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D. A. Lega, N. I. Filimonova, H. M. Dikaya, V. P. Chernykh, L. A. Shemchuk

National University of Pharmacy

IN VITRO ANTIMICROBIAL ACTIVITY EVALUATION OF 2-AMINO-3-R-6-ETHYL-4,6-DIHYDROPYRANO[3,2-c] [2,1] BENZOTHIAZINE 5,5-DIOXIDES WITH 4-ARYL SUBSTITUENT AND SPIROFUSED WITH 2-OXOINDOLINE CORE

Currently, antibiotic resistance is becoming a tremendous threat in treatment of bacterial and fungal infections. Therefore, a topical question is the search of new classes of antimicrobial drugs. The present article is devoted to the study of antimicrobial activity of 2-amino-3-R-6-ethyl-4,6-dihydropyrano[3,2-c][2,1]benzothiazine 5,5-dioxide derivatives comprising 4-aryl substituent and spirocondensed with 2-oxindole core. Such choice is caused by a high level of antimicrobial activity of some 2-amino-4-aryl-4H-pyran and 2-oxindole derivatives as was reported previously. Microbiological screening of 4-aryl-4H-pyrans showed the high antifungal activity for some derivatives, among which 2-amino-3-cyano-4-(4-dimethylaminophenyl)-6-ethyl-4,6-dihydropyrano[3,2-c][2,1]benzothiazine-5,5-dioxide had the lowest value of MIC. Spirocondensed derivatives of 2-oxindole displayed a higher antimicrobial influence as compared to 4-aryl substituted products. Compounds, that possess moderate antimicrobial activity against B. subtilis and P. aeruginosa as well as antifungal activity against C. albicans, were found among spiro-compounds. The lead compound appeared to be spiro[(2-amino-3-cyano-6-ethyl-4,6-dihydropyrano[3,2-c][2,1]benzothiazine-5,5-dioxide)-4,3'-(1'-methyl-indolin-2'-one)], which was effective against B. subtilis, P. aeruginosa and C. albicans. The results of microbiological screening are discussed from a perspective of «structure – antimicrobial activity» relationships.

Key words: 2,1-benzothiazin 2,2-dioxide; antimicrobial activity; benzaldehydes; isatins; active methylene nitriles; 4*H*-pyranes; spiro-compounds; three-component reaction

INTRODUCTION

For the past 70 years, antimicrobial drugs have been successfully used to treat bacterial and infectious diseases. Over time, however, many infectious organisms have adapted to the drugs designed to kill them. Antimicrobial resistance makes it harder to eliminate infections from the body as existing drugs become less effective. As a result, some infectious diseases are now more difficult to treat than they were just a few decades ago. As more microbes become resistant to antimicrobials, the protective value of these medicines is reduced. Due to the high cost of innovative antimicrobial drug development, the flow of new antimicrobials runs low.

According to the Centers for Disease Control and Prevention (CDC) (April 2011), antibiotic resistance in the United States costs an estimated $ 20 billion a year in excess health care costs and more than 8 million additional days that people spend in the hospital [8]. These Centers estimate that antibiotic resistant organisms cause about 2 million infections and 23.000 deaths occur every year in this country [28]. The global burden is staggering – each year, over 25.000 deaths in the European Union, 38.000 deaths in Thailand, and 58.000 deaths in India are attributable to antibiotic resistant infections [23].

Fluconazole-resistant Candida and multidrug-resistant Pseudomonas aeruginosa are among the most spread antibiotic resistance threats. For example, an estimated 51.000 healthcare-associated Pseudomonas aeruginosa infections occur in the United States each year. More than 6,000 (or 13 %) of these are multidrug-resistant, with roughly 400 deaths per year attributed to these infections [7].

Enhancing the role of yeasts in emergence of hospital infections led to the introduction in clinical practice of various antifungal drugs and their wide usage. This became the reason of appearance of antifungal drug resistance infections.

One of the ways to address this growing problem is search and development of new effective antimicrobial compounds among which 2-amino-4*H*-pyranes (**A**) and their hetero-fused derivatives (**B**) (Fig. 1) are one of the promising classes.

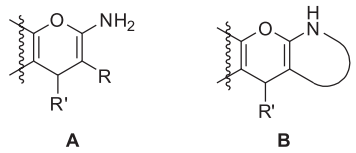


Fig. 1. *The general structure of 2-amino-4H-pyranes and their condensed derivatives*

4*H*-Pyranes and 4*H*-pyran-annulated heterocyclic compounds are well distributed in naturally occurring sources and also possess a broad spectrum of significant biological activities such as anticancer [32], cytotoxic [25], anti-HIV [11], anti-inflammatory [21], antihyperglycemic and antidyslipidemic [17]. Moreover, functionalized 4*H*-pyran derivatives have played increasing roles in synthetic approaches to promising compounds in the field of medicinal industry [10]. Recently, a series of synthetic 2-amino-4*H*-pyrans have been evaluated to possess potent anticancer [16], and antirheumatic [29] properties. Furthermore, antibacterial, antifungal, antiprotozoal and antiviral activities were also determined for a large number of 4*H*-pyran derivatives. This fact allows us to consider 4*H*-pyranes as very promising compounds for development of new antimicrobial chemotherapy drugs based on them. Nonannulated 2-amino-4*H*-pyranes as well as 2-amino-4*H*-pyranes condensed with carbo- and heterocycles (e.g. 5,6,7,8-tetrahydro-4*H*-chromenes, 4*H*-benzo[*h*]chromenes, 4*H*-pyrano[3,2-*c*]pyridines, 2,4-dioxo-2,3,4,8-tetrahydro-1*H*-pyrano[3,2-*d*]pyrimidines) are among derivatives possessing high level of antimicrobial activity. Selected examples of these heterocycles are presented on Fig. 2.

Moreover, pyranoquinoline derivatives are also known to possess various pharmacological and biological activities such as antimalarial [5], antiseptic [26], antihypertensive [15], antiviral effect and act as H_1-antihistamines [6]. Several quinolone derivatives have been reported as antimicrobial agents in the treatment of many infections [20]. Recently series of ethyl 2-amino-4-aryl-5-oxo-5,6-dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carboxylates (Fig. 3) were screened to have antibacterial activity against Gram positive and Gram negative bacteria [9, 22]. The results indicated that these compounds are highly effective against bacterial growth.

Lately, we have synthesized a series of 2-amino-6-ethyl-4,6-dihydropyrano[3,2-*c*][2,1]benzothiazine 5,5-dioxides [19, 27]. The latter are isosteric compounds to the abovementioned 4*H*-pyrano[3,2-*c*]quinolines and can be considered as promising antimicrobial agents (Scheme 1).

2-Amino-4-aryl-4*H*-pyranes (**I**) and 2-amino-4*H*-pyranes spiro-condensed with 2-oxindole core (**II**) are the objects of study of their antimicrobial activity (Fig. 4).

Evaluated compounds (Fig. 4) comprise 2,1-benzothiazine and 2-amino-4*H*-pyran cores. The derivatives of the former are also known to be biologically active compounds, but data about their antimicrobial properties are poor. So, this study allows to expand information related to the such kind of bioactivity of condensed 2,1-benzothiazines.

Variability of the compounds **I** and **II** structure on the position 3 of the 4*H*-pyrane ring as well as various nature of 4-aryl substituent or spiro-combined 2-oxoindoline core allowed us to determine the "structure – antimicrobial activity" relationships for evaluated compounds.

In this article we have tested *in vitro* antibacterial activity of 2-amino-6-ethyl-4,6-dihydropyrano[3,2-*c*][2,1] benzothiazine 5,5-dioxides **I** and **II** against Gram positive (*S. aureus* ATCC 6538 and *B. subtilis* ATCC 6633*)* and Gram negative (*E. coli* ATCC 8739 and *P. aeruginosa* ATCC 9027*)* bacterial strains and also antifungal activity against

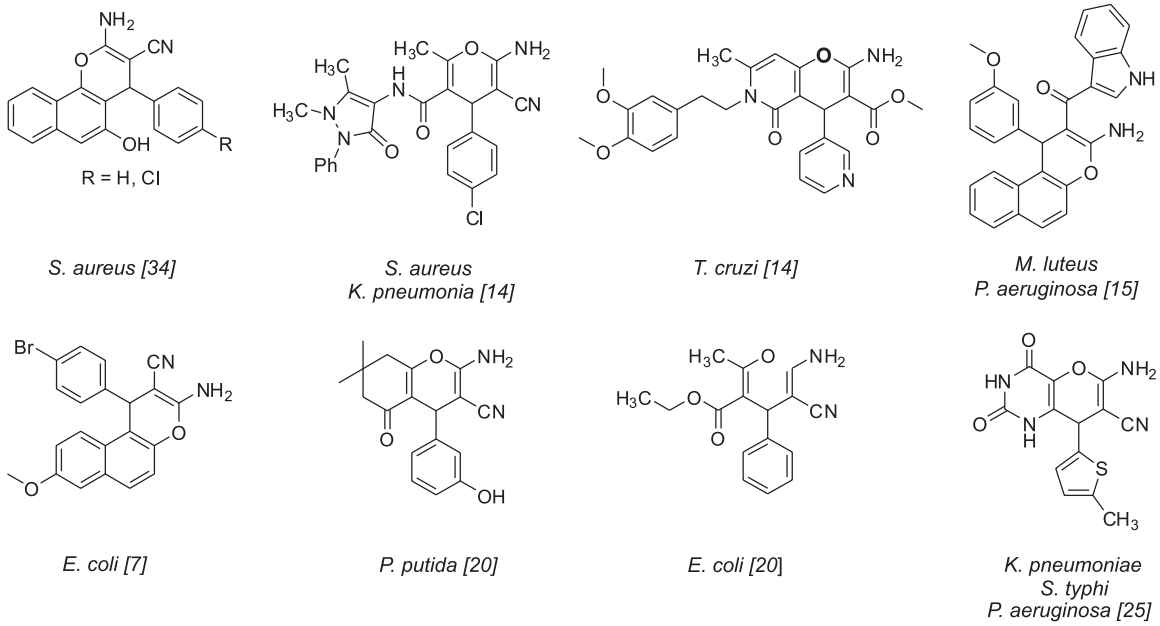


Fig. 2. *Representatives of antimicrobial synthetic 2-amino-4H-pyranes and their the most preferable targets*

the fungal strain of *C. albicans* (ATCC 10231) via the double serial dilution method in liquid growth medium [1]. Stock solutions of the tested compounds with 500, 250, 125, 62.50, 31.25 and 15.62 μg/mL concentrations were prepared using dimethylsulfoxide (DMSO) as solvent. Since DMSO possesses a moderate antimicrobial activity [2], it was used as reference antimicrobial drug. Inoculums of the bacterial and fungal cultures were also prepared and added to the test tubes with solutions of evaluated compounds and with reference drug. The test-tubes were incubated for 24 h at 37 °C (for bacterial strains) or for 48 h at 25 °C (for fungal strain) and carefully observed for the presence of turbidity. The minimum concentration at which no growth was observed was taken as the minimum inhibitory concentration (MIC) value. The comparison of the MICs (μg/mL) of evaluated compounds and reference drug against tested strains are presented in the corresponding tables below.

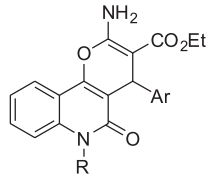
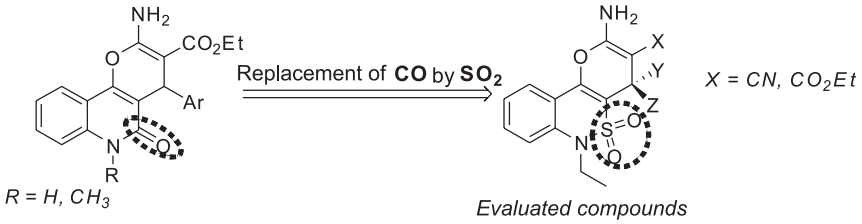


Fig. 3. *The general structure of pyrano[3,2-c]quinolones as promising antibacterial*

Three-component condensation of 1-ethyl-1*H*-2,1-benzothiazin-4(3*H*)-one 2,2-dioxide **1** with active methylene nitriles **2** (malononitrile and ethyl cyanoacetate) and benzaldehydes **3a-g**, which led to the evaluated derivatives **I**, was described previously in our work [19] (Scheme 2).

The detected values of MIC for tested compounds **IAa-Bd** are presented on the Tab. 1. From the activity report (Tab. 1) it was notified that, most of the compounds had no activity or showed slight antimicrobial activity against the bacterial and fungal strains. Furthermore, it was interesting, that compounds containing NO_2-group are almost inactive against used strains. Moreover, the DMSO solutions of examined derivatives (e.g. **IAa, IAe,** IBb, IBc) revealed the higher MIC values against bacterial strains compared to the reference DMSO. This could be due to either the protective action f the compounds **I** toward bacterial cells by the modifying of cell wall constituents or more strong interaction of compounds **I** with molecules of DMSO which prevents the destructive action of DMSO on the biomolecules of microorganisms' cell. At the same time about half of the tested compounds exhibited moderate or high antifungal activity which was much higher compared to their antibacterial influence. The most active derivatives are **IAd, IAg**, **IBa** at that for **IBa** the activity increases significantly when passing from 3-cyano to 3-ethoxycarbonyl derivative. Introducing of the more lipophilic anthracene residue in the molecule



Scheme 1. *Isosteric relations of pyrano[3,2-c][2,1]benzothiazine 5,5-dioxides with 4H-pyrano[3,2-c]quinolones*

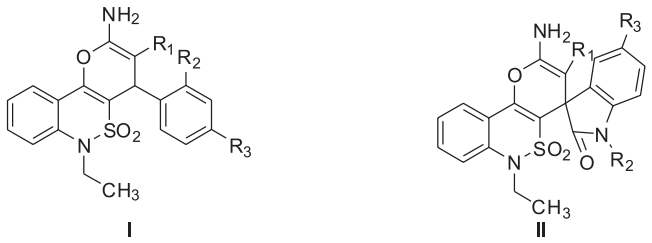
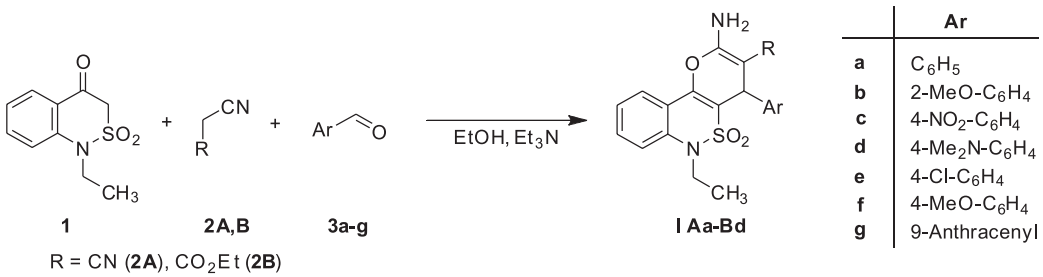


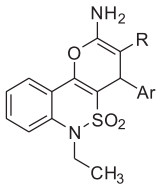
Fig. 4. *Evaluated compounds*



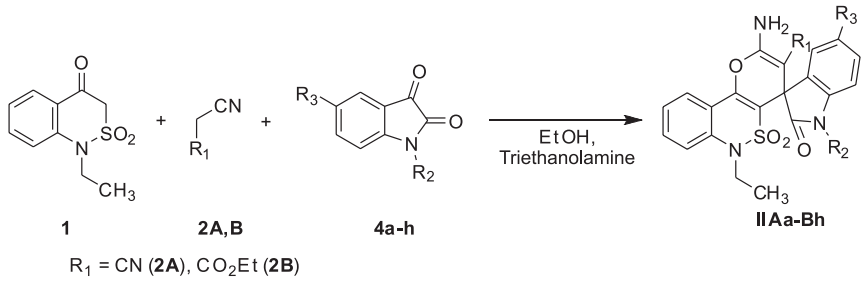
Scheme 2. *Synthesis of tested compounds* **I**

Table 1

MIC VALUES FOR COMPOUNDS I

Compounds			MIC (μg/mL)				
	R	Ar	*S. aureus* (ATCC 6538)	*E. coli* (ATCC 8739)	*B. subtilis* (ATCC 6633)	*P. aeruginosa* (ATCC 9027)	*C. albicans* (ATCC 10231)
IAa	CN	C_6H_5	500	500	500	500	500
IAb	CN	2-MeO-C_6H_4	250	125	125	125	125
IAc	CN	4-NO_2-C_6H_4	250	125	125	125	500
IAd	CN	4-Me_2N-C_6H_4	125	125	125	125	31.25
IAe	CN	4-Cl-C_6H_4	500	500	500	500	500
IAf	CN	4-MeO-C_6H_4	250	125	250	250	125
IAg	CN	9-Anthracenyl	250	250	125	125	62.5
IBa	CO_2Et	C_6H_5	250	250	125	125	62.5
IBb	CO_2Et	2-MeO-C_6H_4	250	500	250	500	500
IBc	CO_2Et	4-NO_2-C_6H_4	500	500	500	125	125
IBd	CO_2Et	4-MeO-C_6H_4	250	125	125	125	125
DMSO*	–	–	275	137.5	137.5	275	550

Note: * – Concentration for DMSO was calculated based on its density value 1.1 mg/mL.



Scheme 3. *Synthesis of tested compounds* **II**

also led to the increase of the antifungal activity. At the same time compound **IAd** showed the highest anti-Candida influence. Most probably, the reason for this is the presence of basic 4-dimethylamino group in structure of **IAd**, since it is known that the optimum of pH for *C. albicans* growth lies in the range of pH = 5.1-6.9 [31].

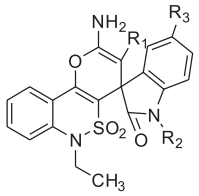
Another class of examined compounds **II** included 2-amino-3-R-6-ethyl-4,6-dihydropyrano[3,2-*c*][2,1]benzothiazine 5,5-dioxide core spirocombined with 2-oxindole nucleus (Fig. 4). Previously we described the synthesis of such compounds via the three-component one-pot interaction of 1-ethyl-1*H*-2,1-benzothiazin-4(3*H*)-one 2,2-dioxide **1** with active methylene nitriles **2A,B** and isatins [27]. In the present work we expand the series of spiro-derivatives **II** due to the application of formerly unutilized N- and 5-substituted isatins **4f-h** under the previously reported conditions. Physical properties, ^{1}H NMR-spectral data, elemental analysis data of new spiro-compounds **II** are presented in *Experimental part* (Scheme 3).

Compounds **II** (Fig. 4) represent spiro-2-oxindole derivatives, which have also drawn tremendous interest of researchers in the area of medicinal chemistry worldwide because they have been reported to possess various types of bioactivity among which anti-HIV [4], antitubercular [30], antimalarial [33] are the common. Combination of both 2-amino-4*H*-pyran and spiro-2-oxindole pharmacophores in one structure could lead to displaying of high bioactivity level including antimicrobial activity.

The detected values of MIC for tested compounds **IIAa-Bh** are presented in the Tab. 2. Microbiological investigations revealed that spiro-derivatives **II**, in general, possess a higher level of antimicrobial effect compared to 4-aryl-4*H*-pyranes **I**. In its turn, within the group of spiro-compounds, 3-cyano derivatives **IIA** turned out to be slightly more effective than 3-ethoxycarbonyl derivatives **IIB**. Among malononitrile derived products **IIA** we observed the next regularity: the bulkier substituent attached to the 2-oxindole nitrogen, the less activity exhibits a com-

Table 2

MIC VALUES FOR COMPOUNDS II

Compounds				MIC (μg/mL)				
	R_1	R_2	R_3	*S. aureus* (ATCC 6538)	*E. coli* (ATCC 8739)	*B. subtilis* (ATCC 6633)	*P. aeruginosa* (ATCC 9027)	*C. albicans* (ATCC 10231)
IIAa	CN	H	H	250	125	125	62.5	62.5
IIAb	CN	CH_2=CH-CH_2	H	250	250	500	500	500
IIAc	CN	H	Br	125	125	125	62.5	62.5
IIAd	CN	C_6H_5-CH_2	H	250	250	250	500	500
IIAe	CN	CH_3	H	125	125	62.5	62.5	62.5
IIAf	CN	C_2H_5	H	250	125	125	125	62.5
IIAg	CN	EtOOC-CH_2	H	250	125	250	125	125
IIBa	CO_2Et	H	H	250	250	500	125	125
IIBb	CO_2Et	CH_2=CH-CH_2	H	500	500	500	250	250
IIBc	CO_2Et	H	Br	125	125	125	125	62.5
IIBd	CO_2Et	C_6H_5-CH_2	H	250	500	250	125	125
IIBe	CO_2Et	CH_3	H	250	125	125	125	62.5
IIBf	CO_2Et	C_2H_5	H	250	125	125	250	500
IIBg	CO_2Et	EtOOC-CH_2	H	250	250	125	125	62.5
IIBh	CO_2Et	H	CH_3	250	125	125	250	125
DMSO*	–	–	–	275	137.5	137.5	275	550

Note: * – Concentration for DMSO was calculated based on its density value 1.1 mg/mL.

pound. Indeed, the most active compounds contain NH, N-methyl and N-ethyl groups in 2-oxindole ring, while allyl, benzyl and ethoxycarbonylmethyl substituents linked to the nitrogen significant decrease the antimicrobial activity. At the same time replacement hydrogen atom in position 5 of 2-oxindole ring with bromine atom has not any noticeable influence on the antimicrobial activity. Among spiro-derivatives **II**, in contrast to the 4-aryl-4*H*-pyrans **I**, we found compounds that showed a high level of antibacterial effect against *B. subtilis* (**IIAe**) and *P. aeruginosa* (**IIAa**, **IIAc**, **IIAe**). These results are of interest to discover a new class of compounds for treatment of multidrug-resistant *P. aeruginosa* strains. However, as for the 4-aryl-4*H*-pyrans **I**, the most prevalent kind of activity for tested derivatives **II** was antifungal effect. Compounds **IAa**, **IIAc**, **IIAe**, **IIAf**, **IIBc**, **IIBe**, **IIBg** displayed the lowest values of MIC (Tab. 2). It was also interesting that replacing of 3-cyano group in **IIAf** by the ethoxycarbonyl group (**IIBf**) led to the complete loss of antifungal activity. To summarize, compound **IIAe** is the most prospective for further study and chemical modification since it possesses antimicrobial activity against Gram positive (*B. subtilis)* and Gram negative (*P. aeruginosa)* bacterial strains and also antifungal activity against *C. albicans*.

EXPERIMENTAL PART

Chemical Part

Starting benzaldehydes, active methylene nitriles and isatins were obtained from commercial suppliers and used without further purification. Starting 1-ethyl-1*H*-2,1-benzothiazin-4(3*H*)-one 2,2-dioxide was obtained according to the previously described procedure [28]. Synthesis of compounds **IAa-Bd**, **IIAa-Ae**,**Ba-Be** as well as their physical and spectral data were described in our previous works [21, 28]. Synthetic procedure for the synthesis of new spiro-derivatives **IIAf**,**Ag**,**Bf-Bh** is presented in this section. The new compounds are characterized by melting points (obtained on a Gallenkamp melting point apparatus, Model MFB-595 in open capillary tubes), ^{1}H NMR-spectroscopic data (recorded on a Varian WXR-400 spectrometer in DMSO-d_6 using TMS as an internal standard, chemical shifts in parts per million) and elemental analysis data (carried out using Carlo Erba CHNS-O EA 1108 analyzer).

The ^{1}H NMR-spectra of new spiro-compounds **IIAf**, **Ag**,**Bf-Bh** feature a characteristic high-intensity singlet of the α-amino group of the pyran ring. For 3-cyano derivatives **IIAf-Ag** it can be found at 7.54-7.63 ppm while for 3-ethoxycarbonyl derivatives **IIBf-Bh** it shifts downfield and can be observed at 8.03-8.13 ppm, which is probably connected with intramolecular hydrogen bond forma-

tion between NH_2 protons and carbonyl oxygen of ester fragment. The corresponding N-non-alkylated compound **IIBh** also features a proton singlet from the NH-group of the 2-oxindole ring in a downfield at 10.33 ppm. Protons of the NH-group of the 2-oxindole ring as well as of the α-amino group of the pyran ring can be easily exchanged in the presence of CD_3OD in DMSO-d_6 solution. It was also interesting that the CH_2 protons of 3-ester group of pyrane moiety in **IIBg** are strongly unequal and appeared in ^{1}H NMR-spectrum as two multiplets (*see spectral data for* ***IIBg***).

General procedure for the synthesis of ***IIAf,Ag,Bf-Bh***

To a solution of 1-ethyl-1*H*-2,1-benzothiazin-4(3*H*)-one 2,2-dioxide **1** (0.225 g, 0.001 Mol), malononitrile **2A** (0.066 g, 0.001 Mol) toward **IIAf,Ag** or ethyl cyanoacetate (0.11 mL, 0.001 Mol) toward **IIBf-Bh** and appropriate isatins **4f-h** (0.001 Mol) in ethanol (5-10 mL), triethanolamine (0.14 mL, 0.001 Mol) was added. The mixture was refluxed during 3 h and cooled to the room temperature; the resulting precipitates were filtered off, washed with ethanol and dried on air to afford the pure products **IIAf,Ag,Bf-Bh**.

Spiro[(2-amino-3-cyano-6-ethyl-4,6-dihydropyrano [3,2-c][2,1]benzothiazine-5,5-dioxide)-4,3'-(1'-ethyl-indolin-2'-one)] ***IIAf***

Yield – 78 %. Colorless prisms. M. p. – 228-230 °C. Anal. Calcd for $C_{23}H_{20}N_4O_4S$, %: C 61.59; H 4.49; N 12.49; S 7.15. Found, %: C 61.32; H 4.73; N 12.28; S 7.44. ^{1}H NMR-spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 0.99 (3H, t, *J* = 6.87, $NCH_2C\underline{H}_3$ indolin-2'-one); 1.16 (3H, t, *J* = 6.87, $NCH_2C\underline{H}_3$ benzothiazine); 3.63-3.90 (4H, m, $NC\underline{H}_2CH_3$ indolin-2'-one, $NC\underline{H}_2CH_3$ benzothiazine); 7.00-7.15 (2H, m, Ar); 7.28-7.50 (3H, m, Ar); 7.54-7.75 (4H, m, Ar, NH_2); 7.96 (1H, d, *J* = 7.32, H-5, benzothiazine).

Spiro[(2-amino-3-cyano-6-ethyl-4,6-dihydropyrano [3,2-c][2,1]benzothiazine-5,5-dioxide)-4,3'-(1'-ethoxycarbonylmethyl-indolin-2'-one)] ***IIAg***

Yield – 53 %. Light yellow powder. M. p. – 226-228 °C. Anal. Calcd for $C_{25}H_{22}N_4O_6S$, %: C 59.28; H 4.38; N 11.06; S 6.33. Found, %: C 58.97; H 4.52; N 11.27; S 5.98. ^{1}H NMR-spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 0.97 (3H, t, *J* = 6.85, $OCH_2C\underline{H}_3$); 1.15 (3H, t, *J* = 7.09, $NCH_2C\underline{H}_3$); 3.72-3.87 (2H, m, $OC\underline{H}_2CH_3$); 4.11 (2H, q, *J* = 6.93, $NC\underline{H}_2CH_3$); 4.46-4.59 (2H, m, NCH_2CO); 7.00-7.12 (2H, m, Ar); 7.29-7.38 (2H, m, Ar); 7.43 (1H, t, *J* = 7.46, Ar); 7.54-7.63 (3H, m, Ar, NH_2); 7.64-7.70 (1H, m, Ar); 7.96 (1H, d, *J* = 8.07, H-5, benzothiazine).

Spiro[(2-amino-3-ethoxycarbonyl-6-ethyl-4,6-dihydropyrano[3,2-c][2,1]benzothiazine-5,5-dioxide)-4,3'-(1'-ethyl-indolin-2'-one)] ***IIBf***

Yield – 57 %. Light grey plates. M. p. – 249-251 °C. Anal. Calcd for $C_{25}H_{25}N_3O_6S$, %: C 60.59; H 5.08; N 8.48; S 6.47. Found, %: C 60.81; H 5.32; N 8.54; S 6.21. ^{1}H NMR-spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 0.64 (3H, t, *J* = 7.17, $OCH_2C\underline{H}_3$); 0.94 (3H, t, *J* = 7.02, $NCH_2C\underline{H}_3$ indolin-2'-one); 1.20 (3H, t, *J* = 6.87, $NCH_2C\underline{H}_3$ benzothiazine); 3.55-3.86 (6H, m, $OC\underline{H}_2CH_3$, $NC\underline{H}_2CH_3$ indolin-2'-one, $NC\underline{H}_2CH_3$ benzothiazine); 6.86-7.01 (2H, m, Ar); 7.11-7.29 (2H, m, Ar); 7.38-7.48 (1H, m, Ar); 7.51-7.71 (2H, m, Ar); 8.03 (1H, d, *J* = 8.24, H-5, benzothiazine); 8.10 (2H, s, NH_2).

Spiro[(2-amino-3-ethoxycarbonyl-6-ethyl-4,6-dihydropyrano[3,2-c][2,1]benzothiazine-5,5-dioxide)-4,3'-(1'-ethoxycarbonylmethyl-indolin-2'-one)] ***IIBg***

Yield – 47%. White needles. M. p. – 187-189 °C. Anal. Calcd for $C_{27}H_{27}N_3O_8S$, %: C 58.58; H 4.92; N 7.59; S 5.79. Found, %: C 58.21; H 5.17; N 7.78; S 5.41. ^{1}H NMR-spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 0.52-0.60 (3H, m, $OCH_2C\underline{H}_3$ pyrane); 0.90-0.98 (3H, m, $NCH_2C\underline{H}_3$ indolin-2'-one); 1.18-1.25 (3H, m, $NCH_2C\underline{H}_3$ benzothiazine); 3.58-3.69 (1H, m, $OC\underline{H}_2CH_3$ pyrane); 3.72-3.84 (2H, m, $NC\underline{H}_2CH_3$ indolin-2'-one); 3.86-3.97 (1H, m, $OC\underline{H}_2CH_3$ pyrane); 4.09-4.23 (2H, m, $NC\underline{H}_2CH_3$ benzothiazine); 4.38-4.52 (2H, m, $NC\underline{H}_2CO$ indolin-2'-one); 6.91-6.98 (2H, m, Ar); 7.17 (1H, d, *J* = 7.58, Ar); 7.20-7.27 (1H, m, Ar); 7.43 (1H, t, *J* = 7.70, Ar); 7.54 (1H, d, *J* = 8.31, Ar); 7.61-7.69 (1H, m, Ar); 7.99-8.05 (1H, m, H-5 benzothiazine), 8.13 (2H, br. s., NH_2).

Spiro[(2-amino-3-ethoxycarbonyl-6-ethyl-4,6-dihydropyrano[3,2-c][2,1]benzothiazine-5,5-dioxide)-4,3'-(5'-methyl-indolin-2'-one)] ***IIBh***

Yield – 41%. Light yellow prisms. M. p. > 250 °C. Anal. Calcd for $C_{24}H_{23}N_3O_6S$, %: C 59.86; H 4.81; N 8.73; S 6.67. Found, %: C 59.52; H 5.11; N 8.94; S 7.01. ^{1}H NMR-spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 0.70-0.83 (3H, m, $OCH_2C\underline{H}_3$); 0.95 (3H, t, *J* = 6.85, $NCH_2C\underline{H}_3$); 2.15 (3H, s, 5'-CH_3); 3.64-3.87 (4H, m, $NC\underline{H}_2CH_3$, $OC\underline{H}_2CH_3$); 6.63 (1H, d, *J* = 7.83, Ar); 6.86-6.98 (2H, m, Ar); 7.42 (1H, t, *J* = 7.58, Ar); 7.55 (1H, d, *J* = 8.07, Ar); 7.60-7.68 (1H, m, Ar); 7.95-8.10 (3H, m, H-5 benzothiazine, NH_2); 10.33 (1H, br. s, NH).

The general technique for the study of the antimicrobial activity *(double serial dilution method in liquid growth medium).*

Into the six test-tubes 1 mL of the broth was added. After that, 1 mL of examined compound solution in DMSO in concentration 1000 μg/mL was added into the first test-tube and the resulted solution was thoroughly mixed. Thereafter 1 mL of the solution was carried from the first test-tube into the second. The solution in the second test-tube was also thoroughly mixed and 1 mL of resulted solution was carried into the third test-tube and so on up to sixth test-tube. 1 mL of the solution was poured out from sixth test-tube to be the equal volume in all of the test-tubes. Thus in test-tubes from the first to the sixth the concentrations of the examined compounds were 500, 250, 125, 62.5, 31.25, 15.62 μg/mL. The dilutions of DMSO as the reference drug were prepared in the similar way without using of the evaluated compounds. Thereby, the concentrations of DMSO in reference solutions were 550, 275, 137.5, 68.75, 34.38, 17.19 μg/mL (taking into account the density of DMSO – 1.1 mg/mL).

Inoculums of the bacterial and fungal cultures were prepared according to optical turbidity standard 0.5 ME from a daily agar culture. Microbe suspension (microbe loading 150 × 10^6 microbes per mL of growth medium) was transferred into the prepared dilutions of the com-

pounds and DMSO in growth medium. The test-tubes with bacterial cultures were kept in thermostat for 24 h at 37 °C and test-tubes containing *C. albicans* culture were kept in thermostat for 48 h at 25 °C and carefully observed for the presence of turbidity. The minimum concentration at which no growth was observed was taken as the MIC value.

CONCLUSIONS

1. The microbiological screening allowed to determine two the most active compounds – 2-amino-3-cyano-4-(4-dimethylaminophenyl)-6-ethyl-4,6-dihydropyrano[3,2-c][2,1]benzothiazine-5,5-dioxide as representative of 2-amino-4-aryl-4H-pyrans and spiro[(2-amino-3-cyano-6-ethyl-4,6-dihydropyrano[3,2-c][2,1]benzothiazine-5,5-dioxide)-4,3'-(1'-methylindolin-2'-one)] as representative of spiro-derivatives.
2. The most prevalent kind of antimicrobial activity for studied compounds appeared to be antifungal effect.
3. On the whole, the spiro-derivatives possess a higher level of antimicrobial effect as compared to 4-aryl-4H-pyrans.
4. For 3-cyano-4-aryl-4H-pyrans increase of antimicrobial activity was observed due to introducing of more lipophilic anthracene residue as well as presence of basic group fixed to 4-aryl residue.
5. Some "structure – activity" relationships were established for malononitrile derived spiro-derivatives:
   - the bulkier substituent attached to the 2-oxindole nitrogen, the less activity exhibits a compound;
   - introducing of a substituent in 5 position of 2-oxindole ring did not change the activity significantly.

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Д. О. Лега, Н. І. Філімонова, О. М. Дика, В. П. Черних, Л. А. Шемчук

ДОСЛІДЖЕННЯ *IN VITRO* АНТИМІКРОБНОЇ АКТИВНОСТІ 2-АМІНО-3-R-6-ЕТИЛ-4,6-ДИГІДРОПІРАНО[3,2-*c*][2,1]БЕНЗОТІАЗИН 5,5-ДІОКСИДІВ, ЩО МІСТЯТЬ 4-АРИЛЬНИЙ ЗАМІСНИК ТА СПІРОКОНДЕНСОВАНІ З ЯДРОМ 2-ОКСІНДОЛУ

На теперішній час антибіотикорезистентність стає великою проблемою при лікуванні бактеріальних та грибкових інфекцій, тому актуальним питанням є пошук нових класів антимікробних засобів. Дана стаття присвячена вивченню антимікробної активності похідних 2-аміно-3-R-6-етил-4,6-дигідропірано[3,2-*c*][2,1]бензотіазин 5,5-діоксиду, які містять 4-арильний замісник та спіроконденсовані з ядром 2-оксіндолу. Даний вибір обумовлений даними щодо високої антимікробної активності деяких похідних 2-аміно-4-арил-4*H*-пірану та 2-оксіндолу, що містяться в літературі. Мікробіологічний скринінг 4-арил-4H-піранів виявив у деяких похідних високу протигрибкову активність, серед яких 2-аміно-3-ціано-4-(4-диметиламінофеніл)-6-етил-4,6-дигідропірано[3,2-*c*][2,1]бензотіазину 5,5-діоксид мав найменше значення МІК. Порівняно з 4-арильними похідними спіроконденсовані похідні 2-оксіндолу виявили вищий антимікробний ефект. Серед них знайдені сполуки, що виявили помірну антимікробну активність проти *B. subtilis* та *P. aeruginosa*, а також протигрибкову активність проти *C. albicans*. Серед протестованих похідних сполукою-лідером виявився спіро[(2-аміно-3-ціано-6-етил-4,6-дигідропірано[3,2-*c*][2,1]бензотіазину 5,5-діоксид)-4,3'-(1'-метил-індолін-2'-он)], що був ефективним проти *B. subtilis*, *P. aeruginosa* та *C. albicans*. Результати мікробіологічного скринінгу обговорені з точки зору залежності «структура – антимікробна активність».

Ключові слова*:* 2,1-бензотіазину 2,2-діоксид; антимікробна активність; бензальдегіди; ізатини; метиленактивні нітрили; 4*H*-пірани; спіросполуки; трикомпонентна взаємодія

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Д. А. Лега, Н. И. Филимонова, Е. М. Дикая, В. П. Черных, Л. А. Шемчук

ИССЛЕДОВАНИЕ *IN VITRO* АНТИМИКРОБНОЙ АКТИВНОСТИ 2-АМИНО-3-R-6-ЭТИЛ-4,6-ДИГИДРОПИРАНО[3,2-*C*][2,1]БЕНЗОТИАЗИН 5,5-ДИОКСИДОВ, СОДЕРЖАЩИХ 4-АРИЛЬНЫЙ ЗАМЕСТИТЕЛЬ И СПИРОКОНДЕНСИРОВАННЫХ С ЯДРОМ 2-ОКСИНДОЛА

На сегодня антибиотикорезистентность становится большой проблемой при лечении бактериальных и грибковых инфекций, поэтому актуальным вопросом является поиск новых классов антимикробных средств. Данная статья посвящена изучению антимикробной активности производных 2-амино-3-R-6-этил-4,6-дигидропирано[3,2-*c*][2,1]бензотиазин 5,5-диоксида, содержащих 4-арильный заместитель и спироконденсированных с ядром 2-оксиндола. Такой выбор обусловлен данными по высокой антимикробной активности некоторых производных 2-амино-4-арил-4*H*-пирана и 2-оксиндола, которые содержатся в литературе. Микробиологический скрининг 4-арил-4*H*-пиранов показал для некоторых производных высокую противогрибковую активность, среди которых 2-амино-3-циано-4-(4-диметиламинофенил)-6-этил-4,6-дигидропирано[3,2-*c*][2,1]бензотиазин 5,5-диоксид имел наименьшее значение МИК. Спироконденсированные производные 2-оксиндола проявили более высокий антимикробный эффект сравнительно с 4-арильными производными 2-амино-4*H*-пирана. Среди них найдены соединения, которые показали умеренную антимикробную активность против *B. subtilis* и *P. aeruginosa*, а также противогрибковую активность относительно *C. albicans*. Среди протестированных соединений веществом-лидером оказался спиро[(2-амино-3-циано-6-этил-4,6-дигидропирано[3,2-*c*][2,1]бензотиазина 5,5-диоксид)-4,3'-(1'-метил-индолин-2'-он)], который был эффективен относительно *B. subtilis*, *P. aeruginosa* и *C. albicans*. Результаты микробиологического скрининга обсуждены с точки зрения зависимости «структура – антимикробная активность».

Ключевые слова*:* 2,1-бензотиазина 2,2-диоксид; антимикробная активность; бензальдегиды; изатины; метиленактивные нитрилы; 4*H*-пираны; спиросоединения; трехкомпонентное взаимодействие

Адреса для листування:
61168, м. Харків, вул. Валентинівська, 4.
Національний фармацевтичний університет

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