ЕКСПЕРИМЕНТАЛЬНА ТА КЛІНІЧНА ФАРМАКОЛОГІЯ

Recommended by Doctor of Biology, professor V.M.Kravchenko

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ANTIMICROBIAL, ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF 2-AMINO-6-ETHYL-4,6-DIHYDROPYRANO[3,2-*c*][2,1]BENZOTHIAZINE 5,5-DIOXIDES AND TRIETHYLAMMONIUM 3-[1-(4-HYDROXY-1-ETHYL-2,2-DIOXIDO-1*H*-2,1-BENZOTHIAZIN-3-YL)-3-(HET) ARYLMETHYL]-1-ETHYL-1*H*-2,1-BENZOTHIAZIN-4-OLAT 2,2-DIOXIDES

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Key words: 2,1-benzothiazine 2,2-dioxide; 2-amino-4H-pyran; antimicrobial activity; anti-inflammatory activity; analgesic activity

The search and development of new bioactive compounds possessing antimicrobial, anti-inflammatory and analgesic activities are the topical issues of the current medicinal chemistry. 2,1-Benzothiazine 2,2-dioxides condensed with the 2-amino-3-R-4-(het)aryl-4H-pyran core and triethylammonium 3-[1-(4-hydroxy-1-ethyl-2,2-dioxido-1H-2,1-benzothiazin-3-yl)-3-(het)arylmethyl]-1-ethyl-1H-2,1-benzothiazin-4-olat 2,2-dioxides have been studied for the antimicrobial activity. The absence of the antibacterial activity in condensed derivatives has been found. Simultaneously, this group of compounds possesses a pronounced antifungal activity against C. albicans. Ethyl 2-amino-4-{2-[2-oxo-2-(N-R-amino)ethoxy]phenyl}-6-ethyl-4,6-dihydropyrano[3,2-c][2,1]benzothiazine-3-carboxylate 5,5-dioxides have been proposed for further synthetic and screening microbiological studies. Among the triethylammonium salts studied triethylammonium 3-[1-(4-hydroxy-1-ethyl-2,2-dioxido-1H-2,1-benzothiazin-3-yl)-3-arylmethyl]-1-ethyl-1H-2,1-benzothiazin-4-olat 2,2-dioxides have displayed a high level of the antimicrobial activity against E. coli, P. aeruginosa and C. albicans. The study of the anti-inflammatory and analgesic activities of compounds belonging to different groups of 2-amino-3-R-4-R'-6-ethyl-4,6-dihydropyrano[3,2-c][2,1]benzothiazine 5,5-dioxides has shown that they possess the anti-inflammatory activity; moreover, 2-amino-3-ethoxycarbonyl-4-(4-chlorophenyl)-6-ethyl-4,6-dihydropyrano[3,2-c][2,1]benzothiazine 5,5-dioxide is as active as the reference drug Piroxicam. The compounds under research were not inferior to the reference drug by the level of the analgesic activity. These facts allow to consider 2-amino-3-R-4-R'-6-ethyl-4,6-dihydropyrano[3,2-c][2,1]benzothiazine 5,5-dioxides as promising compounds for further search of new substances with the anti-inflammatory and analgesic activity among them.

Infectious diseases are a significant threat to human life causing one third of the total number of deaths in the world per year. Infectious diseases and their after-effects are the cause of 30% of deaths in adults; for children this value exceeds 60% [5]. These diseases kill about 20.000 people per year in Ukraine, including 400-600 children [4].

It is known that the excessive and improper use of antibiotics leads to spread of infections caused resistant strains of microorganisms. [15, 20, 22, 26, 33-35]. Appearance of bacterial and fungal strains that are resistant to novel antibiotics causes the continuing need to develop new drugs.

Another actual problem of the current pharmacology is increase the efficiency of treatment of diseases accompanied with inflammation and pain. The most common drugs used for treating such conditions are non-steroidal anti-inflammatory drugs (NSAIDs) [13]. Currently, more than 30 million people worldwide take NSAIDs every day, and 40% of these patients are aged over 60 years; about 20% of inpatients receive these drugs [17, 25]. Over the past decades the number of NSAIDs has significantly increased, and nowadays this group comprises a lot of medicines, which are different from each other by the peculiarities of use and effects on the body. Despite of this, all of them have a large number of contraindications and can provoke numerous adverse effects limiting their use [6, 12, 19].

The present article is devoted to the study of the antimicrobial, anti-inflammatory and analgesic properties of 1*H*-2,1-benzothiazin-4(3*H*)-one 2,2-dioxide derivatives condensed with the 2-amino-4*H*-pyran core **I** and triethylammonium salts of 3-[1-(4-hydroxy-1-ethyl-2,2-dioxido-1*H*-2,1-benzothiazin-3-yl)-3-(het)arylmethyl]-1-ethyl-1*H*-2,1-benzothiazin-4-olat 2,2-dioxides **II** (Fig. 1).

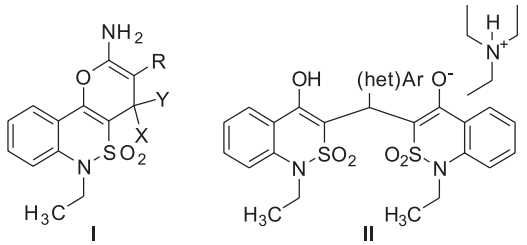


Fig. 1. General structures of the compounds studied.

Such research direction is caused by prospects of development of novel antimicrobial agents among 2-amino-4*H*-pyran derivatives as it was reported in previous works [14, 16, 21]. Moreover, 1*H*-2,1-benzothiazine 2,2-dioxides were considered as quinolone-like scaffolds and proved to be a promising framework for construction of antibacterial drugs [31].

Furthermore, based on the literature data (Fig. 2) it can be assumed that the 2,1-benzothiazine 2,2-dioxide core is promising for developing anti-inflammatory and analgesic agents.

The relevance of developing novel NSAIDs based on 1*H*-2,1-benzothiazine 2,2-dioxides is also due to their isosteric relationships with the core of 2,3-dihydro-4*H*-1,2-benzothiazin-4-one 1,1-dioxide, which is the skeleton of famous NSAIDs related to the oxicam group.

Materials and Methods

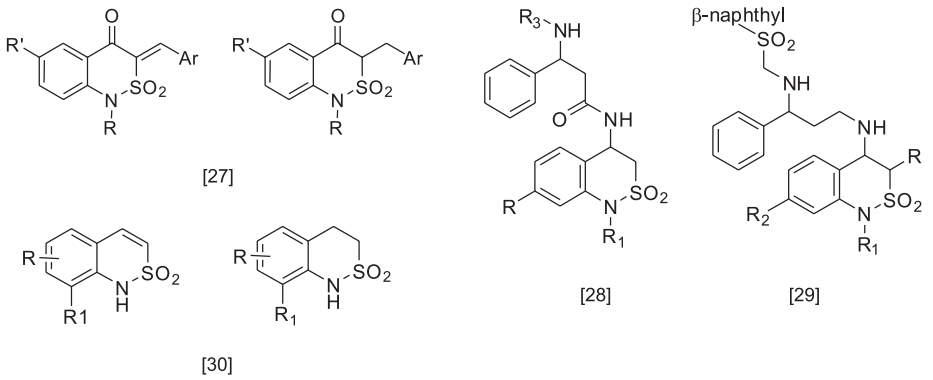
Three groups of 2-amino-3-R-6-ethyl-4,6-dihydropyrano[3,2-*c*][2,1]benzothiazine 5,5-dioxides **I** (Fig. 1) were tested to possess the antimicrobial, anti-inflammatory and analgesic activities. These groups of compounds differed by the residue bound to position 4 of the 2-amino-4*H*-pyran core. Compounds of the first group comprise the 4-heteryl residue (**5a-d**, **6a-d**), the second group contains the 4-aryl residue (**10a,b**, **11c,d**), and the third group comprises a spiro-linked 2-oxindole core (**8**) (Tab. 1). Most of them were previously synthesized [23, 24]. Moreover, the new 2-amino-3-R-6-ethyl-4,6-dihydropyrano [3,2-*c*][2,1]benzothiazine 5,5-dioxides **8, 10a, 10b, 11c** were prepared and studied.

The procedure for the synthesis of spiro[(2-amino-3-cyano-6-ethyl-4,6-dihydropyrano[3,2-*c*][2,1]benzothiazin-5,5-dioxide)-4,3′-(5′-methyl-indolin-2′-one)] 8. To the solution of 1-ethyl-1*H*-2,1-benzothiazin-4(3*H*)-one 2,2-dioxide **1** (0.225 g, 0.001 Mol), 5-methylisatine **7** (0.161 g, 0.001 Mol) and malononitrile **3** (0.066 g, 0.001 Mol) in ethanol (10 mL) add triethanolamine (0.149 g, 0.001 Mol). Heat the mixture at 50-60°C for 4 h and cool to the room temperature. Filter the precipitate formed, wash with ethanol and dry on air. Recrystallize the crude product from the mixture of ethanol/dimethylformamide (1:1) to obtain the pure product **8**. Yield – 0.33 g (77%), a light grey crystalline powder. M. p. – 221-223°C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 0.95-1.12 (3H, m, $NCH_2C\underline{H}_3$); 2.22 (3H, s, 5'-$C\underline{H}_3$); 3.75-3.93 (2H, m, $NC\underline{H}_2CH_3$); 6.71-6.80 (1H, m, Ar); 7.01-7.16 (2H, m, Ar); 7.40-7.49 (1H, m, Ar); 7.51-7.63 (3H, m, Ar, NH_2); 7.64-7.73 (1H, m, Ar); 7.92-8.02 (1H, m, H-5, benzothiazine); 10.58 (1H, s, NH). Found, %: C 61.05; H 4.13; N 12.77; S 7.21. $C_{22}H_{18}N_4O_4S$. Calculated, %: C 60.82; H 4.18; N 12.90; S 7.38.

The procedure for the synthesis of 2-amino-3-R-4-R′-6-ethyl-4,6-dihydropyrano[3,2-*c*][2,1]benzothiazine 5,5-dioxides 10a,b, 11c. To the solution of 1-ethyl-1*H*-2,1-benzothiazin-4(3*H*)-one 2,2-dioxide **1** (0.225 g, 0.001 Mol), active methylene nitrile **3**, **4** (0.001 Mol) and the corresponding benzaldehyde **9a-c** (0.001 Mol) in ethanol (5-10 mL) add the catalytic amount of triethylamine. In the case of aldehyde **9a** heat the mixture at 45-50°C for 1 h, in the case of aldehydes **9b,c** reflux the mixture for 4 h. Cool the mixture to the room temperature; filter the resulting precipitates, wash with ethanol, then dry on air and recrystallize from ethanol to obtain the pure derivatives **10a,b, 11c**.

2-Amino-4-[2-(cyanomethoxy)phenyl]-6-ethyl-4,6-dihydropyrano[3,2-c][2,1]benzothiazine-3-carbonitrile 5,5-dioxide ***10a.*** Yield – 0.38 g (87%), a light grey fine crystalline powder. M. p. – 228-230°C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.03 (3H, t, *J* = 7.00, $NCH_2C\underline{H}_3$); 3.86 (2H, q, *J* = 6.86, $NC\underline{H}_2CH_3$); 4.87 (1H, s, CH pyran); 4.98 (1H, d, *J* = 15.72, $OC\underline{H}_2CN$); 5.11 (1H, d, *J* = 15.72, $OC\underline{H}_2CN$); 6.99 (1H, t, *J* = 7.53, Ar); 7.08 (1H, d, *J* = 8.23, Ar); 7.18 (1H, d, *J* = 7.41, Ar); 7.23-7.31 (3H, m, Ar, NH_2); 7.37 (1H, t, *J* = 7.68, Ar); 7.51 (1H, d, *J* = 8.23, Ar); 7.62 (1H, t, *J* = 7.52, Ar); 7.95 (1H, d, *J* = 7.96, Ar). Found, %: C 60.73; H 4.32; N 13.07; S 7.41. $C_{22}H_{18}N_4O_4S$. Calculated, %: C 60.82; H 4.18; N 12.90; S 7.38.

Ethyl [2-(2-amino-3-cyano-6-ethyl-5,5-dioxido-4,6-dihydropyrano[3,2-c][2,1]benzothiazin-4-yl)phenoxy] acetate ***10b.*** Yield – 0.23 g (47%), a light yellow crystalline powder. M. p. – 179-181°C. ^{1}H NMR (400 MHz,

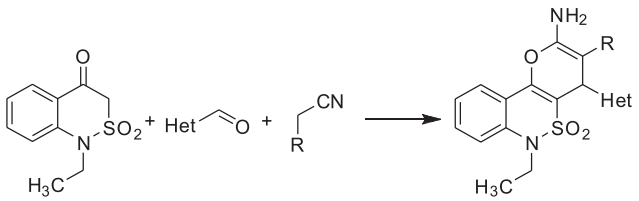


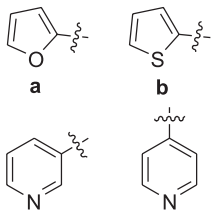
[27] [28] [29] [30]

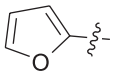
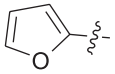
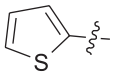
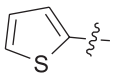
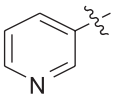
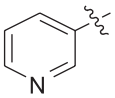
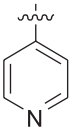
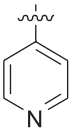
Fig. 2. 2,1-Benzothiazine 2,2-dioxides possessing the anti-inflammatory and analgesic activity.

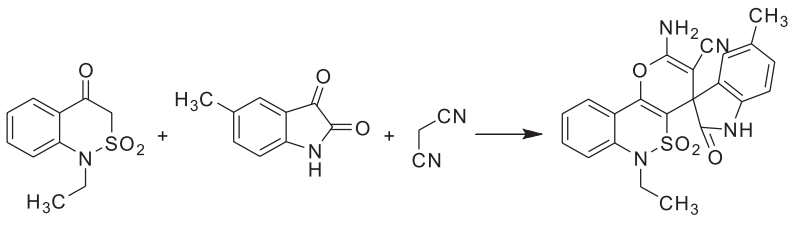
Table 1

The MIC values for 2-amino-3-R-6-ethyl-4,6-dihydropyrano[3,2-*c*][2,1] benzothiazine 5,5-dioxides under research



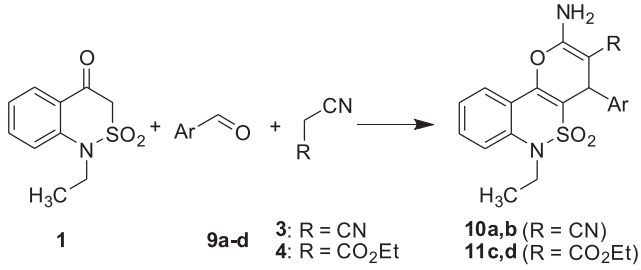


No.	Het	R	MIC (μg/mL)				
			S. aureus (ATCC 6538)	*E. coli* (ATCC 8739)	*B. subtilis* (ATCC 6633)	*P. aeruginosa* (ATCC 9027)	*C. albicans* (ATCC 10231)
5a [23]		CN	250	125	125	125	125
6a [23]		CO_2Et	250	500	250	500	500
5b [23]		CN	250	500	250	125	62.5
6b [23]		CO_2Et	500	250	250	125	125
5c [23]		CN	250	125	growth	growth	62.5
6c [23]		CO_2Et	250	125	125	250	62.5
5d [23]		CN	250	125	125	250	500
6d [23]		CO_2Et	500	250	500	125	125
DMSO			250	125	125	250	500

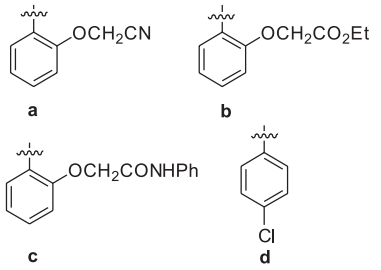


No.	MIC (μg/mL)				
	S. aureus (ATCC 6538)	*E. coli* (ATCC 8739)	*B. subtilis* (ATCC 6633)	*P. aeruginosa* (ATCC 9027)	*C. albicans* (ATCC 10231)
8	125	125	125	125	62.5
DMSO	250	125	125	250	500

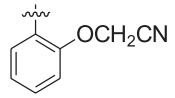
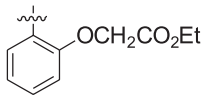
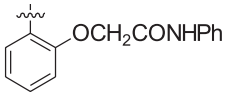
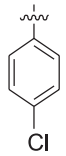
Continuation of Table 1



1 **9a-d** **3**: R = CN, **4**: R = CO_2Et **10a,b** (R = CN), **11c,d** (R = CO_2Et)



a **b** **c** **d**

No.	Het	R	MIC (μg/mL)				
			S. aureus (ATCC 6538)	*E. coli* (ATCC 8739)	*B. subtilis* (ATCC 6633)	*P. aeruginosa* (ATCC 9027)	*C. albicans* (ATCC 10231)
10a		CN	250	125	125	250	250
10b		CN	125	250	125	125	250
11c		CO_2Et	250	125	125	125	62.5
11d [24]		CO_2Et	growth	125	growth	125	250
DMSO			250	125	125	250	500

DMSO-d_6), δ, ppm (*J*, Hz): 1.02 (3H, t, *J* = 6.86); 1.12 (3H, t, *J* = 7.00); 3.86 (2H, q, *J* = 7.00); 4.08 (2H, q, *J* = 6.95); 4.52 (1H, d, *J* = 15.64, $OCH_2COOC_2H_5$); 4.68 (1H, d, *J* = 15.73, $OCH_2COOC_2H_5$); 4.96 (1H, br. s, CH pyran); 6.87-6.86 (2H, m, Ar); 7.11 (1H, d, *J* = 7.14, Ar); 7.14 - 7.23 (3H, m, Ar, NH_2); 7.36 (1H, t, *J* = 7.55, Ar); 7.51 (1H, d, *J* = 8.23, Ar); 7.57-7.65 (1H, m, Ar); 7.92 (1H, d, *J* = 7.68, Ar). Found, %: C 59.77; H 4.88; N 8.54; S 6.81. $C_{24}H_{23}N_3O_6S$. Calculated, %: C 59.86; H 4.81; N 8.73; S 6.67.

Ethyl 2-amino-4-{2-[2-oxo-2-(phenylamino)ethoxy] phenyl}-6-ethyl-4,6-dihydropyrano[3,2-c][2,1]benzothiazin-3-carboxylate 5,5-dioxide ***11c.*** Yield – 0.30 g (53%), a white crystalline powder. M. p. – 225-227°C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 0.96-1.07 (6H, m, NCH_2CH_3, OCH_2CH_3); 3.83-3.95 (4H, m, NCH_2CH_3, OCH_2CH_3); 4.63 (1H, d, *J* = 15.31, OCH_2CONH); 4.69 (1H, d, *J* = 15.32, OCH_2CONH); 5.32 (1H, s, CH pyran); 6.85-6.91 (2H, m, Ar); 7.05 (1H, t, *J* = 7.00, Ar); 7.10-7.16 (2H, m, Ar); 7.24-7.35 (3H, m, Ar); 7.48-7.63 (4H, m, Ar); 7.74 (2H, s, NH_2); 7.98 (1H, d, *J* = 7.96, Ar); 9.34 (1H, s, OCH_2CONH). Found, %: C 62.38; H 5.22; N 7.47; S 5.34. $C_{30}H_{29}N_3O_7S$. Calculated, %: C 62.60; H 5.08; N 7.30; S 5.57.

In addition, the antimicrobial activity of triethylammonium salts **II** (Fig. 1) was studied. The synthesis of salts **II** was described in our previous works [23, 24].

The antimicrobial activity of condensed derivatives **I** and triethylammonium salts **II** (Fig. 1) *in vitro* was studied in accordance with the requirements of the State Pharmacopoeia of Ukraine (ed. 1) by the double serial dilution method in the liquid growth medium. The compounds synthesized were tested against Pharmacopoeial strains of gram negative (*E. coli* – ATCC 8739, *P. aeruginosa* – ATCC 9027) and gram positive (*S. aureus* – ATCC 6538, *B. subtilis* – ATCC 6633) bacteria, as well as against the fungal strain of *C. albicans* (ATCC 10231) [1, 8]. Solutions of the test compounds with the concentrations of 500, 250, 125, 62.5, 31.25, 15.62 μg/mL were prepared using dimethylsulfoxide (DMSO) as a solvent and a broth as the growth medium. DMSO was also used as the reference drug because of its moderate antimicrobial activity [7]. Dilutions of DMSO were prepared by the similar way without using the compounds under research. As the result, concentrations of DMSO in the solutions prepared were 550, 275, 137.5, 68.75, 34.38, 17.19 μg/mL (taking into account the density value of DMSO – 1.1 mg/mL).

Inocula of the bacterial and fungal cultures were prepared according to the optical turbidity standard of 0.5 IU from a daily agar culture. The suspension of microorganisms (the microbial load – 150×10^6 microbes per 1 mL) was transferred into the solutions of the test compounds prepared and the reference test-tubes. The test-tubes

ontaining bacterial cultures were kept in thermostat for 24 h at 37°C, and test-tubes containing the culture with *C. albicans* were kept in thermostat for 48 h at 25°C. The lowest concentration of the compounds under research, at which any turbidity (growth of microorganisms) was not observed, was taken as the Minimum Inhibitory Concentration (MIC) value.

The study of the anti-inflammatory and analgesic activities was carried out in albino adult rats of both sexes weighing 185-254 g. The animals were randomly divided into three groups of equal number (control, experimental and comparison groups). The use of Piroxicam (Sopharma, Bulgaria) as the reference drug was due to its isosteric relationships with the core of 2,1-benzothiazine 2,2-dioxide as it was mentioned above. The anti-inflammatory activity was studied on the model of the carrageenan-induced paw edema, and the analgesic activity was evaluated on the model of the local inflammatory hyperalgesia. Pathology in both cases was reproduced by the intraplantar injection of 0.1 mL of 1% solution of λ-carrageenan ("Fluka", Switzerland) into the right hind limb of rats [3, 21].

The test compounds and the reference drug were introduced orally as fine aqueous suspensions stabilized with Tween-80 (0.5 mL/100 g) one hour prior to the λ-carrageenan injection [3, 21]. The screening dose for Piroxicam was 2 mg/kg, the test compounds were introduced in the doses that were equimolar to the reference drug. The control group received an equivalent amount of Tween-80 water solution.

The initial and final values of the paw edema volume were measured by water displacement method using a digital plethysmometer (IITC Life Science, USA). The final values of the paw edema volume were received 3 h after the phlogogen agent injection. The initial values of the pain threshold were measured using an Ugo Basile 37215 analgesimeter [32], and the final values of the pain threshold were obtained on the inflamed paw 3 h after administration of the test substances.

The anti-inflammatory activity (%) was expressed as percentage of edema inhibition in the animals treated with the test compound and Piroxicam compared to the control rats. The anti-inflammatory activity was calculated according to the formula:

$$AIA = \frac{\Delta V_c - \Delta V_e}{\Delta V_e} \cdot 100\%,$$

where: AIA – is the anti-inflammatory activity, %; ΔV_c – is the average percentage of edema in the control group, %; ΔV_e – is the average percentage of edema in the experimental group (comparison group), %.

The analgesic activity (%) was evaluated by the change of the pain threshold checked on the inflamed paw in the rats received the test compound and the reference drug compared to the animals from the control group. The analgesic activity was calculated according to the formula:

$$AA = \frac{\Delta PT_c - \Delta PT_e}{\Delta PT_e} \cdot 100\%,$$

where: AA – is the analgesic activity, %; ΔPT_c – is the average percentage of the pain threshold decrease in the control group, %; ΔPT_e – is the average percentage of the pain threshold decrease in the experimental group (comparison group), %.

The results of biological tests were also processed by the method of variation statistics using Student's t-criterion and such programs as STATISTICA 7.0, StatPlus 2009 and MS Excel 2007 [8, 10].

The current study was carried out in full compliance with the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on protection of animals used for scientific purposes [9, 11, 18].

Results and Discussion

The MIC values for 2-amino-3-R-6-ethyl-4,6-dihydropyrano[3,2-*c*][2,1]benzothiazine 5,5-dioxides **I** are presented in Tab. 1. As one can see, the most preferable kind of activity for these derivatives is the antifungal effect against the fungal strain of *C. albicans*. 4-Heteryl-4*H*-pyrans **5b**, **6b,c**, spiro derivative **8** and 4-aryl-4*H*-pyran **11c** appeared to be the most active antifungal compounds (Tab. 1). The high antifungal activity of acetamide derivative **11c**, as compared to cyanomethyl **11a** and ethoxycarbonyl **11b** derivatives, opens up great opportunities for searching more effective antifungal agents by varying the substituent in the amide moiety.

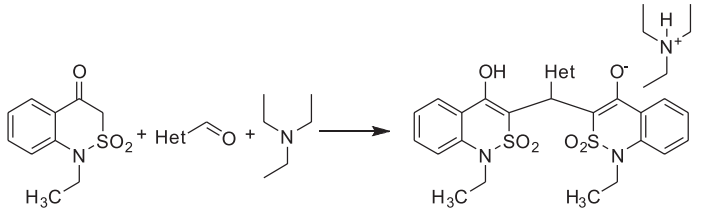
At the same time, the studies have shown that the antibacterial activity is not typical for 2-amino-3-R-6-ethyl-4,6-dihydropyrano[3,2-*c*][2,1]benzothiazine 5,5-dioxides **I**. The MIC values for these derivatives towards the strains of *S. aureus, B. subtilis, E. coli, P. aeruginosa* were almost the same as the MIC values for the reference drug – DMSO. Moreover, some of the derivatives studied showed the higher MICs against bacterial strains as compared to DMSO. It can indicate a protective action of the test compounds towards the microorganisms used.

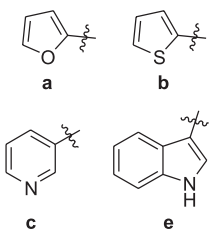
The MIC values for triethylammonium salts **II** (Fig. 1) are given in Tab. 2. The microbiological screening of triethylammonium salts **13**, **14** showed a significant difference in MICs between heterylcarbaldehyde derived products **13** and benzaldehydes derived compounds **14**. Thus, enolates **13a-c,e** appeared to be unpromising for further investigations. Their MIC values were the similar to the reference drug DMSO. At the same time, the interesting results were received in the studies of the antimicrobial activity of triethylammonium salts derived from benzaldehydes **14** (Tab. 2). Compounds **14d-f** revealed the high level of the antifungal activity. Moreover, compounds **14e,f** were active against the strain of *P. aeruginosa*, and compound **14f** showed a moderate activity against the strain of *E. coli*. Therefore, triethylammonium salts of 3-[1-(4-hydroxy-1-ethyl-2,2-dioxido-1*H*-2,1-benzothiazin-3-yl)-3-arylmethyl]-1-ethyl-1*H*-2,1-benzothiazin-4-olat 2,2-dioxides are promising compounds for further microbiological screening.

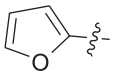
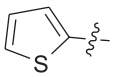
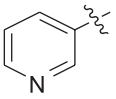
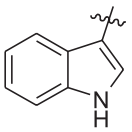
Three representatives referring to different classes of 2-amino-3-R-6-ethyl-4,6-dihydropyrano[3,2-*c*][2,1]benzothiazine 5,5-dioxides were selected for the study of the anti-inflammatory and analgesic activities. These representatives were 4-aryl derivative **11d**, 4-heteryl derivative **5c** and spiro derivative **8** (Tab. 3).

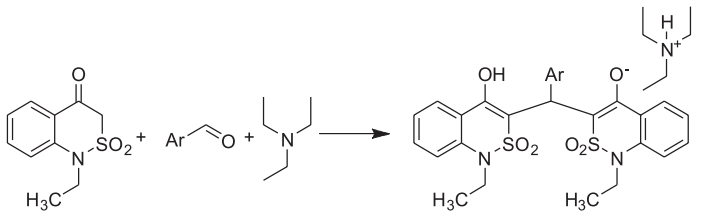
Table 2

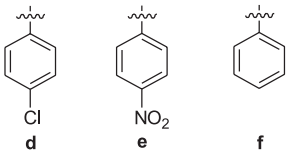
The MIC values for triethylammonium salts of 3-[1-(4-hydroxy-1-ethyl-2,2-dioxido-1*H*-2,1-benzothiazin-3-yl)-3-(het)arylmethyl]-1-ethyl-1*H*-2,1-benzothiazin-4-olat 2,2-dioxides under research





№	Het	MIC (μg/mL)				
		S. aureus (ATCC 6538)	*E. coli* (ATCC 8739)	*B. subtilis* (ATCC 6633)	*P. aeruginosa* (ATCC 9027)	*C. albicans* (ATCC 10231)
13a [23]		250	125	125	250	125
13b [23]		125	250	125	125	125
13c [23]		250	500	250	250	500
13e [23]		250	250	125	125	125
DMSO		275	137.5	137.5	275	500





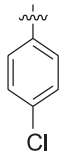
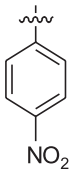
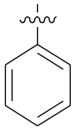
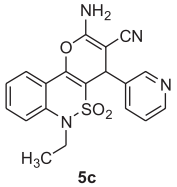
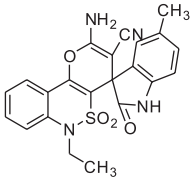
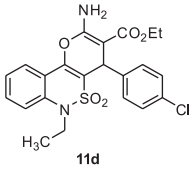
№	Ar	MIC (μg/mL)				
		S. aureus (ATCC 6538)	*E. coli* (ATCC 8739)	*B. subtilis* (ATCC 6633)	*P. aeruginosa* (ATCC 9027)	*C. albicans* (ATCC 10231)
14d [24]		125	125	125	250	62.5
14e [24]		125	125	125	62.5	62.5
14f [24]		250	62.5	125	62.5	62.5
DMSO		250	125	125	250	500

Table 3

The results of studying the anti-inflammatory and analgesic activity

Compound	Dose, mg/kg	The average percentage of edema, %	Anti-inflammatory activity, %	Decrease of the pain threshold, %	Analgesic activity, %
 **5c**	2.3	55.5±6.8	26.2±9.0	30.5±2.7*	52.4±4.3
 **8**	2.6	57.2±3.0**	24.0±4.0**	16.4±4.5**	74.4±7.0**
 **11d**	2.8	38.0±5.6*	49.5±7.5	30.9±2.1*	51.8±3.3
Piroxicam	2.0	40.1±2.6*	46.7±3.5	29.4±3.4*	54.1±5.3
Control	–	75.2±7.8	–	64.1±4.0	–

Note: * – differences were significant at $p<0.05$ compared to the control group; ** – differences were significant at $p<0.05$ compared to Piroxicam.

The results of the screening are given in Tab. 3. As one can see, all the compounds studied had a preventative effect in the applied model of inflammation and decreased edema development compared to the control group. Compounds **5c** and **8** showed the anti-inflammatory activity by 26% and 24%, respectively. These results are of interest since during the screening phase the substantial level of the anti-inflammatory activity is more than 20% [2]. Simultaneously, ethyl 2-amino-4-(4-chlorophenyl)-6-ethyl-4,6-dihydropyrano[3,2-*c*][2,1]benzothiazin-3-carboxylate 5,5-dioxide was as active as the reference drug Piroxicam.

The promising results were received while studying the analgesic activity of compounds **5c**, **8**, **11d**. 4-Heteryl derivative **5c** and 4-aryl derivative **11d** were not inferior to Piroxicam and showed the activity level of about 50%. Spiro derivative **8** appeared to be much more active than the reference drug and showed the analgesic activity level of 74%.

These findings allow considering 2-amino-3-R-6-ethyl-4,6-dihydropyrano[3,2-*c*][2,1]benzothiazine 5,5-dioxides as a novel and promising class of compounds for treating disorders accompanied with inflammation and pain.

CONCLUSIONS

1. The antimicrobial activity of 2-amino-6-ethyl-4,6-dihydropyrano[3,2-*c*][2,1]benzothiazine 5,5-dioxides and triethylammonium 3-[1-(4-hydroxy-1-ethyl-2,2-dioxido-1*H*-2,1-benzothiazin-3-yl)-3-(het)arylmethyl]-1-ethyl-1*H*-2,1-benzothiazin-4-olat 2,2-dioxides has been studied. The most preferable kind of activity for the compounds under research has been found to be the antifungal effect against the fungal strain of *C. albicans*.

2. Triethylammonium salts of 3-[1-(4-hydroxy-1-ethyl-2,2-dioxido-1*H*-2,1-benzothiazin-3-yl)-3-arylmethyl]-1-ethyl-1*H*-2,1-benzothiazin-4-olat 2,2-dioxides have been proposed for further antimicrobial screening as a promising chemical group.

3. Three representatives of 2-amino-6-ethyl-4,6-difydropyrano[3,2-*c*][2,1]benzothiazine 5,5-dioxide have been studied for the anti-inflammatory and analgesic activity. The compounds under research have revealed the high level of the analgesic activity and the moderate level of the anti-inflammatory activity compared to the reference drug Piroxicam.

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АНТИМІКРОБНА, ПРОТИЗАПАЛЬНА ТА АНАЛГЕТИЧНА АКТИВНІСТЬ 2-АМІНО-6-ЕТИЛ-4,6-ДИГІДРОПІРАНО[3,2-*c*][2,1]БЕНЗОТІАЗИН 5,5-ДІОКСИДІВ І ТРИЕТИЛАМОНІЮ 3-[1-(4-ГІДРОКСИ-1-ЕТИЛ-2,2-ДІОКСИДО-1*H*-2,1-БЕНЗОТІАЗИН-3-ІЛ)-3-(ГЕТ)АРИЛМЕТИЛ]-1-ЕТИЛ-1*H*-2,1-БЕНЗОТІАЗИН-4-ОЛАТ 2,2-ДІОКСИДІВ

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***Ключові слова**: 2,1-бензотіазин 2,2-діоксид; 2-аміно-4Н-піран; антимікробна активність; протизапальна активність; аналгетична активність*

Пошук і створення нових біологічно активних сполук, що володіють антимікробною, протизапальною та аналгетичною активністю, є актуальним завданням сучасної медичної хімії. Для виявлення антимікробної активності досліджена серія 2,1-бензотіазин 2,2-діоксидів, конденсованих з ядром 2-аміно-3-R-4-(гет)арил-4Н-пірану, та ряд триетиламонію 3-[1-(4-гідрокси-1-етил-2,2-діоксидо-1Н-2,1-бензотіазин-3-іл)-3-(гет)арилметил]-1-етил-1Н-2,1-бензотіазин-4-олат 2,2-діоксидів. Встановлена відсутність антибактеріальної активності у конденсованих похідних. В той же час для цієї групи сполук є характерним наявність вираженого протигрибкового ефекту проти C. albicans. Для подальших синтетичних та скринінгових мікробіологічних досліджень запропоновані етил 2-аміно-4-{2-[2-оксо-2-(N-R-аміно)етокси]феніл}-6-етил-4,6-дигідропірано[3,2-c][2,1]бензотіазин-3-карбоксилат 5,5-діоксиди. В ряду досліджуваних триетиламонієвих солей високий рівень антимікробної активності відносно E. coli, P. aeruginosa та C. albicans виявили триетиламонію 3-[1-(4-гідрокси-1-етил-2,2-діоксидо-1Н-2,1-бензо-тіазин-3-іл)-3-арилметил]-1-етил-1Н-2,1-бензотіазин-4-олат 2,2-діоксиди. Вивчення протизапальної та аналгетичної активності сполук, що відносяться до різних груп 2-аміно-3-R-4-R'-6-етил-4,6-дигідропірано[3,2-c][2,1]бензотіазин 5,5-діоксидів, показало наявність у них протизапальної активності, причому 2-аміно-3-етоксикарбоніл-4-(4-хлорофеніл)-6-етил-4,6-дигідропірано[3,2-c][2,1]бензотіазин 5,5-діоксид виявив активність на рівні референс-препарату Піроксикаму. За рівнем аналгетичної активності всі досліджувані сполуки не поступалися препарату порівняння, що дозволяє розглядати 2-аміно-3-R-4-R'-6-етил-4,6-дигідропірано [3,2-c][2,1]бензотіазин 5,5-діоксиди як перспективні сполуки для подальшого пошуку серед них нових субстанцій, що володіють протизапальною та аналгетичною активністю.

ПРОТИВОМИКРОБНАЯ, ПРОТИВОВОСПАЛИТЕЛЬНАЯ И АНАЛЬГЕТИЧЕСКАЯ АКТИВНОСТЬ 2-АМИНО-6-ЭТИЛ-4,6-ДИГИДРОПИРАНО[3,2-*c*][2,1]БЕНЗОТИАЗИН 5,5-ДИОКСИДОВ И ТРИЭТИЛАММОНИЙ 3-[1-(4-ГИДРОКСИ-1-ЭТИЛ-2,2-ДИОКСИДО-1*H*-2,1-БЕНЗОТИАЗИН-3-ИЛ)-3-(ГЕТ)АРИЛМЕТИЛ]-1-ЭТИЛ-1*H*-2,1-БЕНЗОТИАЗИН-4-ОЛАТ 2,2-ДИОКСИДОВ

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***Ключевые слова**: 2,1-бензотиазин 2,2-диоксид; 2-амино-4Н-пиран; антимикробная активность; противовоспалительная активность; анальгетическая активность*

Поиск и создание новых биологически активных соединений, обладающих противомикробным, противовоспалительным и анальгетическим действием является актуальной задачей современной медицинской химии. Для определения противомикробной активности исследована серия 2,1-бензотиазин 2,2-диоксидов, конденсированных с ядром 2-амино-3-R-4-(гет)арил-4Н-пирана, и ряд триэтиламмоний 3-[1-(4-гидрокси-1-этил-2,2-диоксидо-1Н-2,1-бензотиазин-3-ил)-3-(гет)арилметил]-1-этил-1Н-2,1-бензотиазин-4-олат 2,2-диоксидов. Установлено отсутствие антибактериальной активности у конденсированных производных. В то же время для этой группы веществ характерно наличие выраженного противогрибкового действия против C. albicans. Для дальнейших синтетических и скрининговых микробиологических исследований предложены этил 2-амино-4-{2-[2-оксо-2-(N-R-амино)этокси]фенил}-6-этил-4,6-дигидропирано[3,2-c][2,1]бензотиазин-3-карбоксилат 5,5-диоксиды. В ряду исследуемых триэтиламмониевых солей высокий уровень противомикробной активности относительно E. coli, P. aeruginosa и C. albicans показали триэтиламмоний 3-[1-(4-гидрокси-1-этил-2,2-диоксидо-1Н-2,1-бензотиазин-3-ил)-3-арилметил]-1-этил-1Н-2,1-бензотиазин-4-олат 2,2-диоксиды. Изучение противовоспалительной и анальгетической активности соединений, относящихся к различным группам 2-амино-3-R-4-R'-6-этил-4,6-дигидропирано[3,2-c][2,1] бензотиазин 5,5-диоксидов, показало наличие у них противовоспалительной активности, причем 2-амино-3-этоксикарбонил-4-(4-хлорфенил)-6-этил-4,6-дигидропирано[3,2-c][2,1]бензотиазин 5,5-диоксид проявил активность на уровне референс-препарата Пироксикама. По уровню анальгетической активности тестируемые вещества не уступали препарату сравнения, что позволяет считать 2-амино-3-R-4-R'-6-этил-4,6-дигидропирано[3,2-c][2,1] бензотиазин 5,5-диоксиды перспективными соединениями для дальнейшего поиска среди них новых субстанций, обладающих противовоспалительным и анальгетическим действием.