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SYNTHESIS, ANALGESIC AND ANTICONVULSANT ACTIVITY OF NOVEL UREAS WITH PYRROLE MOIETY

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The article is devoted to the study of new biologically active compounds based on various derivatives of the novel ureas with pyrrole moiety with different substituents and screening of the activity of synthesized compounds. The preparative method ethyl 2,4-dimethyl-5-{[(R-amino)carbonyl]amino}-1H-pyrrole-3-carboxylate and N,N''-(3,5-dimethyl-1H-pyrrole-2,4-diyl)bis(N'-R-urea) synthesis was developed while brief boiling the corresponding ethyl ester 5-azydocarbonyl-2,4-dymetylpyrrole-3-carboxylic acid or 3,5-dyazydocarbonyl-2,4-dymetylpyrrole in dioxane with aqueous ammonia. The synthesis of novel derivatives of pyrrole was conducted in these conditions for pharmacological screening. The result revealed that the studied new pyrrolyl ureas derivatives exhibit analgesic and anticonvulsant activity.

Key words: analgetic, anticonvulsant, n,n'-substituted ureas, pyrrole, biological activity.

Харченко О., Коваль Д., Воевудский М., Астахина В., Бибик Е., Мелещенко А., Ткаченко Е. Синтез, аналгетическая и противосудорожная активность производных мочевины с пиррольным фрагментом // Український медичний альманах. – 2013. – Том 16, № 4. – С. 57-61.

Статья посвящена изучению новых биологически активных соединений на основе производных впервые синтезированных мочевин с пиррольным фрагментом и изучению фармакологической активности полученных веществ. Было разработано препаративную методику синтеза производных этилового эфира 2,4-диметил-5-{[(R-амино)карбонил]амино}-1Н-пиррол-3-карбоновой кислоты и N,N''-(3,5-диметил-1Н-пиррол-2,4-диил)бис (N'-R-мочевин) при непродолжительном кипячении соответствующего этилового эфира 5-азидокарбонил-2,4-диметилпиррол-3-карбоновой кислоты или 3,5-диазидокарбонил-2,4-диметилпиррола в диоксане с аммиаком или разными аминами. Были проведены комплексные биологические испытания (аналгетическая и противосудорожная активности) новых производных пиррола. В результате исследований соединение 5b (35.1%) проявило активность на среднем уровне в сравнении с анальгином. Все остальные протестованные соединения в соответствующей дозе значительно не снижали количество «корчей» и таким образом не проявили анальгетическую активность. Все синтезированные соединения не рекомендованы для дальнейших испытаний противосудорожной активности, т. к. при их испытании смертность экспериментальных животных составила 100%.

Ключевые слова: анальгетическая, противосудорожная, биологическая активность, n,n'-производные мочевины,пиррол.

Харченко О., Коваль Д., Воєвудський М., Астахіна В., Бибик О., Мелещенко А., Ткаченко Є. Синтез, аналгетична та протисудомна активність похідних сечовини з пірольним фрагментом // Український медичний альманах. – 2013. – Том 16, № 4. – С. 57-61.

Стаття присвячена вивченню нових біологічно активних сполук на основі похідних вперше синтезованих сечовин з пірольним фрагментом та вивченню фармакологічної активності синтезованих сполук. Було розроблено препаративну методику синтезу похідних етилового естеру 2,4-диметил-5-{[(R-аміно)карбоніл]аміно}-1Н-пірол-3-карбонової кислоти та N,N''-(3,5-диметил-1Н-пірол-2,4-диіл)біс(N'-R-сечовин) при короткотривалому кип'ятінні відповідного етилового естеру 5-азидокарбоніл-2,4-диметилпірол-3-карбонової кислоти або 3,5-диазидокарбоніл-2,4-диметилпіролу в діоксані з аміаком або різними амінами. Було проведено комплексні біологічні дослідження (аналгетична та протисудомна активності) нових похідних піролу. В результаті дослідження сполука 5b (35.1%) проявила активність на середньому рівні в порівнянні з анальгином. Всі інші протестовані сполуки у відповідній дозі значительно не знижували кількість «корчів» і таким чином не проявили анальгетичну активність. Всі отримані сполуки не рекомендовані для подальших досліджень їхньої протисудомної активності, т. я. при її дослідженні смертність експериментальних тварин склала 100%.

Ключові слова: анальгетична, протисудомна, біологічна активність, n, n '- похідні сечовини, пірол.

Introduction: Anticonvulsant drugs are widely used in modern neurological and psychiatric practice for the treatment of epilepsy, bipolar disorder, chronic pain syndrome. In all cases the long-term therapy is expected, which can be regarded as a compromise between clinical efficacy, toxicity and cost of treatment [1].

Drugs of the future should be a potentially effective means against different types of epilepsy which relieve convulsive states of various origins as well as means of their prevention. They differ from the first generation anticonvulsants by better tolerability and long-term use safety.

Epilepsy is among the oldest known disorders of the brain. It accompanies the human race throughout its existence. The assets of modern pharmacology made it possible to achieve considerable success in therapy of this disease. However, the requirements for antiepileptic drugs pose more and more challenges for researchers. Treatment of this disease often goes on for years; therefore along with high efficiency the preparations should exercise a minimum of side effects [1]. The duration of treatment and socio-economic conditions of the country for the past 20 years also reveals an important economic aspect of therapy. That is why there is an urgent

problem of development, research and clinical implementation of the next-generation of antiepileptic drugs which are both low-toxic and economically affordable.

At the same time there is a great need for a new generation of analgesic drugs, most of which has a negative effect on the activity of the gastrointestinal tract (non-steroidal anti-inflammatory drugs) and are not permitted for use by children and patients with specific diseases of the stomach and liver [2]. This is due to the fact that the prevalence of pain syndrome among the population is 30% according to the latest information [3], with about 18% of seniors regularly taking analgesics. In the U.S., the cost of treating of back pain was higher than the treatment of cancer patients [4]. The most common cause of chronic pain is a list of diseases of the musculoskeletal system: rheumatoid arthritis, reactive arthritis, psoriatic arthritis, spondylitis, osteoarthritis, gout, osteoporosis, chondropathy et al [5].

Rheumatic pain is multimodal by its mechanism and involves peripheral and central components (inflammatory, mechanical, vascular, neurogenic, psychosomatic), which requires many months and years of continuous therapy in order to improve the quality of life of patients.

It should be noted that chronic pain is accompanied by increasing the level of substance P in the cerebrospinal fluid on the background of reducing the concentration of serotonin, which can lead to a reduction in "pain threshold" [6]. This factor can greatly influence the efficiency of known analgesics used for pain syndromes and reduce their effectiveness in chronic pain treatment. Thus the research for new highly efficient and low-toxic analgesic and anticonvulsant drugs is very important.

The purpose of the research. Make a new synthesis of N-substituted N'-(2,4-dimethyl-3-carboethoxy) pyrrol-5-yl urea and 2,4-dimethyl-3 ,5-dycarbomoilamino pyrroles and to study anticonvulsant and analgesic activity of the obtained derivatives.

Materials and methods used. Synthesis of the substances was performed using reagents provided by "Merck" company (Darmstadt, Germany) and «Sigma-Aldrich» (Missouri, USA). The structure and composition of the synthesized compounds was confirmed by 1H NMR spectroscopy.

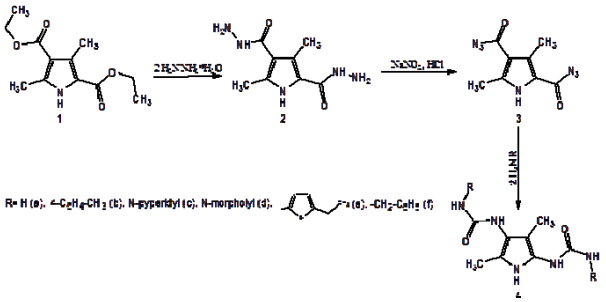
Biological studies were conducted on 72 white mongrel mature rats weighing 170-210g in the autumn-winter period in certified morphological laboratory of the "Lugans'k State Medical University" (certificate number R105/2008, 30.12.2011r.).

During the study animals were kept in a vivarium of SU "Lugansk State Medical University" on a standard diet of no more than six individuals in the cell according to the rules of working with laboratory animals. Animal welfare and manipulation that were performed to them conform to the Law of Ukraine № 3447-IV dated 21.02.06 "On protection of animals from cruelty," and consistent with the basic principles of the "European Convention for the protection of vertebrate animals used for experimental and other scientific purposes" (Strasbourg, 1986), the Declaration "On the humane treatment of animals" (Helsinki, 2000) and the "General ethical principles of animal experiments " National Congress of Bioethics (Kyiv, 2001).

Before the experiment, all animals were carefully inspected, taking into account their weight, age, physical activity and condition of hair cover. After the external examination rats with deviation from the normal rules of behavior where rejected. The experiment began simultaneously with both control group and experimental group of animals.

The number of experimental animals was 6 per group which is a conventional minimum for statistical analysis and allows obtaining reliable results. A minimum number of experimental groups were taken to achieve goals and meet the challenges of the study

Results and discussion. We have previously developed a method for the synthesis of ethyl 2,4-dimethyl-5-{[(R-amino)carbonyl]amino}-1H-pyrrole-3-carboxylate [7] and found that they exhibit the pronounced antioxidant activity in the oxidative modification of proteins *in vitro* in the initiation of free radical oxidation. It was also found that these substances prevent the accumulation of the oxidative modification of proteins products [7]. The next stage of our work was to study other types of biological activity (analgesic and anticonvulsive) of some previously synthesized pyrrole derivatives [7] and novel derivatives of N,N"-(1H-pyrrole-2,4-diyl)diurea obtained by the following scheme (Fig. 1). The ^{1}H NMR spectroscopy data are given in Table 1



Scheme 1

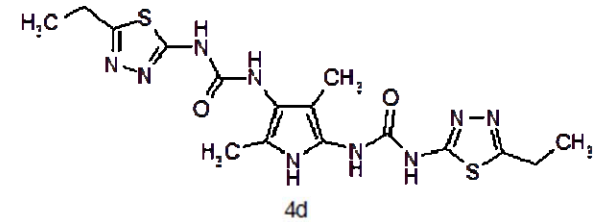
To determine the analgesic and anticonvulsant activities compounds of diurea of pyrrole were selected (4d and 5a). The compounds were synthesized by the method described previously [7].

Analgesic activity of synthesized compounds. Several models of peripheral pain used to study the mechanism of the peripheral analgesic action of drugs: acetic acid, acetylcholine and kaolin "convulsions ", which are based on chemical pain stimuli. Acetic "convulsions" is classical screening model. Intraperitoneal introduction of acetic acid contrib-

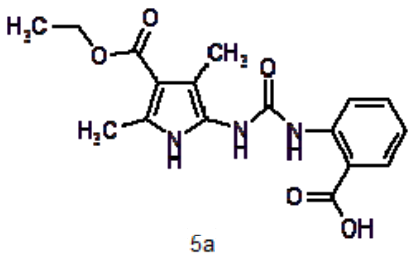
utes to the overall activation of nociceptive local release of bradykinin, histamine, serotonin, prostaglandins and leukotrienes, which leads to uncontrolled reductions of abdominal muscles - "convulsions", accompanied by stretching of hind limbs and arching of a back.

Table 1. Characteristics of the synthesized compounds 4 a-f

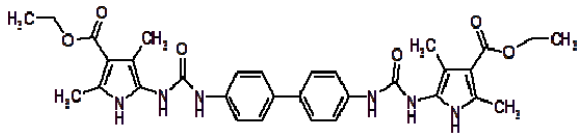
№	Yield	m.p.	^{1}H NMR spectral data			
			2,4-CH_3	NH pyrrole	NHCONH	Other groups
4a	63%	223-225	2,02 m	10,74 s	5,46 s 6,71 s 7,05 s	5,46 s 2H NH_2CONH, 6.71 s 2H NH_2CONH
4b	25%	241-244	2,21 s	11,01 s	7,03 s	1,46 s 3H 4-CH_3-Ph, 1,85 s 3H 4-CH_3-Ph, 7,31 m 6H 4-CH_3-Ph, 7,54 m 6H 4-CH_3-Ph
4c	69%	221-224	1,81 s 1,95 s	10,62 s	7,39 s	1,45 m 20H pyperidyl
4d	71%	230-234	1,62 s 1,90 s	9,95 s	7,41 s 7,90 s	3,55 m 16H morpholyl
4e	55%	169-173	1,68 s 1,98 s	10,42 s	6,98 s 7,70 s 8,24 s	1,24 m 6H 5-C_2N_2S-CH_2-CH_3, 2,83 m 4H 5-C_2N_2S-CH_2-CH_3
4f	85%	205-208	1,63 s 1,96 s	10,20 s	6,16 s 6,42 s 6,97 s 7,54 s	4,19 m 4H -CH_2-Ph, 7,25 m 10H -CH_2-Ph



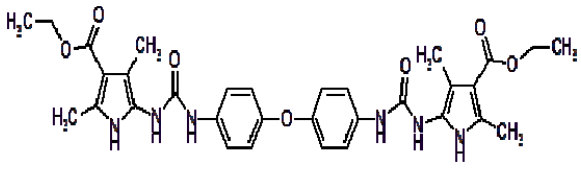
4d



5a



5b



5c

Fig. 1. Structural formulas of compounds selected for analgesic and anticonvulsant activity screening. The structure of the obtained compounds 4d, 5a, 5b, 5c was confirmed by elemental analysis and 1H NMR spectroscopy. Substances 5a, 5b, 5c obtained by the method which is described in detail in [7].

Two types of nerve fibers involved in the transmission of pain: fast myelinated A-delta fibers (acute localized pain) and slow non-myelinated C fibers (conduct delocalized "dull" pain);both conduct impulses through the spinal cord to the thalamus and reticular formation. Then the pain impulses are distributed in the hypothalamus and adjacent cortex of the brain - the frontal and temporal lobes.

Screening studies were conducted on the acetic acid "convulsions", which were modeled by intraperitoneal introduction of 0.6% acetic acid on the basis of 10 ml per 1 kg of body weight. Chemical irritant was introduced 1 hour after introduction of the studied compounds, followed by observation of the animals for 20 minutes and the detection of "convulsions". The control group of animals received an equivalent amount of 0.9% sodium chloride solution. metamizol sodium ("Analgin-Darnytsya" 50% 2 mL) was used intraperitoneally at a dose of 55 mg / kg as a comparison preparation. All test substances were intraperitoneally injected with single dose of 2 mg / kg 1 hour before modeling pathological conditions.

Analgesic activity (AA) was assessed by the ability of the agents to reduce the number of "convulsions" in the experimental group compared with the control one and expressed in percentages.

Screening results of the analgesic activity of the compounds listed in Tab. 2.

Table 2. Analgesic activity of synthesized compounds.

Groups	"Acetic convulsions" (during 20 min)	AA (%)
Control group	74	
Referent Group (analginum 55 mg / kg)	23	68,9
4d	72	2,7
5a	60	18,9
5b	48	35,1
5c	73	5,4

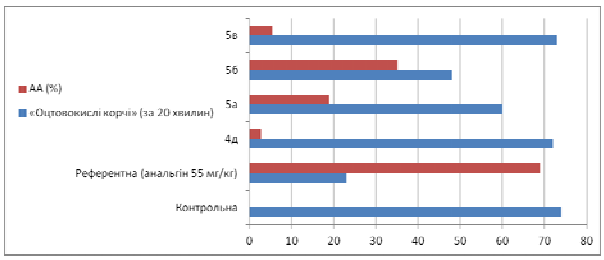


Fig. 2. Determination of analgesic activity of the compounds

As seen from the table data, the most pronounced medium analgesic activity provides a compound 5b (35.1%). In contrast, all other test substances in a similar dose did not significantly reduce the number of "convulsions" recorded and therefore had no analgesic effect.

Anticonvulsant activity of synthesized compounds. It is well known that nociceptive (pain) system and the system of convulsive discharges generation are related to different anatomical structures of the brain and operate with different neurophysiological mechanisms [1]. Thalamus is the collector of all kinds of sensivity, including pain. In the event of convulsion activity the central role belongs to the hippocampus. Moreover, the clinical implementation of convulsion is associated with mobile nerve fibers while pain sensation associated with sensitive nerve fibers.

To determine the anticonvulsant activity of the obtained compounds a research was conducted on the corozal seizures model .

Model of corozal seizures. To study the anticonvulsant effect studied drugs were injected intraperitoneally once only for 30 minutes before entering corozal at a dose of 80 mg / kg. Each series of experiments was began with the introduction of convulsive dose of corazol . When stable seizures has been detected in the control group of animals then study of seizure activity was carried out among rats of experimental groups. The animals were observed during 30 minutes.

Following parameters were recorded: duration of the latent period, the incidence of tonic-clonic seisures, mortality expressed through a percentage of the total number of animals in the group. The absence of seizures indicated the anticonvulsant action of the substance.

Diazepam was used as a comparison drug which belongs to the group of benzodiazepine derivatives, classic tranquilizer with a strong anticonvulsant effect. Diazepam was administered intraperitoneally at a dose of 0.5 mg / kg.

Results obtained from research regarding anticonvulsant activity of these substances were recorded and systematized in a table (Table 3) and as a graph (Fig. 3, Fig. 4).

Table 3. Results of anticonvulsant activity of these substances

Groups	«**Corazol seizures** » (during 30 minutes)	
	duration of the latent period, s	incidence of tonic-clonic seizures, number/min
Control	40-44-45-43-44-45	20-18-22-20-21-22
diasepam	12-18-15-11-20-15	10-16-14-11-11-14
4d	22-28-25-21-28-25	12-23-14-12-15-14
5a	28-38-34-27-40-34	19-22-20-19-22-20
5b	34-40-42-35-39-42	10-12-15-11-12-14
5c	35-38-40-36-39-41	15-14-16-15-15-16

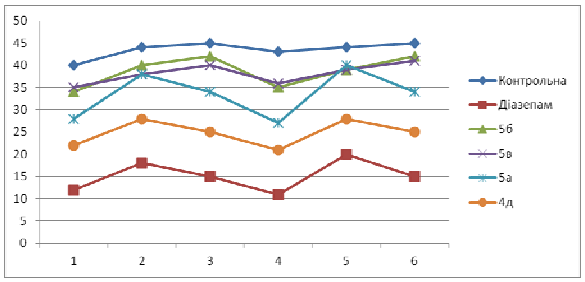


Fig. 3. The duration of the latent period of experimental animals in determination of anticonvulsant activity

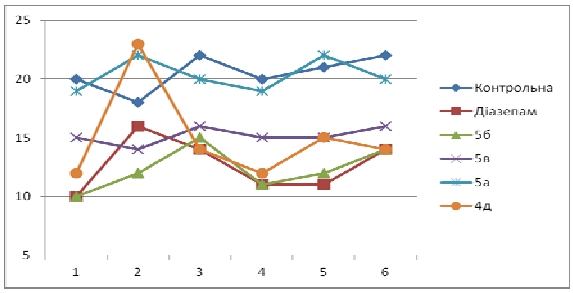


Fig. 4. The incidence of tonic-clonic seizures during screening for anticonvulsant activity

As seen from the data in the table, the compound 5b performed the most pronounced anticonvulsant activity related to effective decrease of the incidence of tonic-clonic seizures, which is slightly inferior to the activity found in classical anticonvulsants diazepam.

Also compounds 5c and 4d displayed anticonvulsant activity of moderate intensity on the effectiveness of reducing the incidence of tonic-clonic seizures.

In contrast to the above compounds, the substance (5a) in the same dosage did not significantly reduced the incidence of tonic-clonic seizures in control group, and therefore had no anticonvulsant effect.

Compared to diazepam all these substances had almost no change in performance relative to the length of the latent period. Only compound 4d showed average efficiency as to the standard of comparison.

Since the mortality (fixed as 100%) of the total number of experimental animals, then the substance with even severe average anticonvulsant activity is not recommended for further study in this area.

Experimental. The progress of reactions and purity of products were controlled by thin-layer chromatography (TLC) method on silicagel plates($60F_{254}$ fromMerck) in a Tol/i-Propanol (10:3,v/v) solvent system at room temperature. The spots on the plates were visualized using iodine vapor. Solid products were purified by the crystallization process. ^{1}H NMR spectra were recorded on a Varian 300VXR. Chemical shifts (δ) are expressed in parts per million (ppm) relative to tetramethylsilane(TMS) as an internalstandard, using DMSO-d_6.

2,4-dimethyl-3,5-bis(2-azidocarbonyl)-1H-pyrrole (3).

20 g (0.095 mol) of 3,5-dimethyl-1H-pyrrole-2,4-dicarbohydrazide was suspended in 200 ml of water. 90 ml of hydrochloric acid was added and the mixture was cooled to t = 0-5 ° C. Then a solution of 13.08 g (0.19 mol) of sodium nitrite in 30 ml of water was added in portions with stirring. The precipitate was filtered, washed with water and dried. Yield 60%.

General method of synthesis of N,N''-(3,5-dimethyl-1H-pyrrole-2,4-diyl)bis(N'-R-urea) (4a – f).

0.5 g (0.002 mol) of 2,4-dimethyl-3,5-bis(2-azidocarbonyl)-1H-pyrrole (3) was dissolved in 10 ml of dry dioxane and heated. After boiling 0.004 mole of the corresponding amine was added and refluxed for 1-2 hours. The reaction mixture was cooled, the precipitate formed wes filtered, washed with petroleum ether, crystallized from acetonitrile.

Synthesis of ureas 5a, 5b and 5c was conducted by the method which was described in detail in [7]. The ^{1}H NMR spectral data of these compounds are given in Table 4

Tab. 4. The characteristic of synthesized compounds 5 a-c.

№	Yieald	m.p.	^{1}H NMR spectral data				
			CH_3CH_2O	2,4-CH_3	NH pyrrole	NHCONH	Other groups
5a	10,3	229-232	1,25 t, 4,15 q	1,88 s, 2,37 s	11,25 s	-	7,23 t 2H Ph, 7,71 t 1H Ph, 7,93 t 1H Ph, 11,63 s OH
5b	74,7	290 p	1,24 t, 4,13 q	2,00 s, 2,34 s	11,06 s	7,87 s, 8,75 s	7,43-7,51 m 8H Ph-Ph
5c	89,2	232-235	1,24 t, 4,12 q	1,99 s, 2,33 s	11,03 s	7,81 s, 8,64 s	6,88 d 4H Ph, 7,41 d 4H Ph

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