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Synthesis and study of biological activity of azometins based on ethyl derivatives 4-acetyl-3,5-dimethyl-1*H*-pyrol-2-carboxylate

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Starting from 4-acetyl-3,5-dimethyl-1*H*-pyrol-2-carboxylate **1**, the derivative of chlorovinylaldehyde 4-[(E)-1-chloro-3-oxoprop-1-enyl]-3,5-dimethyl-1*H*-pyrol-2-carboxylate **2** was synthesized by the Wilsmeier-Haak reaction. Condensation of the aldehyde **2** with aromatic amines (4-substituted anilines and naphthylamine) and with 5-amino-3-(4-bromophenyl(tolyl))-1*H*-pyrazole(s) in absolute diethyl ether at room temperature for several hours afforded a new series of the corresponding azomethines **3**–**10**. The structures of the synthesized compounds were elucidated by ^{1}H NMR and IR spectroscopy and mass spectrometry. It was shown that all the obtained azomethines **3**–**10** were individual substances and not a mixture of isomers. Primary studies were carried out to determine the biological activity of the obtained Schiff bases (compounds **3**–**8**). Antibacterial activity was evaluated on reference test cultures and clinical strains. It was found that the resulting azomethines were effective only against Gram-positive bacteria (not broad spectrum of activity). It was suggested that compounds **5**, **7** have cytotoxic properties and represent a perspective for modification and further investigation of antitumor activity.

Keywords: pyrrole derivatives, 4-acetyl-3,5-dimethyl-1*H*-pyrrole-2-carboxylate, azomethines, β-chlorovinyl aldehydes, 5-aminopyrazoles, pharmacophores, biological activity.

Синтез та дослідження біологічної активності азометинів на основі похідних етил 4-ацетил-3,5-диметил-1*H*-пірол-2-карбоксилату. *О.Й.Міхедькіна, В.В.Анан'єва, О.В.Циганков, Т.П.Осолодченко, С.В.Пономаренко, В.А.Чебанов*

На основі 4-ацетил-3,5-диметил-1*H*-пірол-2-карбоксилату **1** за реакцією Вільсмайра-Хаака синтезовано похідне β-хлорвінілальдегіду і 4-[(E)-1-хлор-3-оксопроп-1-еніл]-3,5-диметил-1*H*-пірол-2-карбоксилат **2**. Конденсацією альдегіду **2** з ароматичними амінами (4-похідні аніліну і нафтиламін) та з 5-аміно-3-(4-бромфеніл(толіл))-1*H*-піразолом(ами) у середовищі абсолютного діетилового ефіру за кімнатної температури впродовж кількох годин отримано нову бібліотеку відповідних азометинів 3–10. Встановлено будову синтезованих сполук за допомогою ЯМР ^{1}Н-, ІЧ- та мас-спектрометрії. Показано, що усі отримані азометини **3**–**10** є індивідуальними речовинами, а не сумішшю ізомерів. Проведено первинні дослідження з визначення біологічної активності отриманих основ Шиффа (сполук **3**–**8**). Оцінено антибактеріальну активність на еталонних тест-культурах та клінічних штамах. Виявлено, що отримані азометини ефективні у відношенні тільки до грампозитивних бактерій (нешироκого спектра дії). Зроблено припущення, що сполуки **5**, **7** мають цитотоксичність та є перспективними для модифікації та подальших досліджень на протипухлинну активність.

На основе 4-ацетил-3,5-диметил-1*H*-пиррол-2-карбоксилата **1** по реакции Вильсмайера-Хаака синтезировано производное β-хлорвинилальдегида-4-[(E)-1-хлор-3-оксопроп-1-енил]-3,5-диметил-1*H*-пиррол-2-карбоксилат **2**. Конденсацией альдегида **2** с ароматическими аминами (4-производные анилина и нафтиламин) и с 5-амино-3-(4-бромфенил (толил))-1*H*-пиразолом(ами) в среде абсолютного диэтилового эфира при комнатной температуре в течение нескольких часов получено новую библиотеку соответствующих азометинов **3**–**10**. Установлено строение синтезированных соединений с помощью ЯМР ^{1}H-, ИК- и масс-спектрометрии. Показано, что все полученные азометины **3**–**10** являются индивидуальными веществами, а не смесью изомеров. Проведены первичные исследования по определению биологической активности полученных оснований Шиффа (соединений **3**–**8**). Оценена антибактериальная активность на эталонных тест-культурах и клинических штаммах. Выявлено, что полученные азометины эффективны по отношению только грамположительных бактерий (неширокого спектра действия). Сделано предположение, что соединения **5**, **7** обладают цитотоксичностью и являются перспективными для модификации и дальнейших исследований на противоопухолевую активность.

1. Introduction

Recently, the objectives of organic chemistry have included not only the directed synthesis of individual substances but also the creation of libraries of structurally complex organic compounds to fill the chemical space and to study it systematically within the concept of the chemistry of molecular diversity and medicinal-oriented synthesis. This is done in particular with the aim of effectively searching for new bioactive substances and new components of functional materials.

Schiff bases (azomethines) are perspective building blocks for the creation of a large number of combinatorial libraries and are also of interest as compounds widely used as dyes, luminophores, components of liquid crystals and optical materials, polymer stabilizers, reagents for the synthesis of vulcanization accelerators [1–5]. It is known that long-chain aromatic azomethines are suitable for the preparation of thermal vacuum deposition films and Langmuir-Blodgett films necessary for the production of nanomaterials [6, 7]. Authors [8–10] presented results of the study on the thermochromic and photochromic effect of azomethines, which allows their use as thermo- and light-sensing nano switches. Moreover, Schiff bases have at least one powerful pharmacophore fragment and are the perspective objects for the study of biological activity for the process of drug discovery [11–13]. Some azomethines are known as antitumor, antiviral, antituberculous, and other biologically active agents [18–21]. On the other hand, the presence of the pyrrole cycle is known to promote the appearance of compounds with antiviral, antituberculous, and antitumor activity [14–16], while the pyrazole fragment, as described in the review [17], is a pharmacologically important moiety that provides a broad spectrum of biological activity.

Combining two active pharmacophores in one structure is a promising approach to achieve improved results in terms of biological activity. Therefore, the introduction of additional pharmacophore fragments into the azomethines can convert them into the perspective drug-like compounds.

Therefore, filling the content of the anatomical-chemical-therapeutic classification (ATC) with novel synthetic druge-like substances containing various combinations of active pharmacophores is certainly one of the important tasks of synthetic organic chemistry and medicinal chemistry. In the future, the obtained experimental results and developed methods can be the basis for creating optimal technological parameters in the production of a new generation of drugs.

Taking into account the mentioned above, one of the actual tasks is the synthesis of imine derivatives of pyrol, for instance, ethyl 4-(1-chloro-3-(4-aryl(heteryl) mino)prop-1-enyl)-3,5-dimethyl-1*H*-pyrol-2-carboxylates and carrying out the primary study of their biological activity using strains of certain types of microorganisms. In addition, such compounds can also be basic functional materials for a wide range of applications.

2. Experimental

^{1}H NMR spectra were recorded on a Varian Mercury VX-200 spectrometer with an operating frequency of 200 MHz and on Varian MR-400 with an operating frequency of 400 MHz (solvents DMSO-d_6 and $CDCl_3$, internal standard — TMS), in all cases δ —

scale of the chemical shifts was used. IR spectra were obtained on SPECORD 75IR spectrophotometer in KBr tablets. Mass spectra were recorded on GS-MS Varian 1200L spectrometers (electron impact ionization, 70 eV, direct entry of the sample into the ionization chamber). The reaction proceeding and purity of the compounds obtained were monitored by TLC on Silufol UV-254 plates (solvents — ethyl acetate, hexane and their mixtures, demonstration in UV and iodine vapor, elluent — mixtures of ethyl acetate and hexane in different ratios).

Ethyl 4-[(E)-1-chloro-3-oxoprop-1-enyl]-3,5-dimethyl-1*H*-pyrol-2-carboxylate **2**. In a 500 ml three-neck flask immersed in a cooling bath (about −10°C) and installed on magnetic stirrer 20,0 ml of $POCl_3$ (0,22 mol) was placed, then 75,0 ml (0,96 mol) of DMF was added dropwise with a rate at which the temperature of the reaction mixture did not rise above 0°C. Then, a solution of 20,0 g (0,10 mol) of acetyl pyrol **1** in 100,0 ml of DMF was added dropwise to the reaction mixture for about 1 h, the temperature in the reaction mixture shall not rise above 0°C. After that, stirring is continued for another 3 h at 40°C. The reaction mixture is neutralized with a saturated solution of sodium acetate (or sodium carbonate) to pH ~ 8. The resulting crystals were filtered and washed with water on the filter, recrystallized from heptane and dried in the air. 19,58 g of product were obtained, 80 % yield, m.p. 120–121°C. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, δ, ppm): 1.36 *t* (3H, $-CH_2-CH_3$, ^3J 8 Hz), 2.37 s (3H, $3-CH_3$), 2.39 s (3H, $5-CH_3$), 4.32 q (2H, $-CH_2-CH_3$, ^3J 8 Hz), 6.09 d (1H, =CH–, ^3J 8 Hz), 9.22 ws (1H, 1H, NH), 10.14 d (1H, C(O)H).

Reaction of ethyl 4-[(E)-1-chloro-3-oxoprop-1-enyl]-3,5-dimethyl-1*H*-pyrol-2-carbo xylate 2 with aromatic amines.

General method. In a 25 ml flask 0,26 g (1,0 mmol) of chlorovinyl aldehyde **2** and 1,0 mmol of the corresponding amine were placed, then 4–6 ml of diethyl ether was added and the flask was installed on a magnetic stirrer at room temperature for about two hours. The resulting precipitate were filtered and washed with diethyl ether on the filter, the reaction was monitored by TLC.

Thus obtained:

Ethyl 4-(1-chloro-3-(4-chlorophenylimino) prop-1-enyl)-3,5-dimethyl-1*H*-pyrol-2-carboxylate 3. Yield 0,30 g (81 %), m.p. 150°C. IR spectrum, ν, cm^{-1}: 760–780 (C–Cl), 1600 (C=C), 1650 (C=N), 1660 (C=O), 3260 (NH). ^{1}H NMR spectrum (200 MHz, DMSO -d_6, δ, ppm): 1.29 t (3H, $-CH_2-CH_3$), 2.31 s (3H, $3-CH_3$),2.33 s (3H, $5-CH_3$), 4.25 q (2H, $-CH_2-CH_3$), 6.55 d (1H, =CH-, ^3J 8 Hz),7.23–7.49 s (4H, C_6H_4), 8.58 d (1H, =CH-, 3*J* 8 Hz), 11.82 s (1H, NH). Obtained, %: C 59.29; H 5.05; N 7.70. m/z 351 (M–15, 37Cl, 66.1 %), 349 (M–15, 35Cl,100 %), 305 (M–15–46, 37Cl, 47.7 %), 303 (M–15–46, 35Cl, 55.3 %), 166 (34.3 %),138 (17.9 %). $C_{18}H_{18}Cl_2N_2O_2$. Calculated, %: C 59.19; H 4.97; N 7.67. M 365.25.

Ethyl 4-(3-(4-bromophenylimino)-1-chloroprop-1-enyl)-3,5-dimethyl-1*H*-pyrol-2 carboxylate **4**. Yield 0.33 g (80 %), m.p. 154°C IR spectrum, ν, cm^{-1}: 760–780 (C–Cl), 1600 (C=C), 1650 (C=N), 1660 (C=O), 3260 (NH). ^{1}H NMR spectrum (200 MHz, DMSO -d_6, δ, ppm): 1.29 t (3H, $-CH_2-CH_3$), 2.31 s (3H, $3-CH_3$), 2.33 s (3H, $5-CH_3$), 4.25 q (2H, $-CH_2-CH_3$), 6.55 d (1H, =CH-, ^3J 8 Hz), 7.17–7.63 s (4H, C_6H_4), 8.57 d (1H, =CH-, ^3J 8 Hz), 11.82 s (1H, NH). Obtained, %: C 52.88; H 4.49; N 6.89. m/z 395 (M–15, 37Cl, 23.3 %), 393 (M–15, 35Cl, 4517.5 %), 349 (M–15–46, 37Cl, 23.3 %), 347 (M–15–46, 35Cl, 19.4 %). $C_{18}H_{18}BrClN_2O_2$. Calculated, %: C 52.77; H 4.43; N 6.84. M 409.70.

Ethyl 4-(1-chloro-3-(4-methoxyphenylimino) prop-1-enyl)-3,5-dimethyl-1*H*-pyrol-2-carboxylate **5**. Yield 0,27 g (74 %), m.p. 142–143°C. IR spectrum, ν, cm^{-1}: 760–780 (C–Cl), 1600 (C=C), 1650 (C=N), 1660 (C=O), 2900–3000 (C–O), 3260 (NH). ^{1}H NMR spectrum (400 MHz, DMSO -d_6, δ, ppm): 1.26 t (3H, $-CH_2-CH_3$), 2.27 s (3H, $3-CH_3$), 2.29 s (3H, $5-CH_3$), 3.74 s (3H, OCH_3), 4.21 q (2H, $-CH_2-CH_3$), 6.48 d (1H, =CH-, ^3J 8 Hz), 6.93 d (2H, C_6H_4), 7.22 d (4H, C_6H_4), 8.56 d (1H, =CH-, ^3J 8 Hz), 11.73 s (1H, NH). Obtained, %: C 63.32; H 5.96; N 7.81. m/z 347 (M–15, 37Cl, 11.0 %), 345 (M–15, 35Cl, 100 %), 301 (M–15–46, 37Cl, 41.8 %), 299 (M–15–, 35Cl, 75.0 %), 159 (5.1 %). $C_{19}H_{21}ClN_2O_3$. Calculated, %: C 63.24; H 5.87; N 7.76. M 360.83.

Ethyl 4-(3-(4-acetylphenylimino)-1-chloroprop-1-enyl)-3,5-dimethyl-1*H*-pyrol-2- carboxylate **6**. Yield 0.36 g (96 %), m.p. 137°C. IR spectrum, ν, cm^{-1}: 760–780 (C–Cl), 1580 (C=C), 1650 (C=N), 1660 (C=O), 1680 (C=O),3260 (NH). ^{1}H NMR spectrum

(200 MHz, DMSO - d_6, δ, ppm): 1.29 t (3H, $-CH_2-CH_3$), 2.33 s (3H, 3–CH_3), 2.35 s (3H, 5–CH_3), 2.58 s (3H, $COCH_3$), 4.25 q (2H, $-CH_2-CH_3$), 6.60 d (1H, =CH-, ^3J 8 Hz), 7.271968.06 m (4H, C_6H_4), 8.58 d (1H, =CH-, ^3J 8 Hz), 11.85 s (1H, NH). Obtained, %: C 64.53; H 5.76; N 7.55. m/z 359 (M–15, 37Cl, 26.2 %), 357 (M–15, 35Cl, 61.0 %), 313 (M–15–46, 37Cl, 38.5 %), 311 (M–15–46, 35Cl, 100 %), 146 (9.4 %). $C_{20}H_{21}ClN_2O_3$. Calculated, %: C 64.43; H 5.68; N 7.51. M 372.85.

Ethyl 4-(1-chloro-3-(4-nitrophenylimino) prop-1-enyl)-3,5-dimethyl-1*H*-pyrol-2-carboxylate **7**. Yield 0.33 g (89 %), m.p. 228°C. IR spectrum, ν, cm^{-1}: 760–780 (C–Cl), 1310, 1560 (NO_2), 1590 (C=C), 1650 (C=N),1660 (C=O), 3260 (NH). ^{1}H NMR spectrum (200 MHz, DMSO -d_6, δ, ppm): 1.30 t (3H, $-CH_2-CH_3$), 2.31 s (3H, 3–CH_3), 2.33 s (3H, 5–CH_3), 4.25 q (2H, $-CH_2-CH_3$), 6.62 d (1H, =CH–, ^3J 8 Hz), 7.35–8.37 m (4H, C_6H_4), 8.59 d (1H, =CH–, ^3J 8 Hz), 11.82 s (1H, NH). Obtained, %: C 57.62; H 4.94; N 11.27 m/z 220 (100 %), 174 (62.2 %). $C_{18}H_{18}ClN_3O_4$. Calculated, %: C 57.53; H 4.83; N 11.18. M 375.81.

Ethyl 4-(1-chloro-3-(naphthalene-1-ylimino) prop-1-enyl)-3,5-dimethyl-1*H*-pyrol-2-carboxylate **8**. Yield 0,32 g (85 %), m.p. 152°C. IR spectrum, ν, cm^{-1}: 760–780 (C–Cl), 1590 (C=C), 1640 (C=N), 1650 (C=O), 3260 (NH). ^{1}H NMR spectrum (200 MHz, DMSO -d_6, δ, ppm): 1.29 t (3H, $-CH_2-CH_3$), 2.34 s (3H, 3–CH_3), 2.36 s (3H, 5–CH_3), 4.24 q (2H, $-CH_2-CH_3$), 6.71 d (1H, =CH–, ^3J 8 Hz), 6.81–8.27 m (7H, Napht), 8.65 d (1H, =CH–, ^3J 8 Hz), 11.82 s (1H, NH). Obtained, %: C 69.33; H 5.66; N 7.43. m/z 367 (M–15, 37Cl, 42.0 %), 365 (M–15, 35Cl, 85.9 %), 321 (M–15–46, 37Cl, 50.6 %), 319 (M–15–46, 35Cl, 100 %), 293 (M–15–74, 37Cl, 2.2 %), 291 (M–15–74, 35Cl, 7.9 %), 154 (11.6 %). $C_{22}H_{21}ClN_2O_2$. Calculated, %: C 69.38; H 5.56; N 7.36. M 380.87.

Reaction of ethyl 4-(E)-1-chloro-3-oxo-prop-1-enyl)-3,5-dimethyl-1*H*-pyrol-2-carboxylate **2** with 5-amino-3-(4-bromophenyl)-1*H*-pyrazole. In a solution of 0,050 g (0,2 mmol) of **2** in 5 ml of absolute diethyl ether at 15–17°C was added 0,048 g (0.2 mmol) of the corresponding 5-aminopyrazole; the reaction mixture was stirred on a magnetic stirrer for about two hours. The resulting precipitate was filtered and washed on the filter with 1 ml of diethyl ether. 0.076 g of azomethine 9 was obtained, yield 81 %, m.p. 181°C (decomp.), ^{1}H NMR spectrum (400 MHz, DMSO-d_6, δ, ppm): 1.26 t (3H, $-OCH_2-CH_3$), 2.28 s (3H, 3–CH_3), 2.31 s (3H, 5–CH_3), 4.21 q (2H, $-OCH_2-CH_3$), 6.49 d (1H, C(Cl)=CH–, *J* 8 Hz), 6.93 s (1H, Pyrazol-H), 7.60 d.–7.74 d (4H, C_6H_4), 8.83 d (1H, N=CH–, ^3J 8 Hz), 11.78 s (1H, Pyrrole-NH).

Reaction of ethyl 4-[(E)-1-chloro-3-oxo-prop-1-enyl]-3,5-dimethyl-1*H*-pyrol-2-carbo xylate **2** with 5-amino-3-(4-tolyl)-1*H*-pyrazole. In a solution of 0,050 (0,2 mmol) g of **2** in 5 ml of absolute diethyl ether at 15–17°C was added 0,035 g (0.2 mmol) of the corresponding 5-aminopyrazole; the reaction mixture was stirred on a magnetic stirrer for about two hours. The resulting precipitate was filtered and washed on the filter with 1 ml of diethyl ether. 0,076 g of azomethine 10 was obtained, yield 88 %, m.p. 171°C (decomp.), ^{1}H NMR spectrum (200 MHz, $CDCl_3$, δ, ppm): 1.36 t (3H, $-OCH_2-CH_3$), 2.36 s (3H, 4–$CH_3C_6H_4$), 2.37 s (3H, 3–CH_3), 2.39 s (3H, 5–CH_3), 4.32 q (2H, $-OCH_2-CH_3$), 6.48 d (1H, C(Cl)=CH–, ^3J 8 Hz), 6.57 s (1H, Pyrazol-H), 7.58–7.24 d (4H, C_6H_4), 8.91 d (1H, N=CH–, ^3J 8 Hz), 9.02 s (1H, Pyrazol-NH).

To study the biological activity of alcohol solutions of ethyl 4-acetyl-3,5-dimethyl-1*H*-pyrol-2-carboxylate derivatives, reference tests of Gram-positive and Gram-negative bacteria were used, which belong to various taxonomic groups [22]: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633, *Proteus vulgaris* ATCC 4636. The antifungal effects of the solutions were studied on reference strain of Candida albicans ATCC 885-653. The labeled set of test strains is commonly used in the primary studies of antimicrobial activity.

The characteristics of the clinical strains used in the studies are listed below:

Staphylococcus aureus No. 24 — clinical strain isolated from patient G. 55 years old from nasal sinus mucosa, diagnosis is chronic sinusitis. The strain is sensitive to ceftriaxone, fortum, cefoperazone, cefepime, azithromycin, gatifloxacin, levofloxacin; weakly sensitive to amoxiclave, 1–2 generation cephalosporins, chlorophilipt. Highly adhesive. Lecithinazopositive, coagulazopositive.

Staphylococcuc saureus No. 38 — clinical strain isolated from patient P. 46 years old from oral mucosa (migdalin), diagnosis is follicular angina. The strain is sensitive to ceftriaxone, fortum, cefoperazone, ce-

fepime, azithromycin, gatifloxacin, levofloxacin; weakly sensitive to 1–2 generation cephalosporins, chlorophilipt. Medium adhesive. Lecithinazopositive, coagulazopositive.

Escherichia coli No. 25 — clinical strain isolated from patient T. 85 years old from ears canals, diagnosis is acute otitis. The strain is sensitive to ceftriaxone, Fortum, cefoperazone, cefepim, gatifloxacin, levofloxacin, ciprofloxacin, phosphomycin, furamag; weakly sensitive to 1–2 generation cephalosporins, ofloxacin, furazolidone, nitroxalin. Medium adhesive. Lecithinazopositive, coagulazopositive.

Pseudomonas aeruginosa No. 16 — clinical strain isolated from patient P. 68 years old from the contents of the skin wound, diagnosis hand injury with deep cut of the soft tissues. The strain is sensitive to cephaperazone, gatifloxacin; not sensitive to 1–2 generation of cephalosporins and fluoroquinolones, gentamicin, furazolidone, nitroxaline. Very adhesive. High membrane-forming capacity.

Pseudomonas aeruginosa No. 89 — clinical strain isolated from patient D. 78 years old from urine, diagnosis is pyelonephritis. The strain is sensitive to cephaperazone, gatifloxacin; not sensitive to 1–2 generation of cephalosporins and fluoroquinolones, gentamicin, furazolidone, nitroxaline. Very adhesive. High membrane-forming ability.

Candida albicans No 23 — clinical strain isolated from patient M. 27 years old from vaginal secretion, diagnosis is bacterial vaginitis. The strain is sensitive to fluconazole, orungal; weakly sensitive to nisoral, nistatin. Medium adhesive.

All test cultures were obtained from the laboratory of medical microbiology with the Museum of Microorganisms of the State Institution "Mechnikov Institute of Microbiology and Immunology of the National Academy of Medical Sciences of Ukraine" (Kharkiv). Agents for cultivation were applied to the type of microorganisms according to the existing methodological developments and recommendations.

Microorganism suspensions with a specific concentration of microbial cells (optical density) were prepared using a turbidity standard (0.5 units on a McFarland scale). Densi-La-Meter (manufactured by PLIVA-Lachema, Czech Republic; wavelength 540 nm) was used. The suspension was prepared in accordance with the instructions for the instrument and "Standardization of Microbial Suspension Preparation" (No. 163–2006), Kyiv. Cultures were synchronized by low temperature (4°C).

The sensitivity of microbial strains to antibacterial drugs was determined according to [23] using the method of wells on Muller-Hinton reagent ("HIMediaLaboratorles" Pvt. Ltd.India). The reagent was prepared according to the manufacturer's instructions. The sensitivity of the fungi was determined on the saburo-dextrose agar reagent. The sensitivity of the study substances was determined on two layers of nutrient reagent poured on the Petri dish. The bottom layer consisted of agar-agar (10 ml). On it, 3–6 sterile metal cylinders with a diameter of 8 mm and a height of 10 mm were placed. A top layer (14 ml of culture reagent + 1 ml of a 0.5 unit microbial solution on a McFarland scale) was poured around the cylinders, which consisted of nutrient agar reagent with an appropriate daily microorganism culture standard. After gelling with sterile forceps, the wells were removed and mixed with the solution of the substance under study (0.3 ml).

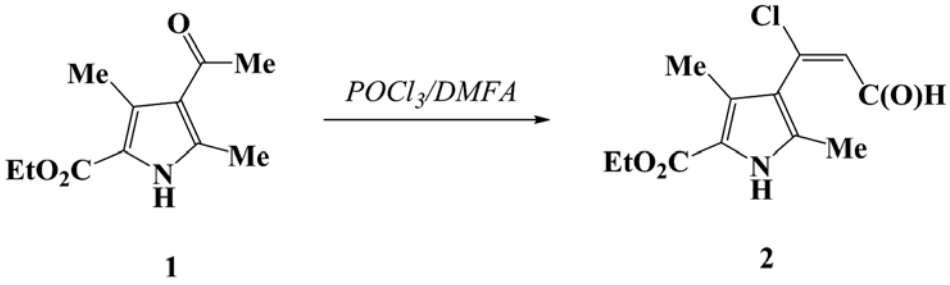
The evaluation of antibacterial activity of the studying substances was carried out by diameter of growth delay zones: 10 mm — microorganism is not sensitive to the studying substance; 10–15 mm — microorganism is weakly sensitive to the studying substance; 15–25 mm — microorganism is sensitive to the studying substance; 25 mm above — microorganism is very sensitive to the studying substance.

3. Results and discussion

Using the Wilsmeier-Haak reaction according to the known method [24], we converted ethyl 4-acetyl-3,5-dimethyl-1*H*-pyrol-2-carboxylate 1 (Fig. 1) into the corresponding derivative of β-chlorovinylaldehyde — ethyl 4-[(E)-1-chloro-3-oxoprop-1-enyl]-3,5-dimethyl-1*H*-pyrol-2-carboxylate **2**.

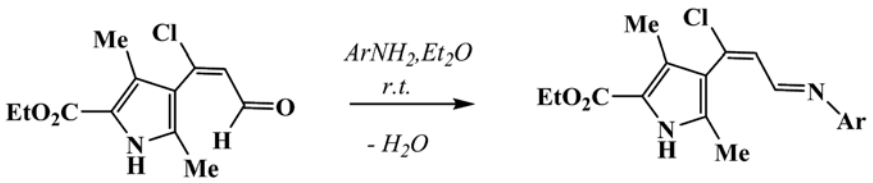
Furthermore, we studied the reaction of β-chlorovinylaldehyde **2** with some aromatic amines (Fig. 2). It was found that the reaction of aldehyde **2** with an equimolar amount of aniline derivatives such as *p*-chloro-, *p*-bromo-, *p*-methoxy-, *p*-acetyl-, *p*-nitroaniline as well as with α-naphthylamine in absolute diethyl ether at room temperature for a few hours led to the formation of the corresponding azomethines **3**–**8** in good-to-excellent yields (74–98 %).

It is known [25] that the reactions of β-chlorovinylaldehydes with aromatic amines can proceed in two ways: nucleophilic addition-elimination to form



1 2

Fig. 1. Synthesis scheme for ethyl 4-[(E)-1-chloro-3-oxoprop-1-enyl]-3,5-dimethyl-1*H*-pyrol-2-carboxylate 2.



2 **3-8** *74-98%*

Ar= 4-Cl-C_6H_4 (**3**), 4-Br-C_6H_4 (**4**), 4-OMe-C_6H_4 (**5**), 4-Ac-C_6H_4 (**6**), 4-NO_2-C_6H_4 (**7**), 1-Naft (**8**)

Fig. 2. Synthesis scheme of ethyl 4-(1-chloro-3-(4-arylimino)prop-1-enyl)-3,5-dimethyl-1*H*-pyrol-2-carboxylate 3-8.

azomethines (Schiff bases) or an attack of the nucleophile simultaneously towards two electrophilic centers — at the carbon atom of the carbonyl group and at the carbon atom of the C–Cl bond to form imin-enamines hydrochlorides, which can further cyclize to quinolines under reaction conditions. In our case, however, even a fourfold increase in the amount of arylamine led only to the isolation of azomethines **3**–**8** (see Fig. 2.); other theoretically possible reaction products were not formed according to thin-layer chromatography (TLC). The excess of arylamine remaining in the reaction mixture was easily separated from the product by recrystallization from ethanol.

The structure of azomethines **3**–**8** was elucidated by ^{1}H NMR and IR spectroscopy and mass-spectrometry. According to TLC and ^{1}H NMR spectra, compounds **3**–**8** are individual isomers (Fig. 3). In the ^{1}H NMR spectra, signals for two protons of the olefin and azomethine fragments are observed as doublets in the range of 6.48–6.71 ppm and 8.57–8.65 ppm, $^3J \approx 8$ Hz; signals for the aromatic protons are observed in the range of 6.81–8.37 ppm; all necessary signals for the protons of the pyrrole fragment are also shown.

We assume that azomethines **3**–**8** most likely have an E-configuration of the C=N bond, as evidenced by the presence of the doublet of the azomethine proton in the 8.57–8.65 ppm range. The signals of similar protons in the spectra of Z-isomers should shift to a weaker field by about ~ 0.5 ppm according to the authors [26, 27] and with spectroscopic studies [28], which is due to their location in the area of deshileding by the aromatic core [26]. The constants of spin-spin interaction for these protons (~ 8 Hz) indicate the possibility of the preferential existence of C=C-C=N fragment of the compounds obtained in **s-cis** conformation [29].

In the IR spectra of compounds **3**–**8**, the band of valence oscillations of the aldehyde group 1680 cm^{-1} which is characteristic for the starting aldehyde **2** disappears, while the band of valence oscillations of C=N appears. In the mass-spectra of azomethines **3**–**8**, the most intense ion is $[M-15]^+$ which corresponds to the fragmentation of the molecule by the elimination of one of the methyl groups at positions 3 or 5 of the pyrrole cycle, and the ion $[M-15-46]^+$, which appears upon the elimination of the ethanol molecule.

Compounds **3**–**8** are sufficiently stable — all attempts to obtain quinoline derivatives gave the compounds unchanged on prolonged heating in high boiling solvents such

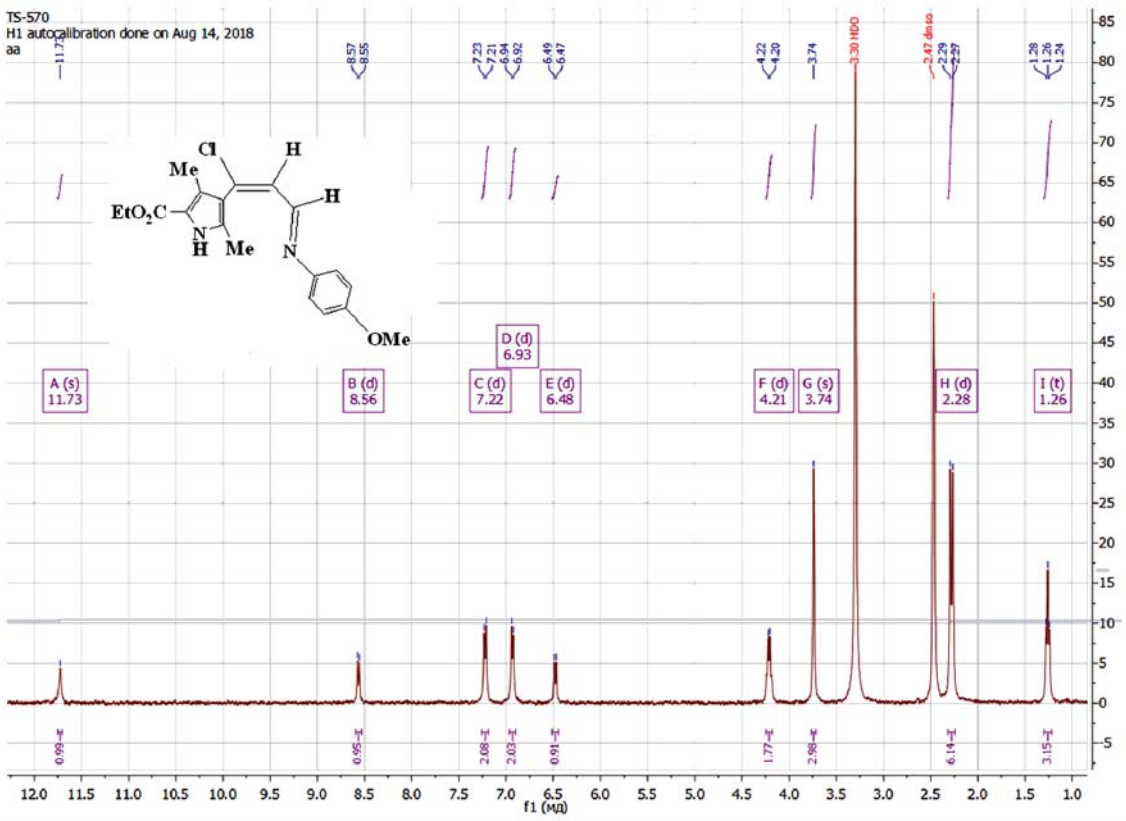


Fig. 3. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) ethyl 4-(1-chloro-3-(4-methoxyphenylimino)prop-1-enyl)-3,5-dimethyl-1*H*-pyrol-2-carboxylate **5**.

as toluene, xylene, dimethylaniline, triethylamine or pyridine.

It was found that the reaction of aldehyde **2** with 5-amino-3-(4-bromophenyl)-1*H*-pyrazole and 5-amino-3-(4-tolyl)-1*H*-pyrazole in absolute diethyl ether at room temperature led to the formation of ethyl 4-(3-(4-bromophenyl)-1*H*-pyrazol-5-yl)imino)-1-chloroprop-1-enyl)-3,5-dimethyl-1*H*-pyr role-2-carboxylate 9 and ethyl 4-(3-(4-tolyl)-1*H*-pyrazol-5-yl)imino)-1-chloroprop-1-enyl) -3,5-dimethyl-1*H*-pyrrole-2-carboxylate **10** in good yields (Fig. 4).

According to TLC and ^{1}H NMR spectra azometines **9** and **10** are individual isomers (Fig. 5). In their ^{1}H NMR spectra in the range 8.8–8.9 ppm the doublet for the azomethine bond proton HC=N is observed, while the doublet of the aldehyde proton (at 10.0 ppm) disappears.

Study of biological activity

The molecules of compounds **3**–**8** have the pyrrole and azomethine fragments in their structure, which may imply the presence of certain types of biological activity. Therefore, we carried out the primary study of the biological activity of azomethines **3**–**8** with strains of some types of microorganisms.

The analysis of the results of the study of antibacterial activity of ethyl-4-acetyl-3,5-dimethyl-1*H*-pyrol-2-carboxylates **3**–**8** on the reference tests and clinical strains (Table 1, 2) showed that all Gram-positive bacteria are very sensitive to compounds **5**, **7** and sensitive to compounds **3**, **4**, **8**. There is no sensitivity of bacteria to compound **6**. The results obtained regarding antibacterial activity are preliminary and require further careful studies in the field. It should also be noted that compounds **5** and **7** are more toxic than the others tested and the biological experiments show that they are more effective than other azomethines in suppressing the growth of microorganisms. We hypothesized that these compounds have cytotoxic properties and are a prospect for further modification and research of just this type of activity.

4. Conclusions

Thus, the reaction of ethyl 4-[(E)-1-chloro-3-oxoprop-1-enyl]-3,5-dimethyl-1*H*-pyrol-2-carboxylate **2** with anilines and 5-

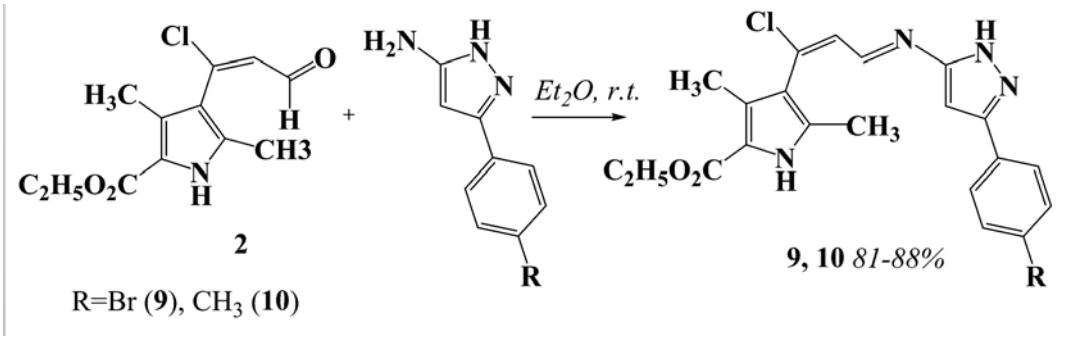


Fig. 4. Scheme of synthesis of azomethines 9 and 10.

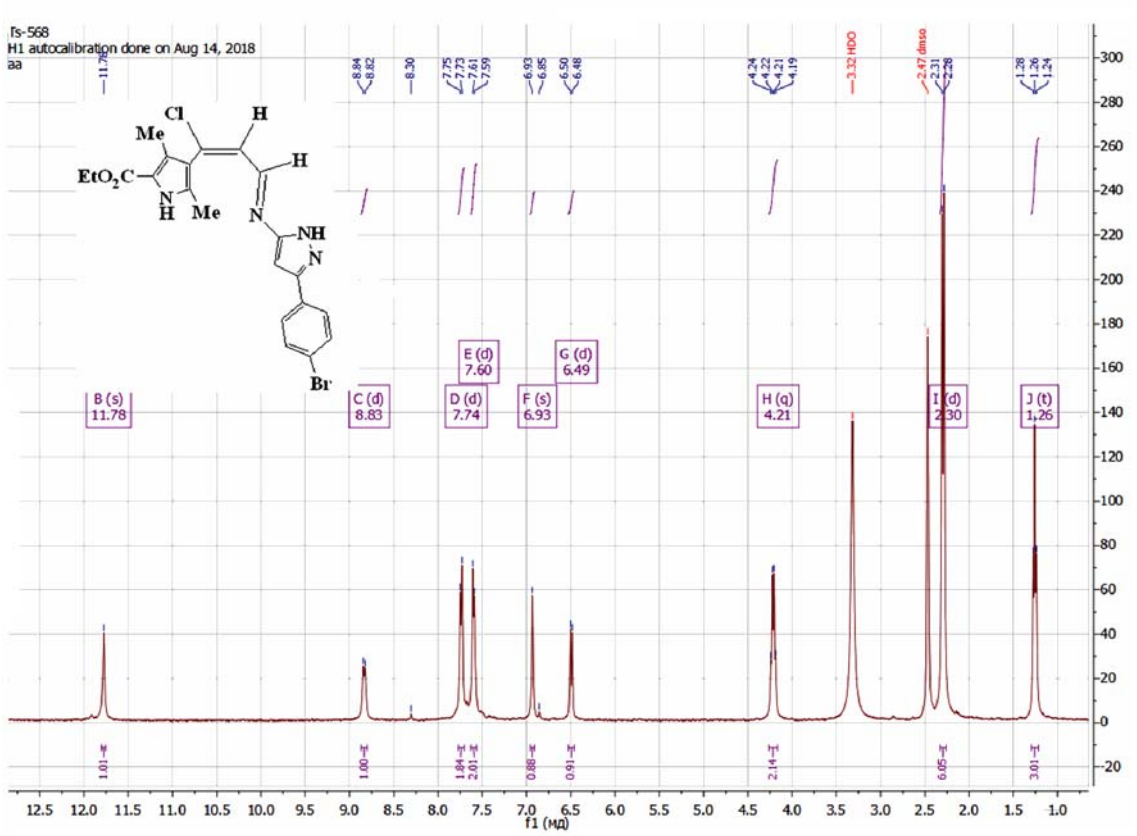


Fig. 5. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) ethyl 4-(3-(4-bromophenyl)-1*H*-pyrazol-5-yl)imino)-1-chloroprop-1-enyl)-3,5-dimethyl-1*H*-pyrol-2-carboxylate **9**.

aminopyrazoles without catalysts at room temperature yielded a new series of azomethines. The structure of all the obtained compounds was elucidated by a complex of spectral methods. The biological activity of some synthesized compounds was studied and their efficiency with respect to Gram-positive bacteria was observed. It was suggested that further modification of two compounds and study of their cytotoxic activity (antitumor activity) are the perspective objects.

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Table 1. Antibacterial activity of ethyl 4-acetyl-3,5-dimethyl-1*H*-pyrol-2-carboxylate derivatives (on reference tests)

Azomethine	Diameters of growth delay zones in mm (M±m) (p≤0.05)					
	Staphylococcus aureus ATCC 25923	Escherichia coli ATCC 25922	Proteus vulgaris ATCC 4636	Pseudomonas aeruginosa ATCC 27853	Basillus subtilis ATCC 6633	Candida albicans ATCC 653/885 (p.sh.)
3	22.5±0.17	15.3±0.16	14.3±0.15	15±0.15	16.7±0.15	14±0.15
4	15±0.15	13±0.16	12±0.15	13.7±0.17	17±0.16	15.3±0,18
5	28,7±0.15	19,3±0.19	18±0.17	19,3±0.16	29.6±0.18	22.7±0.17
6	19.3±0.16	growth	growth	growth	16±0.07	16±0.07
7	29±0.21	19±0.15	16.7±0.16	20±0.16	29.7±0.19	21.7±0.19
8	15.3±0.16	13.3±0.05	12.3±0.16	14±0.18	17±0.19	15,3±0.17

Table 2. Antibacterial activity of ethyl 4-acetyl-3,5-dimethyl-1*H*-pyrol-2-carboxylate derivatives (on clinical strains)

Azomethine	Diameters of growth delay zones in mm (M±m) (p≤0.05)					
	Staphylococcus aureus 24	Staphylococcus aureus 38	Escherichia coli 25	Pseudomonas aeruginosa 16	Pseudomonas aeruginosa 89	Candida albicans 23
3	19.7±0.29	15.7±0.16	15±0.05	growth	growth	growth
4	21.7±0.21	21.3±0.25	13±0.16	growth	growth	growth
5	23.7±0.24	18.3±0.13	15.3±0.16	growth	16.3+–*BR RA* 0.11	15.7±0.16
6	19.3±0.16	growth	growth	growth	16±0.07	16±0.07
7	24.7±0.27	17.3±0.18	14.7±0.17	growth	16.3+–*BR RA* 0.11	15.7±0.16
8	20.3±0.27	18.7±0.18	14.7±0.07	growth	17.3+–*BR RA* 0.21	17.3±0.18

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