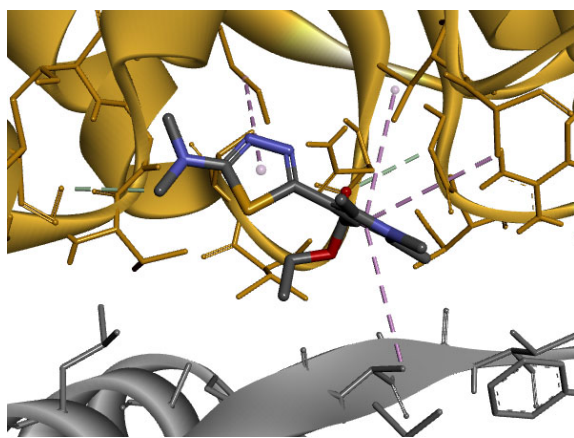
The compound **3 e** is stabilized by a hydrogen bond between the oxygen atom of the carboxylate group of Arg 124 in the B-chain, a weak carbon-hydrogen bond with a Glu 129 residue, and three alkyl interactions with Val 31, Val 100, and Met 192 of the B-chain. There are no bonds between this compound and A-chain.

The compound **3 f** is stabilized by the hydrogen bonds between the oxygen atoms of the carboxylate group with Arg 124 and Cys 200 of the B-chain and a sulfur atom with Gly 197 of the B-chain, by a weak carbon-hydrogen bond with a residue of Glu 129, and by the alkyl and π -alkyl interaction with Met 192 and Val 31 of the B-chain, respectively. There are no bonds between this compound and A-chain.

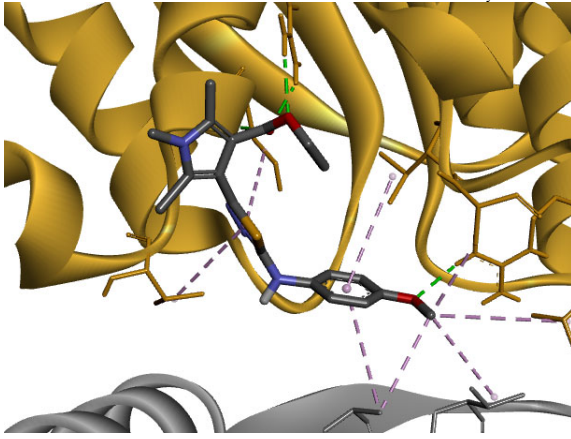
The compound **3 g** is stabilized by the hydrogen bonds between the oxygen atoms of the carboxylate group with Arg 124 and Cys 200 of the B-chain and the alkyl and π -alkyl interaction with Met 192 and Val 31 of the B-chain, respectively. An atom of fluorine is bonded with the B-chain through Gly 71 and Asn 29, while it is bonded with the A-chain only by the π -sulfur interaction through Met 49.

The compound **3 h** is stabilized by the hydrogen bonds between the oxygen atoms of the carboxylate group with Arg 124 and Cys 200, by another weak carbon-hydrogen bond with the residue Glu 129 of the B-chain, by the alkyl interaction with Met 192 and Val 31, and by the π -alkyl interaction with Met 192 and Val 196 of the B-chain. There are no bonds between this compound and A-chain.

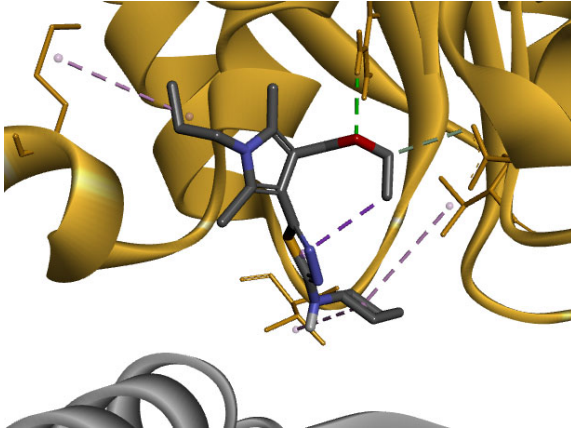
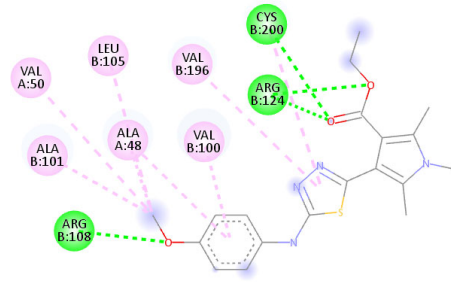
The compound **3 j** is stabilized by a hydrogen bond between the oxygen atoms of the carboxylate group with Arg 124, a weak carbon-hydrogen bond with the residue Glu 129 of the B-chain, the alkyl interaction with Met 192, and π -alkyl interaction with Val 100 of the B-chain. This compound is also stabilized by the alkyl and π -alkyl bonds with Val 50 and Ala 48 of the A-chain.



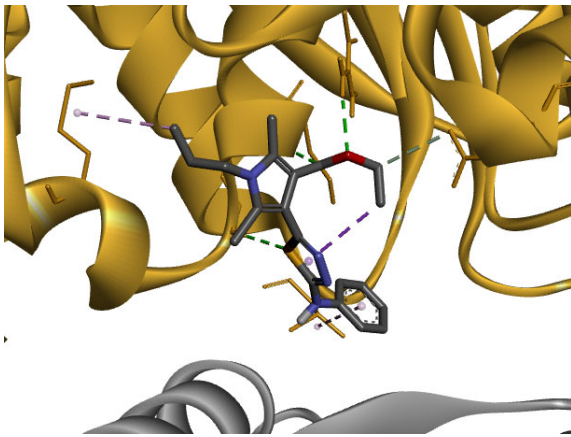
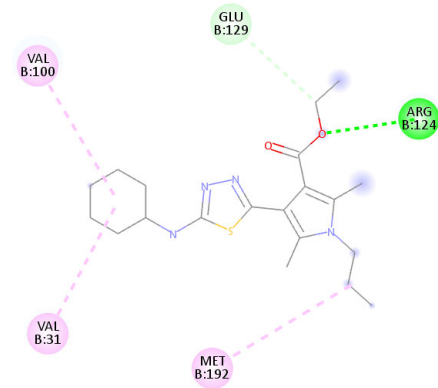
3 a



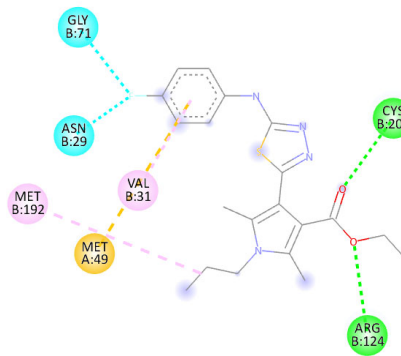
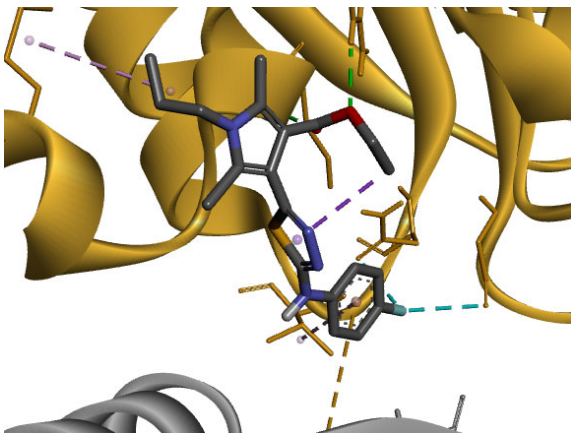
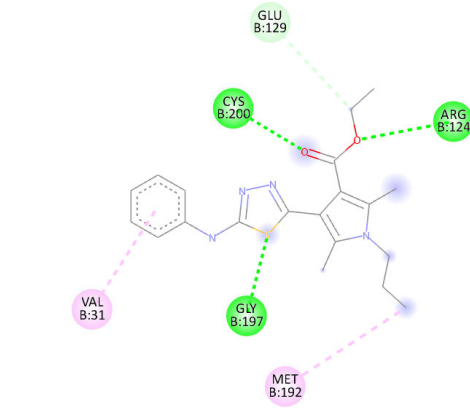
3 c



3 e



3 f



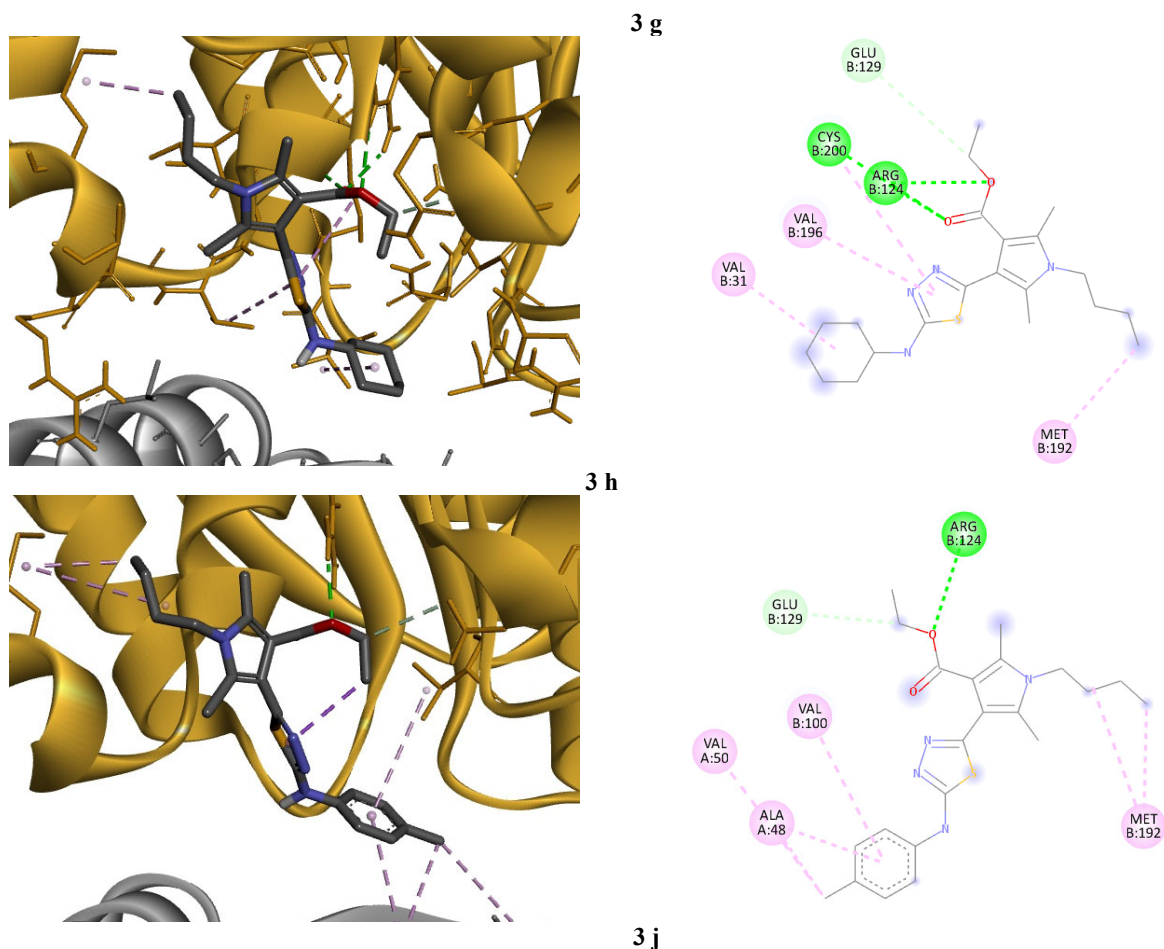


Fig. 6. 3D (left) and 2D (right) schemes of the bonds of **3 a, c, e-h, j** inside the active center of a protein. Chain A (grey) is shown in the lower part of the figures and chain B (yellow) is shown in the upper part

Interactions		
 Conventional Hydrogen Bond	 Halogen (Fluorine)	 Alkyl
 Carbon Hydrogen Bond	 Pi-Sulfur	 Pi-Alkyl

3. Conclusions

As a result of this investigation, some new antimicrobial 1,3,4-thiadiazolopyrroles were obtained, and functional substituents in both cycles of these compounds can further be modified. A synthesis method used in this work is preparative simple and ensures high yields of intermediate and target products. The products' composition has been confirmed by various spectral methods. The antimicrobial activity of the synthesized compounds has also been proved by the screening of their bioactivity. The compounds **3 a-h** inhibit the proliferation of some bacteria and fungi within the range of concentration of 31.25-250 $\mu\text{g/mL}$. The highest inhibition activity was detected against the strain *Klebsiella pneumonia* for 31.25 $\mu\text{g/mL}$ of the compounds **3 a, c, e-h, j**. Their structure, reactivity, and ESP were analyzed by DFT-calculation, while molecular docking was used to assess their affinity to ThiM *Klebsiella pneumoniae* kinase.

Acknowledgements

We would like to thank Enamine Ltd for the material and technical support, and the “European Chemistry School for Ukrainians” (<https://ecpsfu.org/>) for new scientific ideas.

4. Experimental

4.1. Materials and Methods

All chemicals were of analytical grade and commercially available. When performing the synthetic part of the work, the reagents of the company Merck (Germany) and Sigma-Aldrich (USA) were used. All reagents and solvents were used without further purification and drying. All the melting points were determined in an open capillary and left uncorrected. IR spectra were recorded on Bruker Vertex 70 FT-IR spectrometer for samples in KBr pellets. $^1\text{H-NMR}$ spectra were

acquired in pulse Fourier transform mode on a Varian VXR-400 spectrometer (400 MHz) in DMSO- d_6 , while ^{13}C -NMR spectra of all compounds were recorded on a Bruker Avance DRX-500 spectrometer (125 MHz). Mass spectra were recorded on an Agilent LC/MSD SL mass spectrometer; column: Zorbax SB-C18, 4.6×15 mm, $1.8 \mu\text{m}$ (PN 82 (c)75-932); DMSO solvent, atmospheric pressure electrospray ionization. Elemental analysis was performed on a Perkin Elmer 2400 CHN-analyzer. Melting points were determined on a Kofler bench and left uncorrected.

4.2. General procedure

General procedure for the synthesis of 1-alkyl-2-chloro-4-(ethoxycarbonyl)-5-methyl-1H-pyrrole-3-carboxylic acids (2 a-e). 7.20 g of NaH_2PO_4 (60 mmol) was added to a solution of 10 mmol of an aldehyde **1 a-e** in 20 mL of DMSO and 5 mL of water. Then 5.43 g of NaClO_2 (60 mmol) was gradually added to the above solution at constant stirring and the temperature of 10°C . Afterwards, the mixture was stirred at room temperature for the next 30 min, followed by the addition of 10 mL of a 1 M solution of HCl and 10 mL of water. The obtained solid product was filtered, rinsed with water, dried, and recrystallized from isopropanol.

General procedure for the synthesis of ethyl 5-chloro-2-methyl-4-(1,3,4-thiadiazol-2-yl)-1H-pyrrole-3-carboxylate (3 a-k). 5 mmol of a mixture of the acids **2 a-e**, 5 mmol of a mono- or disubstituted thiosemicarbazide, and phosphorus oxychloride (2.30 g, 15 mmol) was boiled for 2 h and cooled to room temperature. Then, some ice was added to the mixture before its neutralization by a solution of ammonium hydroxide. The obtained solid product was filtered, rinsed with water, dried, and recrystallized from acetonitrile.

4.3 Physical and Spectral Data

4.3.1 2-Chloro-4-(ethoxycarbonyl)-1,5-dimethyl-1H-pyrrole-3-carboxylic acid (2 a). Yield 92 % (2.25 g), white solid, m. p. 210-211 $^\circ\text{C}$; IR (KBr, cm^{-1}): 1709 (C=O), 1728 (C=O), 2544-2852 (OH); ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 1.23 (3H, t, $J = 7.2$ Hz, OCH_2CH_3), 2.36 (3H, s, CH_3), 3.49 (3H, s, NCH_3), 4.17 (2H, q, $J = 7.2$ Hz, OCH_2CH_3), 12.46 (1H, s, CO_2H). ^{13}C NMR (125.7 MHz, DMSO- d_6) δ (ppm): 11.0, 13.9, 30.7, 59.8, 111.3, 112.7, 118.2, 134.9, 163.9, 164.0. LCMS $[\text{M}+\text{H}]^+$: 246.7. Anal. Calcd. for $\text{C}_{10}\text{H}_{12}\text{ClNO}_4$ (%): C, 48.89; H, 4.92; N, 5.70. Found: C, 49.02; H, 5.01; N, 5.59.

4.3.2 2-Chloro-4-(ethoxycarbonyl)-1-ethyl-5-methyl-1H-pyrrole-3-carboxylic acid (2 b). Yield 90 % (2.38 g), white solid, m. p. 180-181 $^\circ\text{C}$; IR (KBr, cm^{-1}): 1708 (C=O), 1729 (C=O), 2552-2854 (OH); ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 1.17-1.29 (6H, m, $\text{NCH}_2\text{CH}_3 + \text{OCH}_2\text{CH}_3$), 2.38 (3H, s, CH_3), 3.96 (2H, q, $J = 7.2$ Hz, NCH_2CH_3), 4.17 (2H, q, $J = 7.2$ Hz, OCH_2CH_3), 12.59 (1H, s, CO_2H). ^{13}C NMR (125.7 MHz, DMSO- d_6) δ (ppm): 11.1, 14.4, 15.2, 40.1, 60.3, 112.1, 113.6, 117.7, 133.8, 164.1, 164.5. LCMS $[\text{M}+\text{H}]^+$: 260.7. Anal. Calcd. for $\text{C}_{11}\text{H}_{14}\text{ClNO}_4$ (%): C, 50.88; H, 5.43; N, 5.39. Found: C, 51.02; H, 5.32; N, 5.50.

4.3.3 2-Chloro-4-(ethoxycarbonyl)-5-methyl-1-propyl-1H-pyrrole-3-carboxylic acid (2 c). Yield 89 % (2.44 g), white solid, m. p. 180-181 $^\circ\text{C}$; IR (KBr, cm^{-1}): 1710 (C=O), 1724 (C=O), 2548-2837 (OH); ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 0.87 (3H, t, $J = 7.2$ Hz, $\text{NCH}_2\text{CH}_2\text{CH}_3$), 1.22 (3H, t, $J = 7.2$ Hz, OCH_2CH_3), 1.58-1.64 (2H, m, $\text{NCH}_2\text{CH}_2\text{CH}_3$), 2.37 (3H, s, CH_3), 3.89 (2H, t, $J = 7.2$ Hz, $\text{NCH}_2\text{CH}_2\text{CH}_3$), 4.15 (2H, q, $J = 7.2$ Hz, OCH_2CH_3), 12.57 (1H, s, CO_2H). ^{13}C NMR (125.7 MHz, DMSO- d_6) δ (ppm): 11.2, 11.3, 14.4, 23.1, 45.7, 60.3, 112.1, 113.6, 118.1, 134.1, 164.2, 164.5. LCMS $[\text{M}+\text{H}]^+$: 274.8. Anal. Calcd. for $\text{C}_{12}\text{H}_{16}\text{ClNO}_4$ (%): C, 52.66; H, 5.89; N, 5.12. Found: C, 52.34; H, 6.00; N, 5.20.

4.3.4 1-Butyl-2-chloro-4-(ethoxycarbonyl)-5-methyl-1H-pyrrole-3-carboxylic acid (2 d). Yield 88 % (2.44 g), white solid, m. p. 118-119 $^\circ\text{C}$; IR (KBr, cm^{-1}): 1712 (C=O), 1726 (C=O), 2550-2842 (OH); ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 0.90 (3H, t, $J = 7.2$ Hz, $\text{NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 1.20-1.34 (5H, m, $\text{OCH}_2\text{CH}_3 + \text{NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 1.53-1.60 (2H, m, $\text{NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 2.37 (3H, s, CH_3), 3.92 (2H, t, $J = 7.2$ Hz, $\text{NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 4.15 (2H, q, $J = 7.2$ Hz, OCH_2CH_3), 12.57 (1H, s, CO_2H). ^{13}C NMR (125.7 MHz, DMSO- d_6) δ (ppm): 11.3, 13.9, 14.4, 19.7, 31.8, 40.1, 60.5, 112.1, 113.3, 118.4, 134.3, 164.4, 164.6. LCMS $[\text{M}+\text{H}]^+$: 288.8. Anal. Calcd. for $\text{C}_{13}\text{H}_{18}\text{ClNO}_4$ (%): C, 54.26; H, 6.31; N, 4.87. Found: C, 54.39; H, 6.40; N, 4.74.

4.3.5 1-Benzyl-2-chloro-4-(ethoxycarbonyl)-5-methyl-1H-pyrrole-3-carboxylic acid (2 e). Yield 91 % (2.93 g), white solid, m. p. 180-181 $^\circ\text{C}$; IR (KBr, cm^{-1}): 1709 (C=O), 1725 (C=O), 2549-2841 (OH); ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 1.24 (3H, t, $J = 7.2$ Hz, OCH_2CH_3), 2.32 (3H, s, CH_3), 4.18 (2H, q, $J = 7.2$ Hz, OCH_2CH_3), 5.26 (2H, s, NCH_2Ph), 7.04 (2H, d, $J = 7.4$ Hz, Ph), 7.27 - 7.39 (3H, m, Ph), 12.55 (1H, s, CO_2H). ^{13}C NMR (125.7 MHz, DMSO- d_6) δ (ppm): 11.1, 13.9, 46.9, 60.0, 112.4, 113.5, 118.1, 126.0 (2C), 127.6, 128.9 (2C), 134.1, 136.0, 163.9, 164.1. LCMS $[\text{M}+\text{H}]^+$: 322.8. Anal. Calcd. for $\text{C}_{16}\text{H}_{16}\text{ClNO}_4$ (%): C, 59.73; H, 5.01; N, 4.35. Found: C, 59.94; H, 4.90; N, 4.30.

4.3.6 Ethyl 5-chloro-4-[5-(dimethylamino)-1,3,4-thiadiazol-2-yl]-1,2-dimethyl-1H-pyrrole-3-carboxylate (3 a). Yield 76 % (1.25 g), white solid, m. p. 134-135 $^\circ\text{C}$; IR (KBr, cm^{-1}): 1724 (C=O); ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 1.29 (3H, t, $J = 7.2$ Hz, OCH_2CH_3), 3.13 (6H, s, NCH_3), 3.55 (3H, s, NCH_3), 4.21 (2H, q, $J = 7.0$ Hz, OCH_2CH_3). ^{13}C NMR (125.7 MHz, DMSO- d_6) δ (ppm): 11.5, 13.9, 31.2, 41.3 (2C), 59.4, 109.5, 110.5, 117.2, 136.2, 148.3, 163.1, 171.9. LCMS $[\text{M}+\text{H}]^+$: 329.8. Anal. Calcd. for $\text{C}_{13}\text{H}_{17}\text{ClN}_4\text{O}_2\text{S}$ (%): C, 47.49; H, 5.21; N, 17.04. Found: C, 47.20; H, 5.11; N, 17.19.

4.3.7 Ethyl 4-(5-anilino-1,3,4-thiadiazol-2-yl)-5-chloro-1,2-dimethyl-1H-pyrrole-3-carboxylate (**3 b**). Yield 84 % (1.25 g), white solid, m. p. 212-213 °C; IR (KBr, cm^{-1}): 1727 (C=O), 3285 (N-H); ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 1.09 (3H, t, $J = 7.2$ Hz, OCH_2CH_3), 3.55 (3H, s, NCH₃), 4.07 (2H, q, $J = 7.0$ Hz, OCH_2CH_3), 6.95-7.02 (1H, m, Ph), 7.30-7.37 (2H, m, Ph), 7.59-7.66 (2H, m, Ph). ^{13}C NMR (125.7 MHz, DMSO- d_6) δ (ppm): 11.4, 13.9, 31.2, 59.4, 109.3, 110.6, 117.3, 121.7 (2C), 128.1, 129.0 (2C), 136.3, 140.6, 149.5, 163.1, 165.0. LCMS $[\text{M}+\text{H}]^+$: 377.9. Anal. Calcd. for $\text{C}_{17}\text{H}_{17}\text{ClN}_4\text{O}_2\text{S}$ (%): C, 54.18; H, 4.55; N, 14.87. Found: C, 54.38; H, 4.64; N, 14.99.

4.3.8 Ethyl 5-chloro-4-{5-[(4-methoxyphenyl)amino]-1,3,4-thiadiazol-2-yl}-1,2-dimethyl-1H-pyrrole-3-carboxylate (**3 c**). Yield 79 % (1.63 g), white solid, m. p. 157-158 °C; IR (KBr, cm^{-1}): 1726 (C=O), 3290 (N-H); ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 1.11 (3H, t, $J = 7.1$ Hz, OCH_2CH_3), 3.55 (3H, s, NCH₃), 3.74 (3H, s, OCH₃), 4.08 (2H, q, $J = 7.1$ Hz, OCH_2CH_3), 6.94 (2H, d, $J = 8.9$ Hz, Ph), 7.54 (2H, d, $J = 8.9$ Hz, Ph). ^{13}C NMR (125.7 MHz, DMSO- d_6) δ (ppm): 11.4, 13.9, 31.2, 55.2, 59.4, 109.5, 110.6, 114.3 (2C), 117.1, 119.1 (2C), 134.2, 136.2, 148.7, 154.4, 163.1, 165.7. LCMS $[\text{M}+\text{H}]^+$: 407.9. Anal. Calcd. for $\text{C}_{18}\text{H}_{19}\text{ClN}_4\text{O}_3\text{S}$ (%): C, 53.13; H, 4.71; N, 13.77. Found: C, 52.98; H, 4.64; N, 13.89.

4.3.9 Ethyl 4-(5-anilino-1,3,4-thiadiazol-2-yl)-5-chloro-1-ethyl-2-methyl-1H-pyrrole-3-carboxylate (**3 d**). Yield 92 % (1.80 g), white solid, m. p. 189-190 °C; IR (KBr, cm^{-1}): 1724 (C=O), 3284 (N-H); ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 1.09 (3H, t, $J = 7.0$ Hz, OCH_2CH_3), 1.24 (3H, t, $J = 7.0$ Hz, NCH₂CH₃), 2.49 (3H, s, CH₃), 4.02-4.09 (4H, m, OCH_2CH_3 + NCH₂CH₃), 6.98 (1H, t, $J = 7.1$ Hz, Ph), 7.30 - 7.39 (2H, m, Ph), 7.60 - 7.72 (2H, m, Ph). ^{13}C NMR (125.7 MHz, DMSO- d_6) δ (ppm): 11.0, 13.9, 14.8, 59.4, 109.6, 110.9, 116.4, 117.3 (2C), 121.7, 129.0 (2C), 135.4, 140.6, 149.5, 163.1, 165.0. LCMS $[\text{M}+\text{H}]^+$: 391.9. Anal. Calcd. for $\text{C}_{18}\text{H}_{19}\text{ClN}_4\text{O}_2\text{S}$ (%): C, 55.31; H, 4.90; N, 14.33. Found: C, 55.57; H, 5.00; N, 14.49.

4.3.10 Ethyl 5-chloro-4-[5-(cyclohexylamino)-1,3,4-thiadiazol-2-yl]-2-methyl-1-propyl-1H-pyrrole-3-carboxylate (**3 e**). Yield 88 % (1.80 g), white solid, m. p. 154-155 °C; IR (KBr, cm^{-1}): 1729 (C=O), 3292 (N-H); ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 0.89 (3H, t, $J = 7.3$ Hz, NCH₂CH₂CH₃), 1.15 (3H, t, $J = 7.1$ Hz, OCH_2CH_3), 1.30 - 1.40 (4H, m, NCH₂CH₂CH₃ + 2Hcyclohexyl), 1.54 - 1.72 (6H, m, cyclohexyl), 1.96 (2H, br.s, cyclohexyl), 2.52 (3H, s, CH₃), 3.76 (1H, br.s, cyclohexyl), 3.98 (2H, t, $J = 7.3$ Hz, NCH₂CH₂CH₃), 4.12 (2H, q, $J = 7.0$ Hz, OCH_2CH_3). ^{13}C NMR (125.7 MHz, DMSO- d_6) δ (ppm): 10.7, 11.4, 13.8, 22.6, 23.8, 24.8 (2C), 31.4 (2C), 45.7, 54.5, 59.7, 108.1, 110.7, 117.6, 136.2, 148.2, 162.9, 166.8. LCMS $[\text{M}+\text{H}]^+$: 412.0. Anal. Calcd. for $\text{C}_{19}\text{H}_{27}\text{ClN}_4\text{O}_2\text{S}$ (%): C, 55.53; H, 6.62; N, 13.63. Found: C, 55.78; H, 6.73; N, 13.80.

4.3.11 Ethyl 4-(5-anilino-1,3,4-thiadiazol-2-yl)-5-chloro-2-methyl-1-propyl-1H-pyrrole-3-carboxylate (**3 f**). Yield 93 % (1.88 g), white solid, m. p. 147-148 °C; IR (KBr, cm^{-1}): 1728 (C=O), 3283 (N-H); ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 0.93 (3H, t, $J = 7.3$ Hz, NCH₂CH₂CH₃), 1.10 (3H, t, $J = 7.1$ Hz, OCH_2CH_3), 1.29 - 1.39 (2H, m, NCH₂CH₂CH₃), 2.53 (3H, s, CH₃), 4.00 (2H, t, $J = 7.5$ Hz, NCH₂CH₂CH₃), 4.08 (2H, q, $J = 7.0$ Hz, OCH_2CH_3), 7.00 (1H, t, $J = 7.3$ Hz, Ph), 7.35 (2H, t, $J = 7.9$ Hz, Ph), 7.64 (2H, t, $J = 7.8$ Hz, Ph), 10.36 (1H, s, NH). ^{13}C NMR (125.7 MHz, DMSO- d_6) δ (ppm): 11.2, 13.5, 13.9, 19.2, 43.9, 59.4, 109.6, 110.9, 116.7, 117.2 (2C), 121.7, 129.0 (2C), 135.6, 140.6, 149.5, 163.1, 165.0. LCMS $[\text{M}+\text{H}]^+$: 406.0. Anal. Calcd. for $\text{C}_{19}\text{H}_{21}\text{ClN}_4\text{O}_2\text{S}$ (%): C, 56.36; H, 5.23; N, 13.84. Found: C, 56.07; H, 5.18; N, 14.00.

4.3.12 Ethyl 5-chloro-4-{5-[(4-fluorophenyl)amino]-1,3,4-thiadiazol-2-yl}-2-methyl-1-propyl-1H-pyrrole-3-carboxylate (**3 g**). Yield 81 % (1.71 g), white solid, m. p. 142-143 °C; IR (KBr, cm^{-1}): 1730 (C=O), 3282 (N-H); ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 0.89 (3H, t, $J = 7.3$ Hz, NCH₂CH₂CH₃), 1.09 (3H, t, $J = 7.0$ Hz, OCH_2CH_3), 1.62 - 1.69 (2H, m, NCH₂CH₂CH₃), 2.50 (3H, s, CH₃), 3.96 (2H, t, $J = 7.2$ Hz, NCH₂CH₂CH₃), 4.07 (2H, q, $J = 7.0$ Hz, OCH_2CH_3), 7.18 (2H, t, $J = 8.7$ Hz, Ph), 7.69-7.72 (2H, m, Ph). ^{13}C NMR (125.7 MHz, DMSO- d_6) δ (ppm): 10.7, 11.3, 13.9, 22.7, 45.6, 59.4, 109.4, 110.9, 115.4, 115.6, 116.8, 119.2, 135.7, 137.1, 149.6, 156.2, 158.1, 163.1, 165.0. LCMS $[\text{M}+\text{H}]^+$: 424.0. Anal. Calcd. for $\text{C}_{19}\text{H}_{20}\text{ClFN}_4\text{O}_2\text{S}$ (%): C, 53.96; H, 4.77; N, 13.25. Found: C, 54.17; H, 4.88; N, 13.11.

4.3.13 Ethyl 1-butyl-5-chloro-4-[5-(cyclohexylamino)-1,3,4-thiadiazol-2-yl]-2-methyl-1H-pyrrole-3-carboxylate (**3 h**). Yield 86 % (1.83 g), white solid, m. p. 139-140 °C; IR (KBr, cm^{-1}): 1728 (C=O), 3290 (N-H); ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 0.92 (3H, t, $J = 7.3$ Hz, NCH₂CH₂CH₂CH₃), 1.09 (3H, t, $J = 7.2$ Hz, OCH_2CH_3), 1.16 - 1.38 (7H, m, NCH₂CH₂CH₂CH₃ + CH₂ cyclohexyl), 1.53 - 1.76 (5H, m, NCH₂CH₂CH₂CH₃ + CH₂ cyclohexyl), 1.92 - 2.03 (2H, m, CH₂ cyclohexyl), 2.49 (3H, s, CH₃), 3.42 - 3.57 (1H, m, CH cyclohexyl), 3.97 (2H, t, $J = 7.2$ Hz, NCH₂CH₂CH₂CH₃), 4.05 (2H, q, $J = 7.0$ Hz, OCH_2CH_3), 7.65 (1H, d, $J = 7.2$ Hz). ^{13}C NMR (125.7 MHz, DMSO- d_6) δ (ppm): 11.2, 13.5, 13.8, 19.8, 24.9 (2C), 25.3, 31.5 (2C), 32.1, 43.9, 53.5, 59.3, 110.3, 111.0, 116.3, 135.3, 147.1, 163.2, 168.5. LCMS $[\text{M}+\text{H}]^+$: 426.0. Anal. Calcd. for $\text{C}_{20}\text{H}_{29}\text{ClN}_4\text{O}_2\text{S}$ (%): C, 56.52; H, 6.88; N, 13.18. Found: C, 56.72; H, 7.00; N, 13.09.

4.3.14 Ethyl 1-butyl-5-chloro-2-methyl-4-(5-morpholin-4-yl)-1,3,4-thiadiazol-2-yl)-1H-pyrrole-3-carboxylate (**3 i**). Yield 71 % (1.47 g), yellow oil; IR (KBr, cm^{-1}): 1727 (C=O); ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 0.91 (3H, t, $J = 7.3$ Hz, NCH₂CH₂CH₂CH₃), 1.23 (5H, m, OCH_2CH_3 + NCH₂CH₂CH₂CH₃), 1.56 - 1.62 (2H, m, NCH₂CH₂CH₂CH₃), 2.54 (3H, s, CH₃), 3.41 - 3.50 (4H, m, CH₂ morpholin), 3.68 - 3.77 (4H, m, CH₂ morpholin), 3.98 (2H, t, $J = 7.2$ Hz, NCH₂CH₂CH₂CH₃), 4.22 (2H, q, $J = 7.0$ Hz, OCH_2CH_3). ^{13}C NMR (125.7 MHz, DMSO- d_6) δ (ppm): 11.7, 13.5, 14.2, 19.3, 31.4, 43.6, 49.4 (2C), 59.6, 65.2 (2C), 110.7, 111.8, 117.1, 136.1, 147.0, 163.1, 167.9. LCMS $[\text{M}+\text{H}]^+$: 414.0. Anal. Calcd. for $\text{C}_{18}\text{H}_{23}\text{ClN}_4\text{O}_3\text{S}$ (%): C, 52.36; H, 6.10; N, 13.57. Found: C, 52.58; H, 5.99; N, 13.40.

4.3.15 Ethyl 1-butyl-5-chloro-2-methyl-4-{5-[(4-methylphenyl)amino]-1,3,4-thiadiazol-2-yl}-1H-pyrrole-3-carboxylate (3 j). Yield 89 % (1.93 g), white solid, m. p. 134-135 °C; IR (KBr, cm^{-1}): 1726 (C=O), 3283 (N-H); ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ (ppm): 0.92 (3H, t, $J = 7.3$ Hz, $\text{NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 1.10 (3H, t, $J = 7.0$ Hz, OCH_2CH_3), 1.29 - 1.38 (2H, m, $\text{NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 1.58 - 1.66 (2H, m, $\text{NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 2.26 (3H, s, CH_3), 2.52 (3H, s, CH_3), 4.00 (2H, t, $J = 7.3$ Hz, $\text{NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 4.078 (2H, q, $J = 7.0$ Hz, OCH_2CH_3), 7.15 (2H, d, $J = 8.1$ Hz, Ph), 7.53 (2H, d, $J = 8.2$ Hz, Ph). ^{13}C NMR (125.7 MHz, $\text{DMSO}-d_6$) δ (ppm): 11.2, 13.5, 13.9, 19.2, 20.3, 31.40, 43.9, 59.4, 109.6, 110.9, 116.7, 117.4 (2 C), 129.4 (2C), 130.7, 135.6, 138.3, 149.1, 163.1, 165.2. LCMS $[\text{M}+\text{H}]^+$: 434.0. Anal. Calcd. for $\text{C}_{21}\text{H}_{25}\text{ClN}_4\text{O}_2\text{S}$ (%): C, 58.26; H, 5.82; N, 12.94. Found: C, 57.97; H, 5.88; N, 13.11.

4.3.16 Ethyl 4-(5-anilino-1,3,4-thiadiazol-2-yl)-1-benzyl-5-chloro-2-methyl-1H-pyrrole-3-carboxylate (3 k). Yield 92 % (1.88 g), white solid, m. p. 169-170 °C; IR (KBr, cm^{-1}): 1729 (C=O), 3284 (N-H); ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ (ppm): 1.10 (3H, t, $J = 7.1$ Hz, OCH_2CH_3), 2.45 (3H, s, CH_3), 4.09 (2H, q, $J = 7.1$ Hz, OCH_2CH_3), 5.32 (2H, s, CH_2Ph), 6.98 (1H, t, $J = 7.3$ Hz, Ph), 7.27 - 7.38 (5H, m, Ph), 7.35 (2H, t, $J = 7.9$ Hz, Ph), 7.66 (2H, d, $J = 8.3$ Hz, Ph), 10.51 (1H, s, NH). ^{13}C NMR (125.7 MHz, $\text{DMSO}-d_6$) δ (ppm): 11.5, 13.9, 47.2, 59.6, 110.0, 111.5, 117.2, 117.3, 121.7 (2 C), 126.0 (2C), 127.6 (2C), 128.9 (2C), 129.0, 135.9, 136.1, 140.7, 149.3, 163.1, 165.1. LCMS $[\text{M}+\text{H}]^+$: 454.0. Anal. Calcd. for $\text{C}_{23}\text{H}_{21}\text{ClN}_4\text{O}_2\text{S}$ (%): C, 60.99; H, 4.67; N, 12.37. Found: C, 61.27; H, 4.78; N, 12.12.

4.4 Antimicrobial activity

The antimicrobial activity of the synthesized compounds was investigated by the method of nutrient broth microdilution as recommended by EUCAST (European Committee on antimicrobial susceptibility testing)⁴⁸. According to this method, the minimal inhibitory concentration (MIC) was determined as the concentration of every synthesized compound required to suppress the proliferation of the given microbial culture in the multihole microplate. The stock 1000 $\mu\text{g}/\text{mL}$ solution was prepared by dissolving the required amount of a compound in dimethylsulfoxide (DMSO). Further, diluted solutions with the concentrations from 500 to 3.9 $\mu\text{g}/\text{mL}$ (or from 500 to 0.48 $\mu\text{g}/\text{mL}$ in the case of control drugs) were used to find the MIC values. The sensitivity of every microbial culture to every concentration of the synthesized compounds was tested three times. Besides, the control experiments were carried out to check the proliferation of microbes in the clean broth, in the same broth with an admixture of DMSO, and in the broth with DMSO and the control drugs (Decasaneum⁴⁹ and Clotrimazole⁵⁰) (Table 2). The control clear broth remained sterile and transparent (no proliferation of the microbial cultures), while some proliferation of the cultures has been registered in the case of a mixture of DMSO and the broth.

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