СИНТЕЗ ТА АНАЛІЗ БІОЛОГІЧНО АКТИВНИХ РЕЧОВИН

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The synthesis of mono- and *bis*-derivatives of spiro-2-oxindole[3,3']pyrrole and the study of their antioxidant and anti-inflammatory activities

Aim. To synthesize a series of mono- and *bis*-derivatives of spiro-2-oxindole[3,3']pyrrole and study the antioxidant and anti-inflammatory activity of the compounds obtained.

Materials and methods. Methods of organic synthesis and instrumental methods for determining the structure of organic compounds were used. The antioxidant properties of the compounds synthesized were studied *in vitro* on the model of spontaneous lipid peroxidation (LPO); the model of acute aseptic inflammation (carrageenan edema) was used for determination of the anti-inflammatory (anti-exudative) activity.

Results and discussion. A series of novel spiro-2-oxindole[3,3']pyrrole derivatives was synthesized by means of the three-component cascade interaction of isatin, α-amino acids and dipolarophils based on *bis*-maleimids. In order to broaden the range of compounds obtained nitroso derivatives of ethylene-N,N'-*bis*(spiroindole-3,3'-pyrrolo[3,4-*c*]pyrrole-5'-methyl-2a,5a'-dihydro-2,2',6'(1*H*,1'*H*,5'*H*)-trione) **4** and hexamethylene-N,N'-*bis*(spiroindole-3,3'-pyrrolo[3,4-*c*]pyrrole-5'-benzyl-2a',5a'-dihydro-2,2',6'(1*H*,1'*H*,5'*H*)-trione) **5** were synthesized. The structure of these compounds was reliably confirmed by instrumental methods (^{1}H NMR, IR-spectroscopy and chromatographic mass spectrometry). The screening studies of the anti-inflammatory properties were conducted, they included the study of the antioxidant action of the compounds synthesized *in vitro*. Three of the substances tested revealed the highest antioxidant properties and were subsequently selected for further study of their anti-inflammatory activity. The data of the biological experiments showed their pronounced anti-inflammatory properties on the model of carrageenan edema.

Conclusions. It has been found that the preparatory method of the three-component cascade transformation of isatin, α-amino acids and dipolarophils based on bis-maleimides is effective for the synthesis of spiro-2-oxindole[3,3'] pyrrole mono- and bis-derivatives. A series of hexamethylene- and ethylene-N,N'-*bis*(spiroindole-3,3'-pyrrolo[3,4-*c*] pyrrole-4'-nitroso-2a,5a'-dihydro-2,2',6'(1*H*,1'*H*,5'*H*)-trione) symmetric derivatives have been obtained. The structure of the compounds obtained has been confirmed. When studying the antioxidant activity of the compounds synthesized it has been found that the most active substances are 1'-(hexamethylene-N-maleimido)-5'-benzyl-2a',5a'-dihydro-1'*H*-spiroindole-3,3'-pyrrolo[3,4-*c*]pyrrole-2,2',6'(1*H*,3'*H*,5'*H*)-trione **1**; ethylene-N,N'-*bis*(spiroindole-3,3'-pyrrolo[3,4-*c*]pyrrole-4'-nitroso-5'-methyl-2a,5a'-dihydro-2,2',6'(1*H*,1'*H*,5'*H*)-trione) **6**; hexamethylene-N,N'-*bis*(spiroindole-3,3'-pyrrolo[3,4-*c*] pyrrole-4'-nitroso-5'-benzyl-2a,5a'-dihydro-2,2',6(1*H*,1'*H*,5'*H*)-trione) **7**. Compound **6** has also shown the anti-inflammatory activity at the reference drug level.

Key words: *spiro-2-oxindole; nitroso derivatives; antioxidant activity; anti-inflammatory activity*

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Синтез і дослідження антиоксидантної та протизапальної активності моно- та *біс*-похідних спіро-2-оксіндол[3,3']піролу

Мета роботи – синтез ряду моно- та *біс*-похідних спіро-2-оксіндол[3,3']піролу та дослідження їх антиоксидантної та протизапальної активності.

Матеріали та методи. Методи органічного синтезу, інструментальні методи встановлення будови органічних сполук, метод дослідження антиоксидантних властивостей синтезованих сполук в умовах *in vitro* на моделі спонтанного перекисного окиснення ліпідів (ПОЛ), вивчення протизапальної (антиексудативної) активності на моделі гострого асептичного запалення задньої кінцівки (карагенінового набряку).

Результати та їх обговорення. Використовуючи трикомпонентне каскадне перетворення ізатину з α-амінокислотами та диполярофілами на основі біс-малеїнімідів синтезовано ряд нових похідних спіро-2-оксіндол[3,3']піролу. Для розширення цього ряду здійснені хімічні перетворення етилен-N,N'-*біс*(спіроіндол-3,3'-піроло[3,4-*c*]пірол-5'-метил-2a',5a'-дигідро-2,2',6'(1*H*,1'*H*,5'*H*)-тріону) **4** і гексаметилен-N,N'-*біс*(спіроіндол-3,3'-піроло[3,4-*c*]пірол-5'-бензил-2a',5a'-дигідро-2,2',6'(1*H*,1'*H*,5'*H*)-тріону) **5** та отримані їх нітрозопохідні. Будову одержаних сполук надійно підтверджено інструментальними методами, такими як ^{1}Н ЯМР, ІЧ-спектроскопія та хромато-мас-спектрометрія. Проведені скринінгові дослідження потенційної протизапальної дії, що включало вивчення *in vitro* антиоксидантної дії синтезованих сполук. Відібрані три сполуки, які виявили найбільш потужні антиоксидантні властивості для подальшого вивчення їх протизапальної активності. Дані біологічного експерименту показують виражену протизапальну активність цих сполук на моделі карагенінового набряку.

Висновки. Встановлено, що препаративна методика трикомпонентного каскадного перетворення ізатину з α-амінокислотами та диполярофілами на основі *біс*-малеїнімідів є ефективним методом синтезу моно- та *біс*-похідних спіро-2-оксіндол[3,3']піролу. Отримані симетричні похідні гексаметилен- та етилен-N,N'-*біс*(спіроіндол-3,3'-піроло[3,4-*c*]пірол-4'-нітрозо-2a',5a'-дигідро-2,2',6'(1*H*,1'*H*, 5'*H*)-тріону). Доведено будову отриманих сполук. При вивченні антиоксидантної активності синтезованих сполук встановлено, що найбільш високу антиоксидантну активність виявили сполуки: 1'-(гексаметилен-N-малеїнімідо)-5'-бензил-2a',5a'-дигідро-1'*H*-спіроіндол-3,3'-піроло[3,4-*c*]пірол-2,2',6'(1*H*,3'*H*,5'*H*)-тріон **1**; етилен-N,N'-*біс*(спіроіндол-3,3'-піроло[3,4-*c*]пірол-4'-нітрозо-5'-метил-2a',5a'-дигідро-2,2',6'(1*H*,1'*H*,5'*H*)-тріон) **6**; гексаметилен-N,N'-*біс*(спіроіндол-3,3'-піроло[3,4-*c*]пірол-4'-нітрозо-5'-бензил-2a',5a'-дигідро-2,2',6'(1*H*,1'*H*,5'*H*)-тріон) **7**. Сполука **6** виявила протизапальну активність, що вірогідно не відрізнялася від активності референс-препарату.

Ключові слова: *спіро-2-оксіндол; нітрозопохідні; антиоксидантна активність; протизапальна активність*

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Синтез и исследование антиоксидантной и противовоспалительной активности моно- и *бис*-производных спиро-2-оксиндол[3,3']пиррола

Цель работы – синтез ряда моно- и *бис*-производных спиро-2-оксиндол[3,3']пиррола и исследование их антиоксидантной и противовоспалительной активности.

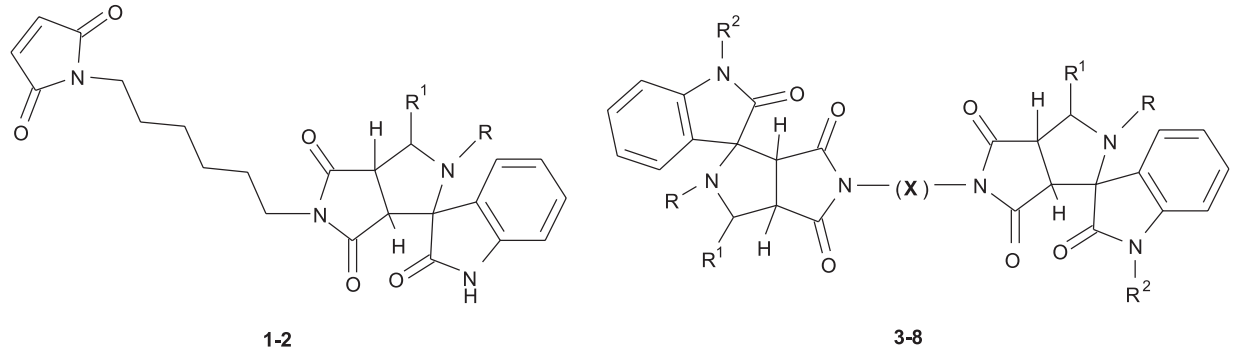
Материалы и методы. Методы органического синтеза, инструментальные методы определения структуры органических соединений, метод исследования антиоксидантных свойств синтезированных соединений в условиях *in vitro* на модели спонтанного перекисного окисления липидов (ПОЛ), изучение противовоспалительной (антиэкссудативной) активности на модели острого асептического воспаления задней конечности (каррагенинового воспаления).

Результаты и их обсуждение. Используя трехкомпонентное каскадное превращение изатина с α-аминокислотами и диполярофилами на основе *бис*-малеинимидов синтезировано ряд новых производных спиро-2-оксиндол[3,3'] пиррола. Для того, чтобы расширить этот ряд, осуществлены химические превращения этилен-N,N'-*бис*(спироиндол-3,3'-пирроло[3,4-*c*]пиррол-5'-метил-2a',5a'-дигидро-2,2',6'(1*H*,1'*H*,5'*H*)-триона) **4** и гексаметилен-N,N'-*бис*(спироиндол-3,3'-пирроло[3,4-*c*]пиррол-5'-бензил-2a',5a'-дигидро-2,2',6'(1*H*,1'*H*, 5'*H*)-триона) **5** и получены их нитрозопроизводные. Структура полученных соединений достоверно доказана инструментальными методами, такими как ^{1}H ЯМР, ИК-спектроскопия и хромато-масс-спектрометрия. Проведены скрининговые исследования потенциального противовоспалительного действия, которые включали изучение *in vitro* антиоксидантного действия синтезированных соединений. Отобраны три соединения, которые проявили наиболее выраженные антиоксидантные свойства для дальнейшего изучения их противовоспалительной активности. Данные биологического эксперимента показывают выраженную противовоспалительную активность этих соединений на модели каррагенинового воспаления.

Выводы. Установлено, что препаративная методика трехкомпонентного каскадного превращения изатина с α-аминокислотами и диполярофилами на основе *бис*-малеинимидов является эффективным методом синтеза моно- и *бис*-производных спиро-2-оксиндол[3,3']пиррола. Получены симметричные производные гексаметилен- и этилен-N,N'-*бис*(спироиндол-3,3'-пирроло[3,4-*c*] пиррол-4'-нитрозо-2a',5a'-дигидро-2,2',6'(1*H*,1'*H*,5'*H*)-триона). Доказана структура полученных соединений. При изучении антиоксидантной активности синтезированных соединений установлено, что наиболее высокую антиоксидантную активность проявили соединения: 1'-(гексаметилен-N-малеинимидо)-5'-бензил-2a',5a'-дигидро-1'*H*-спироиндол-3,3'-пирроло[3,4-*c*] пиррол-2,2',6'(1*H*,3'*H*,5'*H*)-трион **1**; этилен-N,N'-*бис*(спироиндол-3,3'-пирроло[3,4-*c*]пиррол-4'-нитрозо-5'-метил-2a',5a'-дигидро-2,2',6'(1*H*,1'*H*,5'*H*)-трион) **6**; гекса-метилен-N,N'-*бис*(спироиндол-3,3'-пирроло[3,4-*c*]пиррол-4'-нитрозо-5'-бензил-2a', 5a'-дигидро-2,2',6'(1*H*,1'*H*,5'*H*)-трион) **7**. Соединение **6** проявило противовоспалительную активность, которая вероятно не отличается от активности референс-препарата.

Ключевые слова: *спиро-2-оксиндол; нитрозопроизводные; антиоксидантная активность; противовоспалительная активность*

One of the topical issues of modern pharmacology is the increase of the effectiveness of treating concomitant diseases that occurs with inflammation and pain. The most commonly used group of drugs for this case is non-steroidal anti-inflammatory drugs (NSAIDs). Over the past decades, the number of NSAIDs has significantly increased, and nowadays it consists of drugs that differ in their chemical structure, methods of application and the effect on the body. However, they all have a number of contraindications and can provoke adverse effects; therefore, their use is restricted [1]. Thus, the search of new biologically active substances with the anti-inflammatory properties is a promising direction of modern pharmacology. It is known that the spiroindol-3,3'-pyrrole core is in the structure of many natural alkaloids with the pronounced biological activity, in particular with the anti-inflammatory properties. For example, leaves of the Malaysian plant *Hunteria zeylanica* (*Apocynaceae*) are the source of bisindole alkaloid – *bisnicolaterin A* with the antinociceptive, antipyretic and anti-inflammatory activity [2]. From the leaves of the tropical liana of *Gelsemium elegans* (*Loganiaceae*) bisoxindole alkaloids –



1-2 **3-8**

3, 5, 7 X = $(CH_2)_6$; **4, 6** X = $(CH_2)_2$; **8** X = $NH(CO)_2NH$

1, 3, 4, 5, 8 R = H; **2** R = R^1 = -$(CH_2)_3$-; **6, 7** R = NO

1, 5, 7 R^1 = Bn; **3** R^1 = CH_2OH; **4, 6** R^1 = CH_3; **8** R^1 = Ph

3 R^2 = Bn; **4, 5, 6, 7, 8** R^2 = H; **3** R^2 = Bn

Fig. The general structures of the compounds studied

heleganidins B and *C* and *geleganimins A* and *B* that exhibit the cytotoxic, anti-inflammatory and analgesic properties were isolated [3, 4].

The aim of the work was to synthesize a series of mono- and *bis*-derivatives of spiro-2-oxindole[3,3']pyrrole and study their antioxidant and anti-inflammatory activity.

Materials and methods

Chemical part

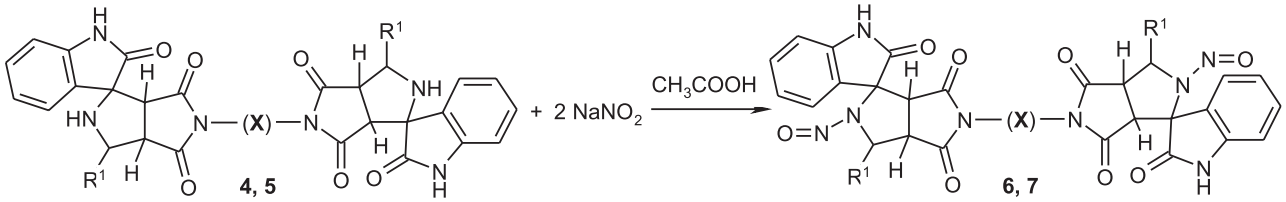
To conduct the first stage of the potential anti-inflammatory activity screening, which included the study of the antioxidant activity *in vitro*, 8 compounds were selected (Fig.).

Four of these compounds were synthesized for the first time in order to expand the range of the substances under research. The synthesis of derivatives of 1'-(hexamethylene-N-maleimido)-2a',5a'-dihydro-1'*H*-spiroindole-3,3'-pyrrolo[3,4-*c*]pyrrole-2,2',6'(1*H*,1'*H*,5'*H*)-trione **1-2**, hexamethylene-N,N'-*bis*(spiroindole-3,3'-pyrrolo[3,4-*c*]pyrrole-5'-hydroxymethyl-4'*H*,1N-benzyl-2a,5a'-dihydro-2,2',6'(1*H*,1'*H*,5'*H*)-trione) **3** and ethylene-N,N'-*bis*(spiroindole-3,3'-pyrrolo[3,4-*c*]pyrrole-5'-methyl-2a,5a'-dihydro-2,2',6'(1*H*,1'*H*,5'*H*)-trione) **4**, their chemical structure and biological activity as inhibitors of protein kinases, as well as their antimicrobial properties were described earlier [5, 6, 7, 8].

The synthesis of compound 5 was performed according to the general procedure [7]. **Hexamethylene-N,N'-spiroindole-3,3'-pyrrolo[3,4-*c*]pyrrole-5'-benzyl-2a',5a'-dihydro-2,2',6'(1*H*,1'*H*,5'*H*)-trione) 5** was obtained with the yield of 92 %. M. p. – 246-248 °C. 1H NMR d, ppm (400 MHz, DMSO): δ 10.27 (s, 2H), 7.33-7.18 (m, 8H), 7.13 (t, *J* = 7.1 Hz, 4H), 6.84 (m, 4H), 6.73 (d, *J* = 7.7 Hz, 2H), 4.33 (m, 2H), 3.54 (d, *J* = 3.7 Hz, 2H), 3.48-3.36 (m, 6H), 3.25 (d, *J* = 7.6 Hz, 2H), 3.20 (dd, *J* = 13.8, 5.5 Hz, 2H), 2.58 (dd, *J* = 14.0, 8.2 Hz, 4H), 1.57 (m, 4H), 1.37 (m, 4H). Found, %: C 71.08; H 5.74; N 10.85. Calculated, %: C 71.12; H 5.71; N 10.82.

The general procedure for the synthesis of symmetric derivatives of ethylene and hexamethylene-N,N'-*bis*(spiroindole-3,3'-pyrrolo[3,4-*c*]pyrrole-4'-nitroso-2a',5a'-dihydro-2,2,6'(1*H*,1'*H*,5'*H*)-trione) 6, 7. Dissolve ethylene-N,N'-*bis*(spiroindole-3,3'-pyrrolo[3,4-*c*]pyrrole-5'-methyl-2a',5a'-dihydro-2,2',6'(1*H*,1'*H*,5'*H*)-trione) 4 (1 mmol) and hexamethylene-N,N'-*bis*(spiroindole-3,3'-pyrrolo[3,4-*c*]pyrrole-5'-benzyl-2a',5a'-dihydro-2,2',6'(1*H*,1'*H*,5'*H*)-trione) **5** (1 mmol) separately in 50 ml of acetic acid (Scheme 1). Cool the solutions to 10 °C and add sodium nitrite to them (3 mmol). In 12 h add 150 ml of water to the solutions, filter the precipitates obtained, wash them with water and recrystallize from water.

Ethylene-N,N'-*bis*(spiroindole-3,3'-pyrrolo [3,4-*c*]pyrrole-4'-nitrozo-5'-methyl-2a',5a'-dihydro-2,2',6'(1*H*,1'*H*,5'*H*)-trione) 6 was obtained with the yield of 88 %. M. p. – 298-300 °C. ^{1}H NMR, δ, ppm (400 MHz, DMSO): δ 10.95 (s, 1H), 7.32-7.06 (t, 1H), 6.92-6.57 (m, 3H), 5.30 (m, 1H), 4.26-4.02 (m, 1H), 3.67 (m, 1H), 3.41 (m, 1H), 3.19 (m, 1H), 1.82 (d, 3H). Found, %: C 57.52; H 4.16; N 17.91. Calculated, %: C 57.51; H 4.18; N 17.88.



4, 5 **6, 7**

4, **6** X = $(CH_2)_2$; R^1 = CH_3 **5**, **7** X = $(CH_2)_6$; R^1 = Bn

Scheme 1

Table 1

The characteristic absorption frequencies in IR-spectra of compounds **4**, **5**, **6**, **7**

Compound	IR, cm^{-1} assignment					
	ν_{NH}	$\nu_{CH(arom)}$	$\nu_{CH(aliph)}$	$\nu_{C=O}$	$\nu_{N=O}$	$\nu_{C=C(arom)}$
4	3269, 3177	3030	2933, 2870	1703	–	1624, 1473
5	3325	3062, 3029	2940, 2862	1702	–	1620, 1453
6	3362	3030	2990, 2947, 2853	1715	1430,	1620, 1474
7	3365	3062, 3030	2941, 2861	1708	1439	1619, 1473

Hexamethylene-N,N'-*bis*(spiroindole-3,3'-pyrrolo[3,4-*c*]pyrrole-4'-nitrozo-5'-benzyl-2a',5a'-dihydro-2,2',6'(1*H*,1'*H*,5'*H*)-trione) 7 was obtained with the yield of 80 %. M. p. – 170-172 °C. ^{1}H NMR, δ, ppm (400 MHz, DMSO): δ 10.93 (s, 2H), 7.33 (d, *J* = 7.1 Hz, 4H), 7.28-7.09 (m, 8H), 6.85 (t, *J* = 7.4 Hz, 4H), 6.75 (d, *J* = 7.5 Hz, 2H), 5.59 (dd, *J* = 14.7, 8.5 Hz, 2H), 4.27 (t, *J* = 9.3 Hz, 2H), 3.81-3.73 (m, 2H), 3.71 (d, *J* = 9.2 Hz, 2H), 3.29-3.20 (m, 4H), 3.19-3.10 (m, 2H), 1.35 (m, 4H), 1.17 (s, 4H). Found, %: C 66.21; H 5.09; N 13.45. Calculated, %: C 66.18; H 5.07; N 13.42. The presence of nitroso group was confirmed by IR-spectra (Tab. 1).

The procedure for the synthesis of oxalylamine-N,N'-*bis*(spiroindole-3,3'-pyrrolo[3,4-*c*]pyrrole-5'-phenyl-2a',5a'-dihydro-2,2',6'(1*H*,1'*H*,5'*H*)-trione) 8. The synthesis was carried out according to Scheme 2. N,N'-*bis*-maleimidoxalylamine a was obtained according to the procedures [9, 10].

The starting isatin **b** and the amino acid phenylglycine **c** were obtained from commercial sources and used without further purification.

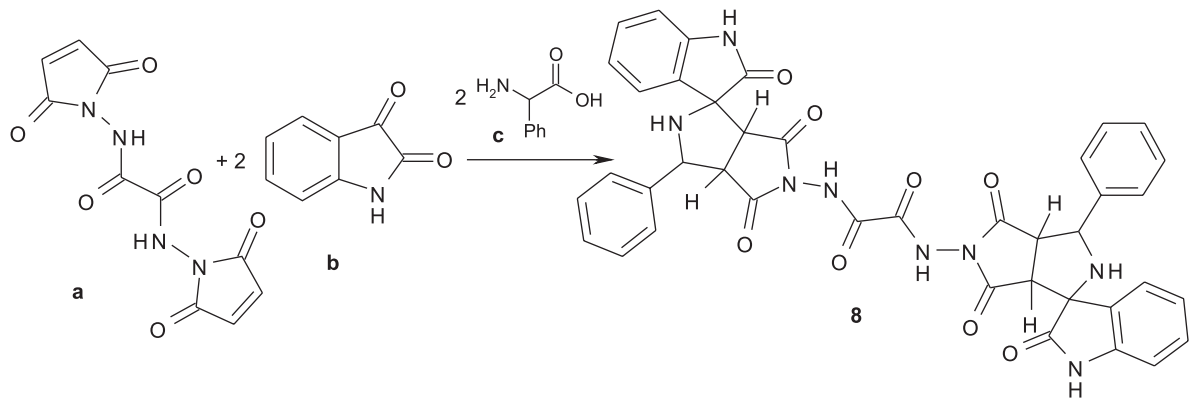
Dissolve the mixture of N,N'-*bis*-maleimidoxalylamine **a** (1 mmol), isatin **b** (2 mmol) and phenylglycine **c** (2 mmol) in the mixture of *i*-PrOH (3 ml) and H_2O (1 ml) and reflux for 4 h, control the course of the reaction by changing the color of the reaction mixture from bright red to dark brown and then to pale yellow. Filter the precipitate obtained, wash with *i*-PrOH and recrystallize from the mixture of i-PrOH (1 ml) and H_2O (1 ml).

Oxalylamine-N,N'-*bis*(spiroindole-3,3'-pyrrolo[3,4-*c*]pyrrole-5'-phenyl-2a',5a'-dihydro-2,2',6'(1*H*,1'*H*,5'*H*)-trione) 8 was obtained with the yield of 83 %. M. p. – 265-266 °C. 1H NMR, d, ppm (400 MHz, DMSO): δ 11.65 (s, 2H), 10.34 (s, 2H), 7.48-7.36 (m, 4H), 7.24 (m, 10H), 6.89 (t, 2H), 6.79 (d, *J* = 7.7 Hz, 2H), 5.50 (dd, *J* = 7.7, 2.7 Hz, 2H), 4.14 (s, 2H), 3.96-3.80 (m, 2H), 3.57 (m, 2H). LCMS, m/z (I, %) = 751(100) [M + 1], 376(5), 101(7). Found, %: C 63.09; H 4.05; N 14.96. Calculated, %: C 64.00; H 4.03; N 14.93.

Melting points were determined on a Gallenkamp melting point apparatus, model MFB-595 in open capillary tubes. The ^{1}H NMR-spectra were recorded on a Varian WXR-400 spectrometer using DMSO-d_6 as a solvent and TMS as an internal standard. The chromatography-mass spectra were recorded on an Agilent 1100 HPLC device with a diode matrix detector. The elemental analysis was carried out using a Carlo Erba CHNS-O EA 1108 analyzer. The IR-spectra were taken on a Brucker Tensor 27 FT-IR 400-4000 cm^{-1} in KBr pellets with the concentration of substances of 1 %.

Biological experiment

The study of antioxidant properties of the compounds synthesized was conducted *in vitro* on the model of spontaneous lipid peroxidation (LPO) in the liver homogenate [11, 12]. In the test tubes 2 ml of the homogenate and 0.1 ml of the suspension of the compounds tested in the concentration of 50 or 100 μmol were introduced. The tubes were incubated for 15 min. Then 1 ml of 30 % CCl_4 solution was added, centrifuged, 2 ml of the supernatant was taken and 2 ml of 0.8 % solution of thiobarbituric acid (TBA) was added. The samples were boiled for 15 min, cooled and measured at the wavelength of 532 nm. In parallel, an intact test tube and the control sample were prepared. By extinction the content of TBA-re-



Scheme 2

actants in the sample was calculated according to the formula:

$$C = A/\varepsilon \cdot 1 \cdot n,$$

where: A – is extinction of the sample; ε – is 0.156 (the recalculation coefficient of molarity for TBA-reactants); n – is the content of the tissue in the sample, g.

Compounds **1, 6, 7** showed the most potent antioxidant properties and were selected for further study of the anti-inflammatory (anti-exudative) activity on the model of acute aseptic inflammation of the hind limb (carrageenan edema) [13, 14]. Indomethacin ("Sofarma", Bulgaria), which was administered in the dose of 5.25 mg/kg, was selected as the reference drug. The anti-inflammatory (anti-exudative) activity was studied in the doses of 5 mg/kg and 7 mg/kg. The compounds studied and the reference drug were administered intragastrically an hour before the phlogogen injection.

The study was carried out in albino adult rats weighing 170-200 g; they were kept under the standard conditions in the vivarium of the Central Research Laboratory at the National University of Pharmacy. The study was carried out in accordance with the "General ethical principles of animal experimentation" (Ukraine, 2001), which are consistent with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and the Code of Ethics of the World Medical Association (Helsinki Declaration, 1964). The animals were divided into 8 groups:

Group 1 – control pathology: for the pathology simulation the experimental animals were subplantarly injected with 0.1 ml of 1 % carrageenan solution. Animals of the control group received distilled water intragastrically.

Groups 2 and 3 – animals that were subplantarly injected with carrageenan (see group 1) and 1 hour before administration of phlogogen compound **1** in the doses of 5 and 7 mg/mg, respectively, was injected intragastrically.

Groups 4 and 5 – animals that were subplantarly injected with carrageenan (see group 1) and 1 hour before administration of phlogogen compound **6** in the doses of 5 and 7 mg/mg, respectively, was injected intragastrically.

Groups 6 and 7 – animals that were subplantarly injected with carrageenan (see group No. 1) and 1 hour before administration of phlogogen compound **7** in the doses of 5 and 7 mg/mg, respectively, was injected intragastrically.

Group 8 – animals that were subplantarly injected with carrageenan (see group 1) and 1 hour before administration of phlogogen the reference drug in the dose of 5.25mg/mg was injected intragastrically.

The size of edema was measured by an onkometer in 1, 2, 3, 4, 5 and 24 hours after the introduction of carrageenan.

The anti-inflammatory (anti-exudative) activity was calculated by the formula:

$$A = 100\ \% - [(P_{experim}/P_{control}) \bullet 100],$$

where: A – is the anti-inflammatory activity, %; $P_{control\cdot}$ – is the average difference in the volume of swollen and healthy paws in the control pathology; $P_{experim.}$ – is the average difference in the volume of swollen and healthy paws in the experimental group.

The experimental data were processed by the method of variation statistics using the standard software package "Statistica 6.0". The data comparison was performed by the ANOVA criterion [15].

Results and discussion

The study of the antioxidant activity *in vitro*

At first the antioxidant activity of compounds **1-8** (Fig., Tab. 2) was studied. The intensity of lipid peroxidation processes was assessed by accumulation of compounds reacting with thiobarbituric acid (TBA-reactants). The decrease of the content of TBA-reactants in of samples indicated the presence of the antioxidant activity of the compounds studied.

The data obtained are presented in Tab. 2. It was found that compounds **4** and **8** did not reveal the antioxidant activity in both concentrations of 50 and 100 μmol. Compounds **2** and **5** reduced the content of TBA-reactants in the concentration of 50 μmol. However, their antioxidant activity did not have the concentration dependence since when 100 μmol of compounds **2, 5** were used in the test a reliable decrease in the content of TBA-reactants compared to the control indicator was not observed. Compound **3** revealed the antioxidant activity only in the concentration of 100 μmol, with 13 % decrease in the TBA-reagent content. In the concentration of 50 μmol compound **3** did not show the significant antioxidant activity.

Compounds **1, 6, 7** reduced the content of TBA-reactants almost to the level of intact indicators. At the same time, there was the concentration dependence in manifestation of the antioxidant activity, i.e. an increase in the concentration of the test compound in the sample led to a more significant decrease in the content of TBA-reactants in the liver homogenate.

Thus, according to the results of the first stage of the experiment compounds **1, 6, 7,** which showed the highest antioxidant activity, were selected for further study of the anti-inflammatory activity.

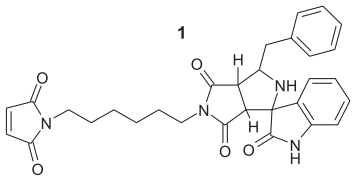
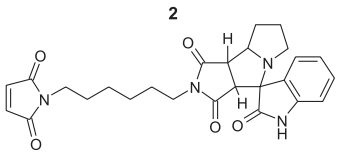
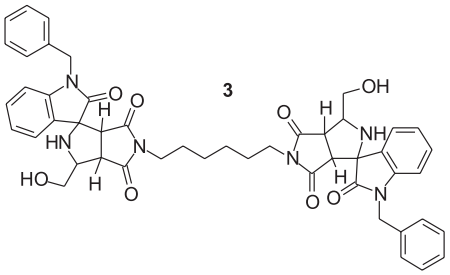
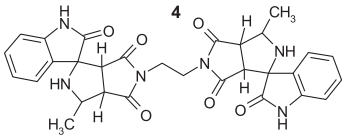
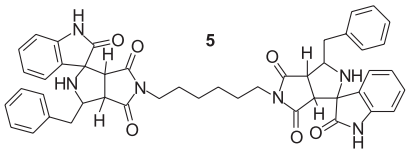
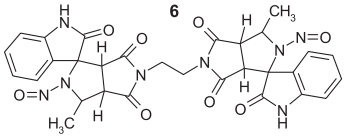
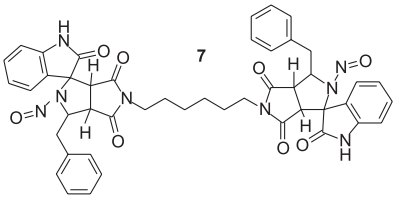
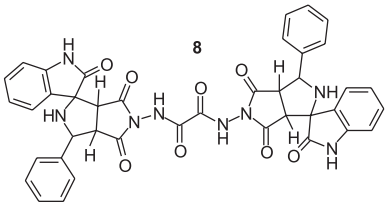
Based on the structure of the compounds under research it was decided to study the potential anti-inflammatory (anti-exudative) properties on the model of carrageenan edema.

The results of the study presented in Tab. 3 and 4 showed that the control group of rats had a gradual increase in inflammation up to 3 h (the prostaglandin phase) and a decrease to 24 h in the experiment.

Compounds **1, 7** in the doses of 5 mg/kg and 7 mg/kg did not show sufficient anti-inflammatory activity throughout the experiment and did not significantly affect the reduction of the edema size compared to the control group. However, in separate control hours of the experiment there were significant differences compared to the control group. Thus, compound **7** showed a moderate anti-inflammatory activity in the dose of 5 mg/kg in 4 h, and in the dose of 7 mg/kg in 3 h, but this activity was significantly lower than the activity demonstrated by indomethacin. The anti-inflammatory activity of compound **1** was revealed as reducing swelling both in the dose of

Table 2

The study of the effect of the test compounds on the content of TBA-reactants in the liver homogenate (mmol/g tissue), $\bar{x} \pm S_x$, n = 5)

Compound	The concentration of the compound, μmol	The content of TBA-reactants in the samples, mmol/g tissue	Confidence
	50	9.35 ± 0.71	*/**
	100	8.52 ± 0.67	**
	50	8.52 ± 0.59	**
	100	12.32 ± 0.88	*
	50	10.80 ± 0.72	*
	100	9.59 ± 0.81	*/**
	50	12.56 ± 0.82	*
	100	13.09 ± 0.97	*
	50	8.37 ± 0.54	**
	100	10.27 ± 0.92	*
	50	8.45 ± 0.47	**
	100	8.29 ± 0.69	**
	50	8.59 ± 0.63	**
	100	8.22 ± 0.45	**
	50	12.94 ± 0.79	*
	100	13.09 ± 0.84	*
Intact	–	8.37 ± 0.37	
Control	–	11.03 ± 0.59	*

Notes: * – significant differences in relation to the intact group ($p \le 0.05$); ** – significant differences in relation to the control group ($p \le 0.05$).

Table 3

The effect of compounds 1, 6, 7 on the dynamics of inflammation compared to indomethacin on the model of carrageenan inflammation of the hind limb in rats (ΔV c.u., M ± m, n = 6)

The time of observation	Control pathology	1		6		7		Indomethacin, 5.25 mg/kg
		5 mg/kg	7 mg/kg	5 mg/kg	7 mg/kg	5 mg/kg	7 mg/kg	
1 h	15.77 ± 0.11	15.37 ± 1.85**	14.37 ± 1.81**	14.70 ± 0.96**	12.97 ± 1.10*	14.23 ± 1.06**	14.32 ± 1.75**	10.12 ± 0.71*
2 h	31.28 ± 2.21	30.87 ± 3.17**	28.78 ± 2.02*/**	25.60 ± 3.55*/**	22.01 ± 0.84*	29.58 ± 1.97**	26.24 ± 2.11**	21.85 ± 2.76*
3 h	62.78 ± 7.30	45.20 ± 2.55*/**	47.19 ± 4.78*/**	29.08 ± 0.65*/**	25.91 ± 0.70*	56.37 ± 2.49**	47.19 ± 4.67*/**	25.29 ± 4.98*
4 h	35.91 ± 1.36	26.47 ± 2.22*/**	24.59 ± 2.14*/**	21.53 ± 1.48*/**	16.40 ± 1.10*	29.98 ± 1.76*/**	20.12 ± 3.01*/**	15.89 ± 0.75*
5 h	27.67 ± 1.15	23.36 ± 2.14*/**	21.47 ± 1.87*/**	15.84 ± 0.43*/**	12.26 ± 0.65*	25.36 ± 1.68**	17.36 ± 1.78*/**	12.20 ± 0.57*
24 h	13.79 ± 0.69	11.24 ± 0.98**	10.45 ± 1.23**	9.68 ± 1.02*/**	6.90 ± 0.53*	11.21 ± 0.49**	10.33 ± 1.32*/**	6.79 ± 0.42*
The average anti-exudative activity, %	–	15.36	19.97	31.86	44.44	11.49	26.07	48.01

Notes: * – significant differences in relation to the control pathology ($p \leq 0.05$); ** – significant differences in relation to indomethacin ($p \leq 0.05$); ΔV – difference between the swollen paw and its initial size, c.u.

5 mg/kg and in the dose of 7 mg/kg in 3 to 5 h of the experiment compared to the control pathology; but, at the same time, it was significantly lower than the activity of the reference drug.

The highest activity at all phases of the inflammatory process was shown by compound **6**, in both doses of 5 mg/kg and 7 mg/kg. However, its effects on the development of carrageenan inflammation depended on the dose administered to animals, and the dose of 7 mg/kg appeared to be more effective. In 1 h of the experiment the introduction of compound **6** in this dose significantly reduced inflammation till the end of the experiment compared to the control pathology. The maximum anti-exudative activity (58.72 %) was observed in 3 h during the period of the action of such inflammatory mediators as prostaglandins. it may indicate the ability to suppress the COX activity. However, the anti-exudative activity was recorded in 1 h – 27.01 % (the phase of biogenic amines), and in 2 h of the experiment – 29.63 % (the kinin phase). Later in 4, 5 and 24 h the anti-inflammatory activity of compound **6** remained rather high (45.66 %, 55.69 % and 49.96 %, respectively).

The anti-inflammatory activity of the reference drug indomethacin was fixed throughout the experiment. The highest reduction of edema (by 2.4 times compared to the control group) was observed in 3 hours (the anti-exudative activity 59.71 %) corresponding to the anticyclooxygenase activity of the drug. In 1 h the size of edema under the effect of indomethacin was reduced by 1.5 times, and in 2 h – by 1.6 times compared to the control group, indicating a moderate effect on such inflammatory mediators as biogenic amines and kinins.

Thus, during the study of the anti-inflammatory (anti-exudative) activity of compounds **1, 6, 7**, it was found that compounds **1, 7** in the doses of 5 and 7 mg/kg revealed a moderate anti-inflammatory activity, which was lower compared to the activity of the reference drug. However, compound **6** in the doses of 5 and 7 mg/kg

Table 4

The anti-exudative activity of compounds **1, 6, 7** compared to indomethacin on the model of carrageenan inflammation of the hind limb in rats

The group of animals	**1**		**6**		**7**		Indomethacin
The time of observation	5 mg/kg	7 mg/kg	5 mg/kg	7 mg/kg	5 mg/kg	7 mg/kg	5.25 mg/kg
1 h	2.53	8.87	6.78	27.01	9.76	9.19	35.82
2 h	1.31	7.99	18.15	29.63	5.43	16.11	30.14
3 h	28.00	24.83	53.67	58.72	10.21	24.83	59.71
4 h	26.28	31.52	40.04	45.66	16.51	43.97	55.75
5 h	15.57	22.40	42.75	55.69	8.34	37.26	55.90
24 h	18.49	24.22	29.80	49.96	18.70	25.09	50.76

showed a pronounced anti-inflammatory effect, which mechanism was probably implemented through the inhibitory effect on the synthesis of prostaglandins and biogenic amines. The analysis of the data obtained showed that the anti-inflammatory activity of compound **6** in the dose of 7 mg/kg and indomethacin did not significantly differ.

CONCLUSIONS

It has been found that the preparatory method of the three-component cascade transformation of isatin, α-amino acids and dipolarophils based on *bis*-maleimides is effective for the synthesis of spiro-2-oxindole[3,3']pyrrole mono- and *bis*-derivatives. A series of hexamethylene- and ethylene-N,N'-*bis*(spiroindole-3,3'-pyrrolo[3,4-*c*]pyrrole-4'-nitroso-2a,5a'-dihydro-2,2',6'(1*H*,1'*H*,5'*H*)-trione) symmetric derivatives have been obtained. The structure of the compounds obtained has been confirmed. When studying the antioxidant activity of the compounds synthesized it has been found that the most active substances are 1'-(hexamethylene-N-maleimido)-5'-benzyl-2a',5a'-dihydro-1'*H*-spiroindole-3,3'-pyrrolo[3,4-*c*]pyrrole-2,2',6'(1*H*,3'*H*,5'*H*)-trione **1**; ethylene-N,N'-*bis*(spiroindole-3,3'-pyrrolo[3,4-*c*]pyrrole-4'-nitroso-5'-methyl-2a,5a'-dihydro-2,2',6'(1*H*,1'*H*,5'*H*)-trione) **6**; hexamethylene-N,N'-*bis*(spiroindole-3,3'-pyrrolo[3,4-c] pyrrole-4'-nitroso-5'-benzyl-2a,5a'-dihydro-2,2',6 (1*H*,1'*H*,5'*H*)-trione) **7**. Compound **6** has also shown the anti-inflammatory activity at the reference drug level.

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

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