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# Synthesis and the antimicrobial activity of hexamethylene-N-maleinimidospiroindole-3,3'-pyrrolo[3,4-*c*]pyrrole derivatives

**Aim**. To synthesize a series of hexamethylene-N-maleinimidospiroindole-3,3'-pyrrolo[3,4-*c*]pyrrole derivatives, study the antimicrobial activity of the compounds synthesized and compare their antimicrobial activity with the antimicrobial activity of the *bis*-analogs previously synthesized.

**Materials and methods.** The methods of organic synthesis, instrumental methods for determination of the molecular structure of organic compounds, agar well diffusion method were used.

**Experimental part.** The interaction of isatins with α-amino acids and 1,6-bismaleinimidohexane in the equimolar ratio led to formation of 1′-(hexamethylene-N-maleinimido)-2a′,5a′-dihydro-1′*H*-spiroindol-3,3′-pyrrolo[3,4-*c*]pyrrol-2,2′,6′(1*H*,3′*H*,5′*H*)-trion derivatives. The structure of the compounds synthesized was reliably proven by the instrumental methods. Data of the microbiological screening showed a high level of the antimicrobial activity against *Staphylococcus aureus* and *Candida albicans* fungi.

**Conclusions.** It has been determined that the three-component condensation reaction of isatins with α-amino acids and 1,6-bismaleinimidohexane in the equimolar ratio is an efficient synthetic method of 1′-(hexamethylene-N-maleinimido)-2a′,5a′-dihydro-1′*H*-spiroindol-3,3′-pyrrolo[3,4-*c*]pyrrol-2,2′,6′(1*H*,3′*H*,5′*H*)-trion derivatives, which reveal a high level of the antimicrobial activity against *Staphylococcus aureus* and *Candida albicans* fungi. 1'-(Hexamethylene-*N*-maleinimido)-5'-methyl-2a',5a'-dihydro-1'*H*-spiroindol-3,3'-pyrrolo[3,4-*c*]pyrrol-2,2',6'(1*H*,3'*H*,5'*H*)-trione has shown the highest antimicrobial activity among derivatives of hexamethylene-N-maleinimidospiroindol-3,3'-pyrrolo[3,4-*c*]pyrrols.

***Key words:*** isatin; α-amino acids; bis-maleimide; spiro-2-oxindole; antimicrobial activity

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**Синтез і антимікробна активність гексаметилен-N-малеїнімідопохідних спіроіндол-3,3'-піроло[3,4-*c*]піролу**

**Мета роботи** – синтез ряду похідних гексаметилен-N-малеїнімідо-спіроіндол-3,3'-піроло[3,4*c*]піролу, дослідження та порівняння їх антибактеріальної активності з антимікробною дією раніше синтезованих *біс*-аналогів.

**Матеріали та методи**. Методи органічного синтезу, інструментальні методи встановлення будови органічних сполук, метод дифузії в агар у модифікації колодязів.

**Експериментальна частина.** При взаємодії еквімолярного співвідношення ізатинів, α-амінокислот і 1,6-бісмалеїнімідогексану було отримано ряд похідних 1'-(гексаметилен-*N*-малеїнімідо)-2a',5a'-дигідро-1'*H*-спіроіндол-3,3'-піроло[3,4-*c*]пірол-2,2',6'(1*H*,3'*H*,5'*H*)-триону. Будову одержаних сполук надійно підтверджено інструментальними методами. Дані мікробіологічного скринінгу показують високу біологічну дію синтезованих сполук відносно грампозитивних бактерій *Staphylococcus aureus* і грибів *Candida albicans*.

**Висновки.** Встановлено, що реакція трикомпонентної конденсації при еквімолярному використанні ізатинів, α-амінокислот і 1,6-бісмалеїнімідогексану є ефективним методом синтезу 1'-(гексаметилен-*N*-малеїнімідо)-2a',5a'-дигідро-1'*H*-спіроіндол-3,3'-піроло[3,4-*c*]пірол-2,2',6' (1*H*,3'*H*,5'*H*)-тріонів, які проявляють високу біологічну дію відносно грампозитивних бактерій *Staphylococcus aureus* і грибів *Candida albicans.* Найбільшу активність серед похідних гексаметилен-N-малеїнімідо-спіроіндол-3,3'-піроло[3,4-*c*]піролу проявив 1'-(гексаметилен-*N*-малеїнімідо)-5'-метил-2a',5a'-дигідро-1'*H*-спіроіндол-3,3'-піроло[3,4-*c*]пірол-2,2',6'(1*H*,3'*H*, 5'*H*)-трион.

***Ключові слова:*** ізатин; α-амінокислоти; біс-малеїнімід; спіро-2-оксіндол; антимікробна активність

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**Синтез и антимикробная активность гексаметилен-N-малеинимидопроизводных спироиндол-3,3'-пирроло[3,4-*c*]пиррола**

**Цель работы** – синтез ряда производных гексаметилен-N-малеин-имидоспироиндол-3,3'-пирроло[3,4-*c*]пиррола, исследование и сравнение их антибактериальной активности с антимикробным действием ранее синтезированных *бис*-аналогов.

**Материалы и методы**. Методы органического синтеза, инструментальные методы определения структуры органических соединений, метод диффузии в агар в модификации колодцев.

**Экспериментальная часть.** При взаимодействии эквимолярного соотношения изатинов, α-аминокислот и 1,6-бисмалеинимидогексана был получен ряд производных 1'-(гексаметилен-*N*-малеинимидо)-2a',5a'-дигидро-1'*H*-спироиндол-3,3'-пирроло[3,4-*c*]пиррол-2,2',6'(1*H*,3'*H*,5'*H*)-триона. Структура полученных соединений достоверно доказана инструментальными методами. Данные микробиологического скрининга показывают выраженное биологическое действие синтезированных соединений относительно грамположительных бактерий *Staphylococcus aureus* и грибов *Candida albicans*.

**Выводы.** Установлено, что реакция трехкомпонентной конденсации при эквимолярном использовании изатинов, α-аминокислот и 1,6-бисмалеинимидогексана является эффективным методом синтеза 1'-(гексаметилен-*N*-малеинимидо)-2a',5a'-дигидро-1'*H*-спироиндол-3,3'-пирроло[3,4-*c*]пиррол-2,2',6'(1*H*,3'*H*,5'*H*)-трионов, которые проявляют выраженное биологическое действие относительно грамположительных бактерий *Staphylococcus aureus* и грибов *Candida albicans.* Наибольшую активность среди производных гексаметилен-N-малеинимидоспироиндол-3,3'-пирроло[3,4-c]пиррола проявил 1'-(гексаметилен-*N*-малеинимидо)-5'-метил-2a',5a'-дигидро-1'*H*-спироиндол-3,3'-пирроло[3,4-*c*]пиррол-2,2',6'(1*H*,3'*H*,5'*H*)-трион.

***Ключевые слова:*** изатин; α-аминокислоты; бис-малеинимид; спиро-2-оксиндол; антимикробная активность

Spirooxindoles are very attractive group of organic compounds for scientists working in the area of organic, medicinal chemistry and chemistry of natural compounds since their natural and synthetic derivatives reveal a broad spectrum of biological activities [1]. The spirooxindole core is a base of many alkaloids, such as horsfiline, helsemin, pteropodine, spirotriprostatines A, B, etc., showing the antibacterial, anticancer, anti-inflammatory activity [2-5]. A lot of synthetic spirooxindoles are known as antiviral agents and also as cholinesterase, protease and kinase inhibitors [6-9] (Fig. 1).

Thus, the search of new antimicrobial agents among spiro[2-oxindole-3,1'(3')-pyrrole] derivatives is a promising route for creation of drugs possessing such type of bioactivity. With this purpose the synthesis of hexamethylene-N,N'-bis-spirooxindole-3,1'-pyrrolo[3,4-*c*]pyrrole derivatives [8] and spiroindole-3,3'-pyrrolo[3,4-*c*]pyrroles containing the maleinimide linker was carried out. To reveal the pharmacological potential the antimicrobial activity of the compounds synthesized against standard strains of microbes were studied. A comparative analysis of the antimicrobial activity for two groups of these compounds was conducted.

## Materials and methods

The starting isatins and α-amino acids were obtained from commercial sources and used without further purification. Melting points were obtained using a Gallenkamp melting point apparatus, model MFB-595 in open capillary tubes. The 1H NMR spectra were recorded on a Varian WXR (400 MHz) spectrometer using DMSO-d6 as a solvent and TMS as an internal standard (the chemical shift in ppm). Data for chromato-mass spectrometry were obtained on an Agilent 1100 HPLC device. Mass-spectra were taken using a Varian 1200L DIP (EI, 70 eV) device. The elemental analysis was performed using a Carlo Erba CHNS-O EA 1108 analyser.

1,6-Bismaleinimidohexane **1** was synthesized by the method described in the work [10].

**The general procedure for the synthesis of 1'-(hexamethylene-*N*-maleinimido)-2a',5a'-dihydro-1'*H*-spiriindole-3,3'-pyrrolo[3,4-*c*]pyrrole-2,2',6'(1*H*,3'*H*,5'*H*)-triones (4.1-4.9)**

Dissolve the mixture of the corresponding isatins **3.1-3.3** (1 mmol), an α-amino acids **2.1-2.7** (1 mmol) and 1,6-bismaleinimidohexane **1** (1 mmol) in the mixture of *i*-PrOH (3 ml)/$H_2O$ (1 ml) and reflux for 2 h. The reaction was monitored by the change of the mixture color from bright red to yellow or colorless. Cool the solution to -5°C and keep at this temperature for 24 h (**4.1-4.2, 4.5, 4.7-4.8**). In the case of **4.4, 4.6** the precipitate was formed when refluxing. To obtain solid products **4.3** and **4.9** pour the reaction mixture into ice water. Filter the resulting precipitate, wash with *i*-PrOH and recrystallize from the mixture of DMF/EtOH (1 : 1).

**The study of the antimicrobial activity**

The microbiological studies were performed at the Department of Microbiology, Virology and Immunology of the National University of Pharmacy according to the WHO recommendations [11, 12] and recommendations of the Ministry of Health of Ukraine [13, 14]. *Staphylococcus aureus* (*ATCC 25923*), *Escherichia coli* (*ATCC 25922*), *Pseudomonas aeruginosa* (*ATCC 27853*), *Bacillus subtilis* (*ATCC 6633*) and *Candida albicans* (ATCC 885-653) were used as test-strains.

The antimicrobial activity of the compounds synthesized was studied *in vitro* using the agar well diffusion method.

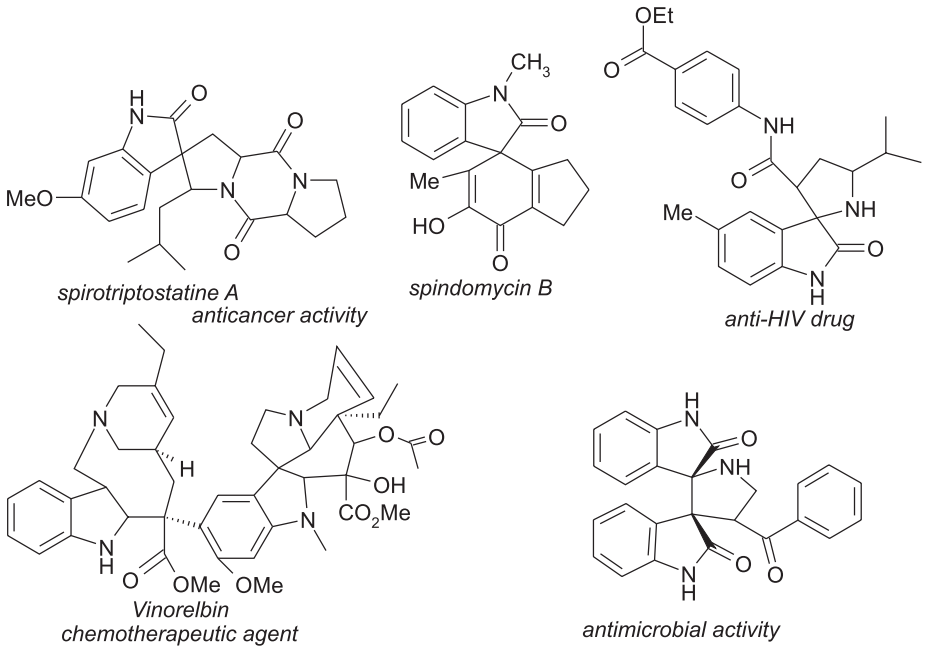
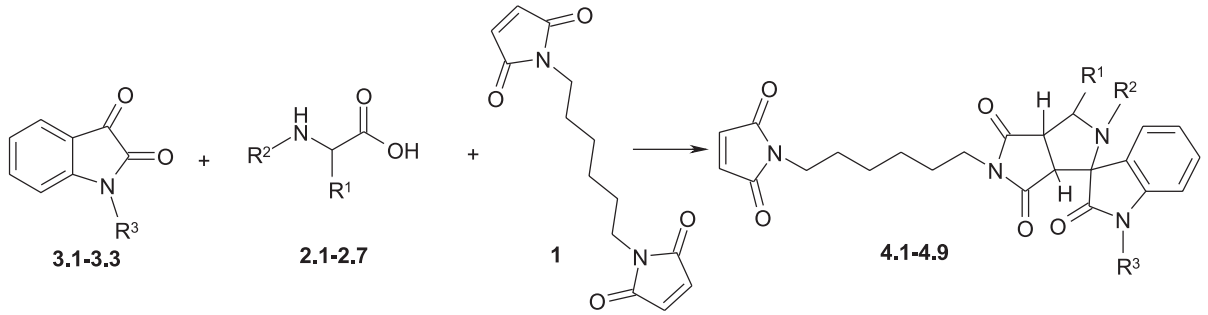



Fig. 1. Some bioactive spirooxindoles




**4.1** $R^1$=$CH_3$,$R^2$=$R^3$=H; **4.2** $R^1$=$CH_3$,$R^2$=H,$R^3$=$CH_3$; **4.3** $R^1$=i-Pr,$R^2$=$R^3$=H; **4.4** $R^1$=Ph,$R^2$=$R^3$=H; **4.5** $R^1$=$CH_2$-Ph,$R^2$=$R^3$=H; **4.6** $R^1$=$CH_2OH$,$R^2$=H,$R^3$=$CH_2$-Ph; **4.7** $R^1$=H,$R^2$=$CH_3$,$R^3$=$CH_2$-Ph; **4.8** $R^1$=$R^2$=$CH_2$-$CH_2$-$CH_2$,$R^3$=H; **4.9** $R^1$=$R^2$=$CH_2$-$CH_2$-$CH_2$,$R^3$=$CH_2$-Ph

Scheme 1

The studies were carried out by two stages. At the first stage the antimicrobial activity of the compounds was studied using Cefalexinum and Fluconazole (for *Candida albicans* strain) as the reference drugs. The weighed quantity of each compound (1 mg) was dissolved in 1 mL of DMSO. From the resulting suspension 0.3 mL was taken and introduced into wells on Petri dishes with microbial strains. The level of the antimicrobial activity was determined by the diameter of the growth inhibition zones around the well with the compound studied compared to the control.

The second stage was to study the antimicrobial activity of the compounds synthesized against *Staphylococcus aureus* (*ATCC 25923*) and *Candida albicans* (*ATCC 885-653*) by the broth dilution method in the liquid growth medium [13]. The Minimum Inhibitory Concentration (MIC) value recorded is defined as the lowest concentration of the antimicrobial agent that inhibits the visible growth of the microorganism tested, and it is expressed in mg/mL. To determine the Minimum Bactericidal Concentration (MBC) the broth with MIC of the test compound was incubated with the growth medium for 24, 48 or 72 h, controlling the growth of bacterial strains.

## Experimental part

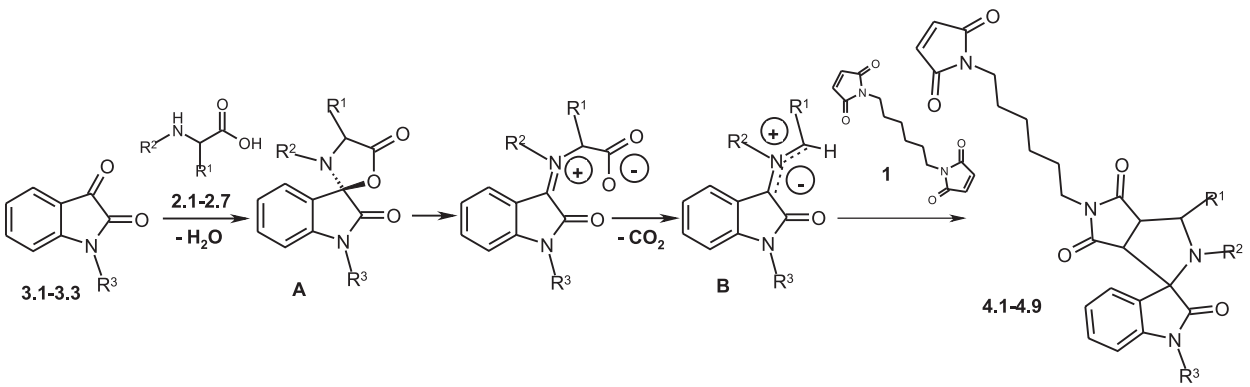
The new asymmetric derivatives of 1'-(hexamethylene-N-maleimidido)-2a',5a'-dihydro-1'*H*-spiroindole-3,3'-pyrrolo[3,4-*c*]pyrrole-2,2',6'(1*H*,3'*H*,5'*H*)-trione **4.1-4.9** were synthesized (Scheme 1).

According to the literature data [15] we can propose the following mechanism of this interaction (Scheme 2):

At the first stage the a-amino group of compounds **2.1-2.7** was added to b-carbonyl of isatins **3.1-3.3** followed by elimination of the water molecule resulted in formation of adduct **A**. Further the ring-opening of the lactone cycle followed by decarboxylation occurred. The next stage was the interaction of dipole **B** with dipolarophile **1** proceeded as [3+2]-cycloaddition reaction and led to target compounds **4.1-4.9**. It is possible because of the presence of π-bond activated by the electron-acceptor substituent. In this case, the direction of the reaction depended on the ratio of reagents. Thus, the symmetric *bis*-derivatives could only be obtained with a double excess of isatins and amino acids [8]. Asymmetric derivatives were obtained using the equimolar ratio of the starting reagents.

The structure of compounds **4.1-4.9** was confirmed by $^1$H NMR spectroscopy and elemental analysis (Tab. 1, Fig. 2). The structure of compound **4.7** was additionally confirmed by mass-spectrometry, while the structure of compound **4.8** was also confirmed by chromato-mass spectrometry.

The $^1$H NMR-spectra of compounds **4.1-4** had a characteristic signals of ABCD-system protons of the 2-oxindole fragment in the region of aromatic protons, a singlet signal of NH proton at δ 10.27…10.55 ppm, the NH signal of the arylamide fragment at δ 9.43…10.42 ppm,




Scheme 2

**Table 1**

The properties and $^1$H NMR-spectral data for derivatives of 1′-(hexamethylene-N-maleimidido)-2a′,5a′-dihydro-1′*H*-spiroindole-3,3′-pyrrolo[3,4-c]pyrrole-2,2′,6′(1*H*, 3′*H*,5′*H*)-trione **4.1-4.9**

| Compounds | M. m.<br>M. p.,°C<br>Yield % | $^1$H NMR, δ, ppm (*J*, Hz) | Elemental analysis %<br>Found<br>Calculated | | |
|---|---|---|---|---|---|
| | | | C | H | N |
| **4.1**<br>$C_{24}H_{26}N_4O$ | 450,50<br>100-102<br>75 | 1.10-1.45 (m, 11H); 3.22 (d, *J* = 6.86, 1H); 3.31 (br. s, 2H); 3.34-3.37 (m, 2H); 3.61 (br. s, 1H); 3.75 (d, *J* = 5.76, 1H); 4.26 (br. s, 1H); 6.70-6.89 (m, 3H); 6.96 (br. s, 2H); 7.16 (br. s, 1H); 10.30 (br. s, 1H) | 64.03<br>64.00 | 5.79<br>5.78 | 12.46<br>12.44 |
| **4.2**<br>$C_{25}H_{28}N_4O_5$ | 464,53<br>102<br>60 | 1.09-1.27 (m, 7H); 1.39-1.54 (m, 4H); 3.05 (s, 3H); 3.21 (d, *J* = 7.43, 1H); 3.34 (d, *J* = 6.65, 5H); 3.63 (br. s, 1H); 4.31 (d, *J* = 4.30, 1H); 6.84 (br. s, 1H); 6.89-6.99 (m, 4H); 7.22-7.30 (m, 1H) | 64.62<br>64.64 | 6.10<br>6.08 | 12.09<br>12.06 |
| **4.3**<br>$C_{26}H_{30}N_4O_5$ | 478,55<br>134-136<br>70 | 0.80-0.89 (m, 3H); 1.10 (d, *J* = 6.22, 3H); 1.19-1.38 (m, 4H); 1.45 (d, *J* = 5.49, 3H); 1.80 (br. s, 1H); 3.24 (d, *J* = 7.69, 1H); 3.29-3.40 (m, 5H); 3.48 (t, *J* = 7.14, 1H); 3.65 (br. s, 1H); 3.74 (d, *J* = 9.89, 1H); 6.71-6.90 (m, 3H); 6.98 (s, 2H); 7.10-7.22 (m, 1H); 10.30 (s, 1H) | 65.26<br>65.27 | 6.31<br>6.28 | 11.74<br>11.72 |
| **4.4**<br>$C_{29}H_{28}N_4O_5$ | 512,57<br>138-140<br>86 | 1.13-1.33 (m, 4H); 1.34-1.53 (m, 4H); 3.24 (dd, *J* = 16.04, 8.22, 2H); 3.33-3.42 (m, 2H); 3.63-3.70 (m, 1H); 3.75 (br. s, 1H); 4.10 (br. s, 1H); 5.45 (d, *J* = 4.30, 1H); 6.79 (d, *J* = 7.83, 1H); 6.91 (d, *J* = 10.96, 1H); 6.93-7.02 (m, 3H); 7.15-7.27 (m, 4H); 7.31-7.39 (m, 2H); 10.37 (s, 1H) | 67.98<br>67.97 | 5.49<br>5.47 | 10.97<br>10.94 |
| **4.5**<br>$C_{30}H_{30}N_4O_5$ | 526,50<br>112-114<br>66 | 1.23-1.61 (m, 8H); 3.17-3.28 (m, 3H); 3.35-3.45 (m, 4H); 3.53 (br. s, 1H); 4.31 (d, *J* = 3.91, 2H); 6.73 (d, *J* = 7.83, 1H) 6.81-6.87 (m, 2H); 6.96 (s, 1H); 7.14 (d, *J* = 6.65, 2H); 7.18-7.31 (m, 5H); 10.27 (s, 1 H) | 68.45<br>68.44 | 5.72<br>5.70 | 10.68<br>10.65 |
| **4.6**<br>$C_{31}H_{32}N_4O_5$ | 556,62<br>118-120<br>90 | 1.13-1.36 (m, 4H); 1.46 (dt, *J* = 14.48, 7.24, 4H); 3.34 (dd, *J* = 11.54, 6.85, 6H); 3.48-3.56 (m, 1H); 3.76 (br. s, 1H); 3.84 (d, *J* = 8.22, 1H); 4.31 (br. s, 1H); 4.55 (br. s, 1H); 4.74-4.91 (m, 2H); 6.73-7.03 (m, 5H); 7.12-7.20 (m, 1H); 7.25 (d, *J* = 7.04, 1H); 7.32 (q, *J* = 7.70, 4 H) | 66.87<br>66.89 | 5.80<br>5.79 | 10.09<br>10.07 |
| **4.7**<br>$C_{31}H_{32}N_4O_5$ | 540,62<br>95<br>82 | 1.08-1.62 (m, 8H); 1.89 (s, 3H); 3.20-3.47 (m, 7H); 3.58 (d, *J* = 5.80, 1H); 4.76-4.97 (m, 2H); 6.73-6.80 (m, 1H); 6.86-7.00 (m, 2H); 7.17-7.39 (m, 8H); EI-MS $[M+1]^+$ 540.0 | 68.88<br>68.89 | 5.95<br>5.93 | 10.40<br>10.37 |
| **4.8**<br>$C_{26}H_{28}N_4O_5$ | 476,54<br>140-142<br>78 | 1.24-1.40 (m, 4H); 1.56-1.94 (m, 8H); 2.97 (t, 2H); 3.56 (1H); 3.73-3.82 (m, 2H); 6.91 (d, *J* = 8.0, 2H); 7.28 (d, *J* = 8.4, 1H); 7.45 (d, *J* = 10.2, 2H); 7.81 (d, *J* = 8.0, 1H ); 10.55 (s, 1H) *m/z* ($I_{rel}$, %): 477,2 (1.4) $[M+1]^+$, 476,1 (100) $[M]^+$, 339.1 (98) $[M-137]^+$, 157.2 (25) $[M-319]^+$ | 65.54<br>65.55 | 5.89<br>5.88 | 11.78<br>11.76 |
| **4.9**<br>$C_{33}H_{34}N_4O_5$ | 566,66<br>85<br>75 | 1.12-1.55 (m, 8H); 1.79 (br. s, 4H); 2.24 (br. s, 1H); 2.33 (br. s, 1H) 3.17-3.30 (m, 2H); 3.36 (d, *J* = 7.04, 2H); 3.52 (d, *J* = 7.83, 1H); 3.63 (d, *J* = 7.04, 1H); 4.25 (br. s, 1H); 4.77-4.93 (m, 2H); 6.83-6.99 (m, 4H); 7.15-7.36 (m, 7H) | 69.97<br>69.96 | 6.03<br>6.01 | 9.91<br>9.89 |

a doublet signal of the maleinimide link at 6.95 ppm, signals of protons of the pyrolo-pyrrolidinedionic and hexamethylene fragments in the strongly-stained region of the spectrum. The typical spectrum of the compounds synthesized is shown in Fig. 2.

The data of the elemental analysis are in agreement with the calculated data, molecular ions in mass-spectra coincide with theoretical data (Tab. 1).

The antibacterial properties of substances **4.1-4.9** against different types of microorganisms (gram-positive and gram-negative) were studied. Almost all compounds showed a significant antibacterial activity against *Staphylococcus aureus.* For compounds **4.1, 4.3** the minimum bactericidal concentration (MBC) was equal to MBC for Cefalexin. The growth inhibition zones for all compounds studied against *Candida albicans* exceeded almost twice compared to the reference drug. The minimum fungicidal concentration (MFC) for compounds **4.1, 4.2** was equal to those of Fluconazole. The results of studying the antimicrobial activity for compounds **4.1-4.9** are given in Tab. 2.

The antimicrobial activity for compounds **4.1-4.9** was compared to those for the corresponding symmetric *bis*-analogs synthesized according to procedure [8]. Derivatives of hexamethylene-N-maleimido-spiroindol-3,3′-pyrrolo[3,4-*c*]pyrrole were generally much more active than hexamethylene-N,N′-*bis*-derivatives of spiroindole-3,1′-pyrrolo[3,4-*c*]pyrrole. Thus, the corresponding *bis*-analogs of compounds **4.1-4.5, 4.8, 4.9** exhibited very little antimicrobial activity against *Staphylococcus aureus* and were completely inactive in relation to *Candida albicans* [8]. The activity of compound **4.7** was found to be at the level of activity of the corresponding hexamethylene-N,N′-*bis*-derivative. However, MBC for *Staphylococcus aureus* and MFC for compound **4.6** were eight-times

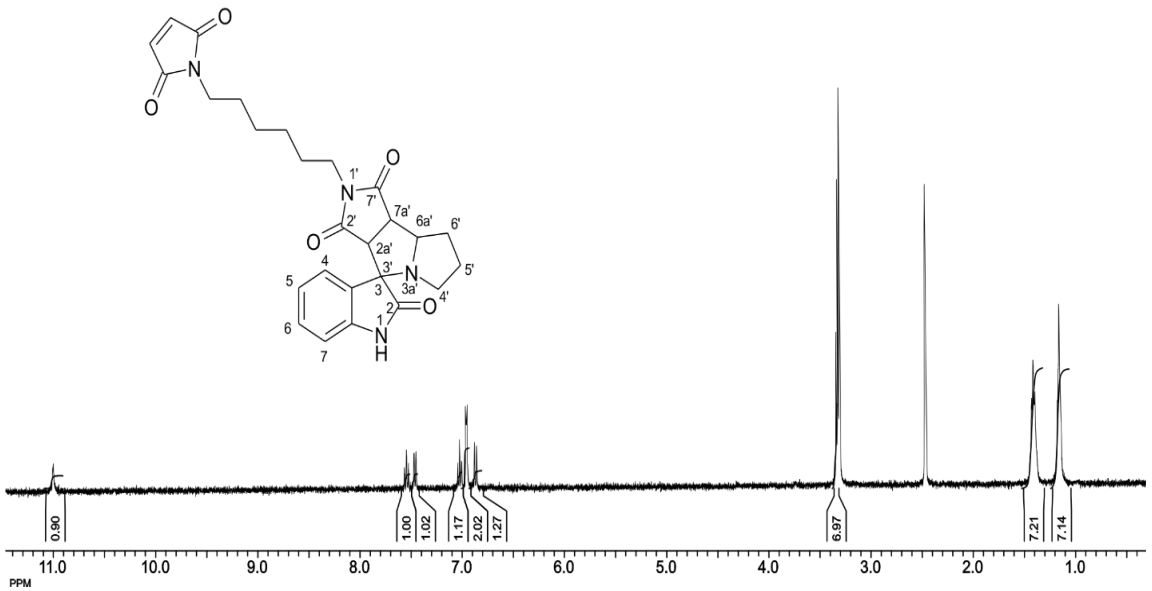



Fig. 2. The $^1$H NMR spectrum of 1'-(hexamethylene-N-maleimidido)-2a',7a'-dihydro-1'*H*-spiroindole-3,3'-pyrrolo[3,4-*c*]pyrrolizidine-2,2',7'(1*H*,3'*H*,5'*H*)-trione **4.8**

**Table 2**

The antimicrobial activity of 1'-(hexamethylene-N-maleimidido)-2a',5a'-dihydro-1'*H*-spiroindole-3,3'-pyrrolo[3,4-c]pyrrole-2,2',6'(1*H*, 3'*H*,5'*H*)-triones **4.1-4.9**

| Compound | Diameter of the growth inhibition zones*, mm | | | | |
|---|---|---|---|---|---|
| | Gram-positive bacteria | | Gram-negative bacteria | | Fungi |
| | *B. subtilis* | S. aureus<br>MBC | *E. coli* | *P. aeruginosa* | C. albicans<br>MFC |
| **4.1** | 25 | 31<br>*12,5* | 18 | growth | 31<br>*3.125* |
| **4.2** | 24 | 30<br>*growth* | 18 | growth | 31<br>*3.125* |
| **4.3** | 27 | 31<br>*12,5* | 14 | growth | 35<br>*6.25* |
| **4.4** | 25 | 29<br>*50* | 16 | growth | 24<br>*growth* |
| **4.5** | 26 | 29<br>*50* | 15 | growth | 30<br>*growth* |
| **4.6** | 24 | 30<br>*50* | 17 | growth | 32<br>*growth* |
| **4.7** | 26 | 31<br>*50* | 15 | growth | 35<br>*growth* |
| **4.8** | 25 | 27<br>*growth* | 20 | growth | 25<br>*growth* |
| **4.9** | 26 | 27<br>*growth* | 21 | growth | 28<br>*growth* |
| **Control** | growth | growth | growth | growth | growth |
| **Cefalexinum** | 32 | 32<br>*12,5* | 35 | 36 | n/t |
| **Fluconazole** | n/t | n/t | n/t | n/t | 16<br>*3.125* |

Notes: * – the data presented are the average values for three experiments relative to each microorganism culture.

higher than the corresponding *bis*-derivative previously synthesized. According to the results of microbiological tests substances **4.1, 4.3, 4.6, 4.7** are promising for further studies against *Staphylococcus aureus*, while compounds **4.1, 4.2, 4.3** are prospective as anti-*Candida* substances.

## Conclusions

It has been found that the dipolar cycloaddition of 1,6-bis-maleimidohexane to azometinilide formed *in situ* from isatin and α-amino acids (with the use of the equimolar ratio of the starting reagents) is an effective synthetic approach towards 1'-(hexamethylene-N-maleimido)-2a',5a'-dihydro-1'*H*-spiroindole-3,3'-pyrrolo[3,4-c]pyrrole-2,2',6'(1*H*, 3'*H*, 5'*H*)-trions showing a high biological activity against *Staphylococcus aureus* and *Candida albicans*. In general, the antimicrobial activity of hexamethylene-N-maleimidopropindol-3,3'-pyrrolo[3,4-*c*]pyrrole derivatives appeared to be significantly higher than the activity of the corresponding symmetrical *bis*-analogs. 1'-(Hexamethylene-N-maleimido)-5'-methyl-2a',5α'-dihydro-1'*H*-spiroindole-3,3'-pyrrolo[3,4-c]pyrrole-2,2',6' (1H, 3'H, 5'H)-trione has shown the highest biological activity among derivatives of hexamethylene-N-maleimidospiroindol-3,3'-pyrrolo[3,4-*c*]pyrrole.

**Conflict of Interests:** authors have no conflict of interests to declare.

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