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ANTINOCICEPTIVE AND ANTIMICROBIAL ACTIVITY OF PRODUCTS FROM REACTIONS OF PYRROLOBENZOXAZINETRIONES WITH THIOSEMICARBAZONES OF AROMATIC AND HETEROAROMATIC ALDEHYDES

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Previously, a series of hetareno[*e*]pyrrole-2,3-diones was found to include compounds with high antimicrobial and antinociceptive activity and low toxicity, so that new hetareno[*e*]pyrrole-2,3-dione derivatives were expected to have these types of activity. The reactions of pyrrolobenzoxazinetriones with a series of aromatic aldehyde thiosemicarbazones and nicotinic aldehyde thiosemicarbazone are reported. Results from studies of the antinociceptive (hot-plate test) and antimicrobial activity (*Staphylococcus aureus* No. 906, *Escherichia coli* No. 1257) are given. The most active compounds were effective on the level of the reference drugs. The target of the antinociceptive activity of the tested compounds was considered nerve endings because signs of central action were not observed in the behavior of animals under the influence of the compounds. The selective antimicrobial activity against Gram-positive bacteria *S. aureus* was presumably associated with disruption of the cytoplasmic membrane permeability and bacterial RNA synthesis.

Keywords: pyrrolobenzoxazinetriones, thiosemicarbazones, antinociceptive and antimicrobial activity.

Hetareno[*e*]pyrrole-2,3-diones and their derivatives include compounds exhibiting antimicrobial, analgesic, anti-inflammatory, and antihypoxic activity [1 – 4]. The presence and structure of the pharmacophoric fragments are known to be largely responsible for the pharmacological effects of synthetic drugs. Considering the antituberculosis, antimicrobial, and antitumor activity of thiosemicarbazide derivatives [5 – 8], it seemed promising to synthesize new hetareno[*e*]pyrrole-2,3-diones containing a thiosemicarbazone fragment.

In continuation of research on the pharmacological activity of hetareno[*e*]pyrrole-2,3-diones, we studied reactions of pyrrolobenzoxazinetriones with a series of aromatic and heteroaromatic aldehyde thiosemicarbazones, i.e., benzaldehyde, *o*-fluoro and *o*-nitrobenzaldehydes, *o*- and *p*-hydroxybenzaldehydes [9], and nicotinic aldehyde thiosemicarbazone [10]. The structures of the synthesized compounds were studied using x-ray crystal structure analysis (XSA). Their antinociceptive and antimicrobial activities were also studied.

Reactions of 3-aroylpyrrolo[1,2-*c*][4,1]benzoxazine-1,2,4-triones **I-V** with aromatic aldehyde thiosemicarbazones in a 1:1 ratio in refluxing anhydrous MeCN for 1.5 – 2 h produced 9-aroyl-8-hydroxy-2-{2-[2-benzylidene(pyrid-3-ylmethylene]hydrazono}-6-(2-hydroxyphenyl)-1-thia-3,6-diaz aspiro[4.4]non-8-en-4,7-diones **VI-XXII** (Scheme 1) [11].

Reactions of 3-aroylpyrrolo[1,2-*c*][4,1]benzoxazine-1,2,4-triones with nicotinic aldehyde thiosemicarbazone were studied first. The reactions of 3-aroylpyrrolo[1,2-*c*]-[4,1]benzoxazine-1,2,4-triones **I-V** with nicotinic aldehyde thiosemicarbazone in a 1:1 ratio with stirring in dioxane at 60°C for 6 – 8 h produced 9-aroyl-8-hydroxy-2-(pyrid-3-ylmethylene)hydrazono-6-(2-hydroxyphenyl)-1-thia-3,6-diaza-spiro[4.4]non-8-ene-4,7-diones **XXIII-XXVII** (Scheme 1).

Compounds **VI-XXVII** were colorless or light-yellow high-melting crystalline compounds that melted with decomposition that were soluble in DMSO, DMF, Me_2CO, 1,2-dichloroethane, and EtOAc; slightly soluble in aromatic hydrocarbons and $CHCl_3$; and insoluble in alkanes and H_2O. They

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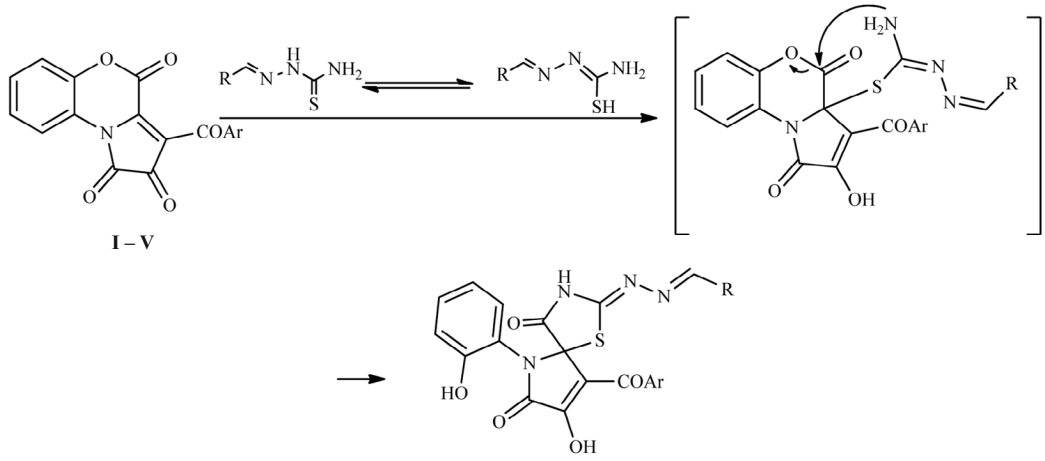
Scheme 1



I – V

VI – XXVII

Ar = Ph (**I**), C_6H_4Me-4 (**II**), C_6H_4Br-4 (**III**), C_6H_4Cl-4 (**IV**), $C_6H_4NO_2$ – 4 (**V**); Ar = Ph, R=C_6H_4OH-2 (**VI**); Ar = Ph, R=C_6H_4OH-4 (**VII**); Ar = C_6H_4Me-4, R=Ph (**VIII**); Ar = C_6H_4Me-4, R=C_6H_4OH-2 (**IX**); Ar = C_6H_4Me-4, R=C_6H_4OH-4 (**X**); Ar = C_6H_4Me-4, R=C_6H_4OMe-4 (**XI**); Ar = C_6H_4Me-4, R=$C_6H_4NO_2$-4 (**XII**); Ar = C_6H_4Me-4, R=C_6H_4F-2 (**XIII**); Ar = C_6H_4Br-4, R=C_6H_4OH-2 (**XIV**); Ar = C_6H_4Br-4, R=C_6H_4OMe-4 (**XV**); Ar = C_6H_4Br-4, R=C_6H_4F-2 (**XVI**); Ar = C_6H_4Cl-4, R=Ph (**XVII**); Ar = C_6H_4Cl-4, R=C_6H_4OH-2 (**XVIII**); Ar = C_6H_4Cl-4, R=C_6H_4F-2 (**XIX**); Ar = C_6H_4Cl-4, R=C_6H_4OMe-4 (**XX**); Ar = C_6H_4Cl-4, R=$C_6H_4NO_2$-4 (**XXI**); Ar = $C_6H_4NO_2$-4, R=C_6H_4OMe-4 (**XXII**); Ar = Ph, R=Py (**XXIII**), Ar = C_6H_4Me-4, R=Py (**XXIV**), Ar = C_6H_4Br-4, R=Py (**XXV**), Ar = C_6H_4Cl-4, R=Py (**XXVI**); Ar = $C_6H_4NO_2$-4, R=Py (**XXVII**)

gave positive tests (wine-red color) for enol and phenol hydroxyl groups with ethanolic $FeCl_3$.

IR spectra of **VI-XXVII** showed bands for OH and NH stretching vibrations (3400 – 3595 cm^{-1}), lactam carbonyls C^4=O and C^7=O (1696 – 1775), and aroyl ketone carbonyl (1611 – 1643).

PMR spectra of **VI-XXVII** showed resonances for the aromatic-ring protons and the groups bonded to them, a singlet for the amide NH proton in the region of aromatic protons or after it (6.77 – 8.88 ppm), a singlet for the methine proton (8.19 – 8.44), a singlet for the phenol OH proton (9.64 – 9.77), and a broad singlet for the enol OH proton (12.22 – 12.62). PMR spectra of **VI**, **VII**, **IX**, **X**, **XIV**, and **XVIII** also exhibited a singlet for the hydroxybenzylidene OH proton (9.94 – 10.54).

The structures of the synthesized compounds were elucidated by an XSA of **IX** [11].

Apparently, **VI-XXVII** formed via initial addition of the thiolimide SH of salicylaldehyde thiosemicarbazone to the C^{3a} atom of pyrrolidones **I-V** followed by closing of the thiazole ring as a result of intramolecular attack of the thiolimide NH_2 at the lactone carbonyl of the benzoxazine ring and its opening at the C^4–O^5 bond.

The direction of the reaction was not affected if various substituents were introduced on the thiosemicarbazone aryl fragment. Table 1 presents the characteristics of the synthesized compounds.

EXPERIMENTAL CHEMICAL PART

PMR spectra were recorded with HMDS internal standard on Bruker Avance III HD 400 spectrometers (400 and 100 MHz, respectively). IR spectra were recorded from mineral-oil mulls on a PerkinElmer Spectrum Two spectrophotometer. Elemental analyses were obtained on a vario Micro cube analyzer. The purity of the synthesized compounds were confirmed by TLC on Sorbfil and Merck silica gel 60 F254 plates using MeOH–EtOAc (1:1) + 1% formic acid and toluene–EtOAc (5:1) eluents with detection by I_2 vapor and UV light at 254 nm. Starting pyrrolidones **I-V** were synthesized from the corresponding enamines and oxalyl chloride using the literature method [12].

8-Hydroxy-6-(2-hydroxyphenyl)-2-[2-(4-methoxybenzylidene)hydrazono]-9-(4-methylbenzo)-1-thia-3,6-diazaspiro[4.4]non-8-ene-4,7-dione (XI). A solution of **II** (0.5 mmol) and anisaldehyde thiosemicarbazone (0.5 mmol) in anhydrous MeCN (10 mL) was stirred and refluxed for 2 h and cooled. The resulting precipitate was filtered off. Yield 64%, mp 212 – 214°C (MeCN). IR spectrum, ν, cm^{-1}: 3500 br (OH, NH), 3180 br (OH_{enol}), 1740 (C^7=O), 1711 (C^4=O), 1625 (COC_6H_4Me-4). PMR spectrum, δ, ppm: 2.43 (s, 3H, OMe), 6.85 – 7.73 (gr.s, 13H, H^{Ar}, $3C_6H_4$ + NH_{amid}), 8.26 (s, 1H, CH), 9.73 (s, 1H, OH_{PhOH}), 12.38 (br.s, 1H, OH_{enol}). $C_{28}H_{22}N_4O_6S$.

Compounds **XV** and **XVII-XXII** were synthesized analogously. Compounds **VI-X**, **XII-XIV**, and **XVI** were previously reported [11].

9-Benzoyl-8-hydroxy-6-(2-hydroxyphenyl)-2-(pyrid-3-ylmethylene)hydrazono-1-thia-3,6-diazaspiro[4.4]non-8-ene-4,7-dione (XXIII). A solution of **I** (0.5 mmol) and nicotinic aldehyde thiosemicarbazone (0.5 mmol) in anhydrous 1,4-dioxane (10 mL) was stirred, refluxed for 7 – 8 h, and cooled. The resulting precipitate was filtered off. Yield 79%, mp 208 – 210°C (dioxane). IR spectrum, ν, cm^{-1}: 3340 br (OH, NH), 3188 (OH_{enol}), 1746 (C^7=O), 1707 (C^4=O), 1637 (COPh). PMR spectrum, δ, ppm: 6.86 – 8.62 (gr.s,

13H, Ph + $2C_6H_4$), 8.42 (s, 1H, CH), 8.82 (s, 1H, NH_{amid}), 9.75 (s, 1H, OH_{PhOH}), 12.49 (br.s, 1H, OH_{enol}). $C_{25}H_{17}N_5O_5S$.

Compounds **XXIV-XXVII** were synthesized analogously.

EXPERIMENTAL BIOLOGICAL PART

Antinociceptive activity of the synthesized compounds was determined using laboratory female white mice (18 – 22 g) and thermal irritation of paws (hot plate test of

TABLE 1. Physicochemical and Spectral Characteristics of **VI**–XXVII

Compound	mp, °C	Empirical formula	Yield, %	PMR, δ, ppm
VI	208 – 210	$C_{26}H_{18}N_4O_6S$	63 [11]	6.84 – 7.84 (gr.s, 14H, H^{Ar}, Ph + $2C_6H_4$ + NH_{amid}), 8.55 (s, 1H, CH), 9.77 (s, 1H, OH_{PhOH}), 10.50 (s, 1H, OH_{PhOH}), 12.59 (br.s, 1H, OH_{enol})
VII	190 – 191	$C_{26}H_{18}N_4O_6S$	65 [11]	6.78 – 7.81 (gr.s, 14H, H^{Ar}, Ph + $2C_6H_4$ + NH_{amid}), 8.20 (s, 1H, CH), 9.70 (s, 1H, OH_{PhOH}), 9.94 (s, 1H, OH_{PhOH}), 12.35 (br.s, 1H, OH_{enol})
VIII	211 – 213	$C_{27}H_{20}N_4O_5S$	69 [11]	2.40 (s, 3H, Me), 6.85 – 7.73 (gr.s, 14H, H^{Ar}, Ph + $2C_6H_4$ + NH_{amid}), 8.33 (s, 1H, CH), 9.72 (s, 1H, OH_{PhOH}), 12.46 (br.s, 1H, OH_{enol})
IX	229 – 231	$C_{27}H_{20}N_4O_6S$	67 [11]	2.40 (s, 3H, Me), 6.85 – 7.82 (gr.s, 13H, H^{Ar}, $3C_6H_4$ + Nh_{amid}), 8.56 (s, 1H, CH), 9.75 (s, 1H, OH_{PhOH}), 10.48 (s, 1H, OH_{PhOH}), 12.57 (br.s, 1H, OH_{enol})
X	205 – 207	$C_{27}H_{20}N_4O_6S$	63 [11]	2.40 (s, 3H, Me), 6.77 – 7.72 (gr.s, 13H, H^{Ar}, Ph + $2C_6H_4$ + OH_{PhOH}), 8.19 (s, 1H, CH), 9.70 (s, 1H, OH_{PhOH}), 9.94 (s, 1H, NH), 12.34 (br.s, 1H, OH_{enol})
XI	212 – 213	$C_{28}H_{22}N_4O_6S$	64	2.40 (s, 3H, Me), 3.72 (s, 3H, MeO), 6.85 – 7.73 (gr.s, 13H, H^{Ar}, $3C_6H_4$ + NH_{amid}), 8.26 (s, 1H, CH), 9.73 (s, 1H, OH_{PhOH}), 12.38 (br.s, 1H, OH_{enol})
XII	238 – 239	$C_{27}H_{19}N_5O_7S$	80 [11]	2.40 (s, 3H, Me), 6.85 – 8.04 (gr.s, 13H, H^{Ar}, $3C_6H_4$ + NH_{amid}), 8.62 (s, 1H, CH), 9.72 (s, 1H, OH_{PhOH}), 12.60 (br.s, 1H, OH_{enol})
XIII	194 – 196	$C_{27}H_{19}FN_4O_5S$	74 [11]	2.40 (s, 3H, Me), 6.85 – 7.78 (gr.s, 13H, H^{Ar}, $3C_6H_4$ + NH_{amid}), 8.43 (s, 1H, CH), 9.72 (s, 1H, OH_{PhOH}), 12.52 (br.s, 1H, OH_{enol})
XIV	213 – 215	$C_{26}H_{17}BrN_4O_6S$	68 [11]	6.85 – 7.74 (gr.s, 13H, H^{Ar}, $3C_6H_4$ + NH_{amid}), 8.56 (s, 1H, CH), 9.72 (s, 1H, OH_{PhOH}), 10.49 (s, 1H, OH_{PhOH}), 12.56 (br.s, 1H, OH_{enol})
XV	228 – 230	$C_{27}H_{20}BrN_4O_6S$	61	3.78 (s, 3H, MeO), 6.78 – 7.75 (gr.s, 13H, H^{Ar}, $3C_6H_4$ + NH_{amid}), 8.26 (s, 1H, CH), 9.66 (s, 1H, OH_{PhOH}), 12.46 (br.s, 1H, OH_{enol})
XVI	206 – 208	$C_{26}H_{16}BrFN_4O_5S$	69 [11]	6.85 – 7.78 (gr.s, 13H, H^{Ar}, $3C_6H_4$ + NH_{amid}), 8.43 (s, 1H, CH), 9.72 (s, 1H, OH_{PhOH}), 12.49 (br.s, 1H, OH_{enol})
XVII	212 – 213	$C_{26}H_{17}ClN_4O_5S$	67	6.86 – 7.83 (gr.s, 14H, H^{Ar}, Ph + $2C_6H_4$ + NH_{amid}), 8.35 (s, 1H, CH), 9.69 (s, 1H, OH_{PhOH}), 12.62 (br.s, 1H, OH_{enol})
XVIII	236 – 238	$C_{26}H_{17}ClN_4O_6S$	60	6.86 – 7.80 (gr.s, 13H, H^{Ar}, $3C_6H_4$ + NH_{amid}), 8.57 (s, 1H, CH), 9.72 (s, 1H, OH_{PhOH}), 10.48 (s, 1H, OH_{PhOH}), 12.57 (br.s, 1H, OH_{enol})
XIX	212 – 214	$C_{26}H_{16}ClFN_4O_5S$	68	6.86 – 7.83 (gr.s, 13H, H^{Ar}, $3C_6H_4$ + NH_{amid}), 8.44 (s, 1H, CH), 9.71 (s, 1H, OH_{PhOH}), 12.56 (br.s, 1H, OH_{enol})
XX	212 – 214	$C_{27}H_{20}ClN_4O_6S$	54	3.78 (s, 3H, MeO), 6.85 – 7.82 (gr.s, 13H, H^{Ar}, $3C_6H_4$ + NH_{amid}), 8.27 (s, 1H, CH), 9.68 (s, 1H, OH_{PhOH}), 12.41 (br.s, 1H, OH_{enol})
XXI	218 – 219	$C_{26}H_{17}ClN_5O_7S$	71	6.86 – 8.06 (gr.s, 13H, H^{Ar}, $3C_6H_4$ + NH_{amid}), 8.63 (s, 1H, CH), 9.69 (s, 1H, OH_{PhOH}), 12.48 (br.s, 1H, OH_{enol})
XXII	223 – 224	$C_{27}H_{20}N_5O_8S$	57	3.78 (s, 3H, MeO), 6.85 – 8.36 (gr.s, 13H, H^{Ar}, $3C_6H_4$ + NH_{amid}), 8.33 (s, 1H, CH), 9.73 (s, 1H, OH_{PhOH}), 12.36 (br.s, 1H, OH_{enol})
XXIII	208 – 210	$C_{25}H_{17}N_5O_5S$	79	6.86 – 8.62 (gr.s, 13H, H^{Ar}, Ph + $2C_6H_4$), 8.42 (s, 1H, CH), 8.82 (s, 1H, NH_{amid}), 9.75 (s, 1H, OH_{PhOH}), 12.49 (br.s, 1H, OH_{enol})
XXIV	206 – 208	$C_{26}H_{19}N_5O_5S$	88	2.40 (s, 3H, Me), 6.85 – 8.60 (gr.s, 12H, H^{Ar}, $3C_6H_4$), 8.40 (s, 1H, CH), 8.82 (s, 1H, NH_{amid}), 9.73 (s, 1H, OH_{PhOH}), 12.56 (br.s, 1H, OH_{enol})
XXV	214 – 216	$C_{25}H_{16}BrN_5O_5S$	79	6.85 – 8.63 (gr.s, 12H, H^{Ar}, $3C_6H_4$), 8.40 (s, 1H, CH), 8.83 (s, 1H, NH_{amid}), 9.71 (s, 1H, OH_{PhOH}), 12.48 (br.s, 1H, OH_{enol})
XXVI	221 – 223	$C_{25}H_{16}ClN_5O_5S$	69	6.85 – 8.62 (gr.s, 12H, H^{Ar}, $3C_6H_4$), 8.42 (s, 1H, CH), 8.82 (s, 1H, NH_{amid}), 9.71 (s, 1H, OH_{PhOH}), 12.53 (br.s, 1H, OH_{enol})
XXVII	224 – 225	$C_{25}H_{16}N_6O_7S$	81	6.85 – 8.66 (gr.s, 12H, H^{Ar}, $3C_6H_4$), 8.44 (s, 1H, CH), 8.88 (s, 1H, NH_{amid}), 9.73 (s, 1H, OH_{PhOH}), 12.45 (br.s, 1H, OH_{enol})

Eddy and Leimbach) [13] (Table 2). Animals with an initial onset time of a defensive reflex of ≤15 sec were used in the test. The nociceptive parameter was the residence time in seconds of the animal on the hot plate until licking of hind paws, shaking them, or attempts to jump when placed on a metal plate heated to 55°C. The effect was assessed 0.5, 1, and 2 h after administration of the compounds before onset of the nociceptive response (latent period). Each compound was tested in six animals. Results were assessed from the increase of onset time of a defensive reflex as compared to the initial data.

The tested compounds were injected i.p. at a dose of 50 mg/kg according to methodical recommendations for experimental (preclinical) studies of new drugs [14] as suspensions in starch paste (2%) 0.5 h before placing the animal on the instrument. The reference drugs were commercially available metamizole sodium (analgin) drug substance (Farmkhimkomplekt OOO) at a dose of 93 mg/kg, which corresponded to ED_{50} for i.p. injection [15, 16], and ibuprofen (Pharmaceutical Secondary Standard, Supelco) at a dose of 50 mg/kg.

Control animals were injected with the corresponding volume of starch paste (2%). Experimental results were statistically processed using the Student *t*-criterion. An effect was considered statistically significant for $p \le 0.05$ vs. the control and reference drugs [17].

Acute toxicities of the compounds were determined in mice of both sexes (18 – 22 g) with 10 animals in a group per dose. Tested compounds were injected i.p. in starch paste (3%) in the range 100 – 2,000 mg/kg. Animals were observed for 10 d. The external appearance (fur and skin condition), color of mucous membranes, behavior, demand for feed and water, and change of body mass during this time were noted. The number of deceased animals was recorded. The toxicity parameter was the mean lethal dose (LD_{50}) causing the death of 50% of the animals at the end of the test. Control animals were injected i.p. with starch paste (3%). Acute toxicity was calculated according to recommendations of the State Pharmacological Committee for studies of general toxicity of biologically active compounds [14].

Test animals were kept under vivarium conditions (with natural lighting at 22 – 24°C and relative humidity 40 – 50%) on a standard diet (GOST R 50258-92). Experiments were conducted according to good laboratory practice (GLP) rules for preclinical studies in the RF (GOST 3 51000.3-96 and 1000.4-96) and rules of the *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes* (1986). Animals were quarantined for 10 – 14 d before the experiments.

TABLE 2. Antinociceptive Activity of **VI-XXVII** in the Hot Plate Test

Compound	Latent period of defensive reflex, s
Control, 2% starch paste	10.30 ± 0.60
Metamizole sodium	16.33 ± 3.02 $p < 0.1$
Ibuprofen	23.50 ± 1.12
VI	24.60 ± 1.11*
VII	23.10 ± 0.93*
VIII	21.20 ± 1.02*
IX	22.10 ± 1.08*
XI	22.00 ± 0.63*
XVI	19.80 ± 0.66*
XVII	20.40 ± 0.75*
XVIII	21.60 ± 0.58*
XIX	19.06 ± 0.34*
XX	22.20 ± 0.58*
XXI	20.70 ± 0.77*
XXII	24.10 ± 0.64*
XXIII	21.20 ± 0.73*
XXVI	19.80 ± 0.37*
XXVII	24.60 ± 1.17*

* Statistically significant vs. control, $p < 0.05$.

TABLE 3. Antimicrobial Activity of **VI-XXVII**

Compound	Antimicrobial activity, μg/mL			
	S. aureus		*E. coli*	
	* MIC	** MBC	MIC	MBC
VII	62.5	+	+	+
VIII	125.0	1000.0	+	+
IX	62.5	500.0	+	+
X	31.2	+	+	+
XXII	31.2	1000.0	+	+
XIII	31.2	+	+	+
XIV	7.8	500.0	+	+
XVI	31.2	500.0	+	+
XVII	125.0	500.0	+	+
XX	62.5	500.0	+	+
XXII	500.0	1000.0	+	+
XXIII	+	+	+	+
XXIV	125.0	1000.0	1000.0	+
XXVI	+	+	+	+
XXVII	500.0	500.0	500.0	+
Dioxidine	62.5	1000.0	3.9	62.5
Culture growth control	+	+	+	+

Note: * MIC is minimum inhibitory concentration; ** MBC, minimum bactericidal concentration; "+", microorganism growth at tested concentrations.

The results showed that all tested compounds had LD_{50} values >2,000 mg/kg, which corresponded to the marginally toxic class according to the Sidorov classification [18].

Experimental results for antinociceptive activity (Table 2) were analyzed and showed that all 15 tested compounds at a dose of 50 mg/kg increased the onset time of a defensive reflex in mice that exceeded that of metamizole sodium and had activities comparable to that of ibuprofen. Compounds **VI**, **XXII**, and **XXVII** were most active with onset times of defensive reflexes of 24.60, 24.10, and 24.60 sec, respectively.

Antimicrobial activity of the synthesized compounds was determined using double serial dilutions in growth broth [14,19] with various concentrations of the tested compounds.

The test cultures contained conditionally pathogenic microorganisms *Staphylococcus aureus* No. 906 and *Escherichia coli* No.1257. Initial bacterial dilutions were prepared in normal saline using one-day agar cultures according to the McFarland standard and a densitometer. The final cell concentration after a series of dilutions was 2.5×10^5 microbes/mL.

Cultures (150 μL) were inoculated into the prepared series of dilutions of the tested compounds dissolved in DMSO (150 μL) in the microplate wells containing the particular concentrations of the tested compounds. The last rows contained equal volumes of growth medium and culture (controls). The maximum tested concentration of the compounds was 1,000 μg/mL.

The microplates were cultivated at 37 ± 1°C in a thermostat for 24 h and 7 d and placed into an Epoch spectrophotometer. The optical density (OD) of the culture fluid was measured at 540 nm.

The dependence of the OD on microorganism cell growth at the various concentrations of the compounds was found using Gen 5 software of the Epoch microplate spectrophotometer. The last well with growth inhibition in a series corresponded to the minimum inhibitory concentration of the compound.

Table 3 shows the analyzed results and indicates that Gram-positive *S. aureus* was more sensitive to the action of the studied compounds. The cultures were moderately sensitive to **VIII**, **XVII**, **XXII**, **XXIV**, and **XXVII**, the bacteriostatic activities of which fell in the range 125 – 500 μg/mL.

Compounds **VII**, **IX**, **X**, **XII**, **XIII**, **XVI**, and **XX** had inhibitory concentrations for *S. aureus* of 31.2 – 62.5 μg/mL, which was comparable to that of dioxidine. However, the bactericidal activity of the compounds started at a concentration ≥500 μg/mL.

Compound **XIV** had the highest antimicrobial activity and inhibited growth of *S. aureus* culture at a concentration of 7.8 μg/mL with MBC of 500 μg/mL, which was more active than dioxidine.

Thus, the biological activities of the synthesized compounds confirmed that a search for new compounds among pyrrolobenzoxazinetrione derivatives was advisable.

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