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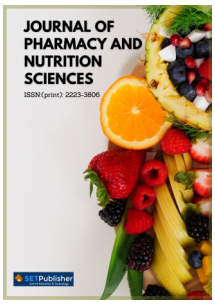

Antimicrobial Activity Derivatives 2*H*-pirano[2,3-*c*]piridines against Pathogens of Intestinal Yersiniosis

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Abstract:

Background: An important aspect in the treatment of patients with intestinal yersiniosis is the administration of effective antibiotic therapy. Performed research aimed to determine the spectrum and level of antimicrobial activity of 2*H*-pyrano[2,3-*c*]pyridine derivatives on the museum and clinical strains of gram-negative microorganisms *Yersinia enterocolitica*.

Methodology: The object of the study was 28 synthetic derivatives of 2*H*-pyrano[2,3-*c*]pyridine. The compounds were studied according to their chemical structure. We used the method of serial dilutions in Muller-Hinton liquid nutrient medium with a museum's and clinical strains of *Y.enterocolitica*.

Results: Studies indicate the promise of further study of the properties of 2*H*-pyrono[2,3-*c*]pyridine to create an effective antimicrobial medicine. According to the results of studies on action of antimicrobial compounds synthesized on the basis of 2*H*-pyrano[2,3-c]pyridine derivatives, it was found that the MIC of compounds for all *Y. enterocolitica* strains was 100.0 μg/ml. The MB_CC of most cultures of *Yersinia* (72.3 %) was 200.0 μg/ml. Compound 2{3} had a pronounced antiyersiniotic activity, the inhibitory effect of which was manifested at a concentration of 25.0 μg/ml. Retarding the growth of most *Yersinia strains* (95.3%) with a MIC of 50.0 μg/ml, the MIC of compounds ranged from 50.0 to 200.0 μg/ml. After statistical data processing, pyridine derivatives (compounds 2{3} and 3{5}) were identified, possessing an effective bacteriostatic and bactericidal effect on *Y. enterocolitica* strains.

Conclusions: The results of the research showed a high antimicrobial activity of 2*H*-pyrano[2,3-*c*]pyridine derivatives. The highest activity against *Y. enterocolitica* was found for 2-N_2-arylimino-5-hydroxy-methyl-8-methyl-2*H*-pyrano[2,3-*c*]pyridine-3-N_1-aricarboxamide derivatives.

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INTRODUCTION

Pandemic diseases are of global concern in the present era, causing gigantic morbidity and transience, regardless of, extensive medical facilities [1, 2] with significant changes in medical service [3] and science [4].

Intestinal yersiniosis, the causative agents of which are various serotypes of *Yersinia enterocolitica*, is registered in many world countries. Intestinal yersiniosis can be found in almost all administrative territories, mostly in the form of sporadic cases [5, 6]. Clinical diagnostics of the intestinal pathology is hard [7, 8] but for yersiniosis especially due to the polymorphism of symptoms, which can be taken for various diseases of both infectious and non-infectious nature. The expressed polymorphism of clinical manifestations and the difficulties of early laboratory diagnostics lead to diagnostic errors, recommending inadequate antibiotic therapy and, as a result, to the development of the disease complications with subsequent disability of the patient [9, 10]. In this connection, Yersiniosis causes not only significant socio-economic losses but also constitute an important problem for human health [11, 12]. An important aspect in the treatment of patients with intestinal yersiniosis is the administration of effective antibiotic therapy [13]. However, even with the use of two courses of antibiotic therapy, the development of chronic forms and complications of the disease is still possible [14]. Therefore, today there is an urgent need to find new antibacterial drugs with antiyersinic activity [15].

Under the modern rapid development of science, experts are constantly searching for new antimicrobial compounds through the directional synthesis of antibiotic substances. Among the most common synthetic drugs, there are heterocyclic compounds, such as pyridine and pyrimidine cycles with a wide range of pharmacological effects [16]. Among them, trimethoprim has a high antimicrobial activity, which suppresses the dihydrofolate reductase of the bacterial cell, and leads to a disturbance of the conversion of bacterial dihydrofolate to tetrahydrofolate [17, 18]. Promising in terms of finding highly active compounds with antimicrobial properties are synthetic derivatives of 2*H*-pirano[2,3-*c*]pyridine.

Preclinical studies of new pharmaceutical substances require mandatory testing of their effectiveness with the participation of comparison drugs. Comparative drugs must comply with the general requirements for drugs obtained in the synthesis process. These include the achievement of a positive clinical effect in an *in vitro* experiment on laboratory animals. They should also be of low or no toxicity and exhibit physicochemical properties similar to the sample/composition under study. In our case, in relation to *Yersinia*, the absence of allergic reactions to the substance was the actual antimicrobial activity.

The drug we have synthesized (2*H*-pirano[2,3-*c*]pyridine or 7-azacumarins) was tested taking these requirements into account. Since the drug we received is a new substance that has no chemical analogues, we used to test, as is recommended in such cases, a drug analogue in clinical action of trimethoprim (2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine) that is a bacteriostatic antibiotic active against gram-negative (*Escherichia coli, Yersinia, Proteus, Klebsiella*) and some gram-positive microorganisms.

Performed research aimed to determine the spectrum and level of antimicrobial activity of 2*H*-pyrano[2,3-*c*]pyridine derivatives on the museum and clinical strains of gram-negative microorganisms *Yersinia enterocolitica*.

MATERIALS AND METHODS OF RESEARCH

The object of the research is 28 synthetic derivatives of 2*H*-pyrano[2,3-*c*]pyridines, synthesized in the laboratory of the Department of Organic Chemistry of the Kharkiv National Pharmaceutical University. The compounds studied were chemically and structurally divided into 4 groups: **I** - 5-hydroxymethyl-2-imino-8-methyl-2*H*-pyrano[2,3-*c*]pyridine-3-*N*-arylcarboxamid (1{1} - 1{7}); **II** - 2-N_2-arylimino-5-hydroxy-methyl-8-methyl-2*H*-pyrano[2,3-*c*]pyridine-3-N_1-aricarboxamides (2{1} - 2{7}); **III** - 2-N_2-arylimino-3-N_1-arylcarboxamido-8-methyl-2*H*-pyrano-[2,3-*c*] pyridin-5-yl) - methylacetates (3{1} - 3{7}); **IV** - 2-*N*-arylimino-5-hydroxymethyl-8-methyl-2*H*-pyrano[2,3-*c*]pyridine-3-carboxamides (4{1} - 4{7}).

Connections have their own codes depending on the radicals they contain. The study of the antimicrobial activity of the substances under research was carried out using the serial dilutions method [19] in the liquid nutrient medium by Muller-Hinton [20, 21].

As a solvent, polypropylene glycol was used in the experiments. The initial solutions of which with the test substances were adjusted to a concentration of 1 mg/ml. The spectrum and the level of antimicrobial

Table 1: Biotypes and Serotypes of *Y. enterocolitica* Isolated from Humans, Rodents, Environmental Objects

Research material	Total cultures		Serotypes *Y. enterocolitica* biotypes 1A								Serotypes *Y. enterocolitica* biotypes 4			
			O:4,32		O:5,27		O:6,30		Not typed		O:3		O:6,30	
	n	%	N	%	N	%	n	%	n	%	n	%	n	%
From patients	5	20	-	-	-	-	-	-	-	-	5	20	-	-
From small rodents	8	32	1	4	1	4	3	12	1	4	1	4	1	4
From vegetables	11	44	-	-	2	8	4	16	1	4	2	8	2	8
Flushes from the inventory of vegetable storage	1	4	-	-	-	-	-	-	1	4	-	-	-	-
Total	25	100	1	4	3	12	7	28	3	12	8	32	3	12

activity of the newly synthesized compounds were studied using 25 strains of *Y. enterocolitica*, isolated from patients with intestinal larynx and bacteriological examination of environmental objects. The microbial strain load was 10^6 and 10^7 colony-forming units per 1 ml of nutrient medium (CFU/ml).

Minimum inhibitory and bactericidal concentrations (MIC and MBC) were determined. As a control, trimethoprim was used. In addition, control of nutrient media and solvent has been carried out in accordance with generally accepted methods. All studies were conducted in five replicates. The results of the study were processed on a computer using the statistical software package "Statistica, Version 13" (Copyright1984-2018 TIBCO Software Inc. All rights reserved. License No. JPZ8041382130ARCN 1-J).

RESULTS

Results of the MIC and MBC of compounds synthesized from substituted 2*H*-pyrano[2,3-*c*]-pyridines with regard to strains *Y.enterocolitica* are presented in Table **2** and Table **3** accordingly. Influence of 2*H*-pyrano[2,3-*c*]-pyridines derivatives on the formation of resistance to them for *Y.enterocolitica* is presented in Figure **1**.

Table 2: Results of the Minimum Bactericidal Concentration of Compounds Synthesized from Substituted 2*H*-pyrano[2,3-*c*]-pyridines with Regard to Strains *Y. enterocolitica*, n=25

The concentration of the drug, μg/ml	Substance code / Number of sensitive strains, %							
	1{1}	2{1}	2{3}	3{5}	4{1}	4{2}	4{3}	4{4}
200,0	72,3 %	28,6 %	38,1 %	33,3 %	72,3 %	90,5 %	33,3 %	33,3 %
100,0	28,6 %	72,3 %	61,9 %	34,6 %	28,6 %	9,5 %	61,9 %	66,4 %
50,0	-	-	66,4 %	65,4 %	-	-	-	-
25,0	-	-	4,8 %	-	-	-	-	-
M±m	214,3±12,3	160,7±12,3	172,6±13,4	160,7±12,3	214,3±12,3	238,1±8,1	163,7±13,7	166,7±13,0

Table 3: Results of the Minimum Inhibitory Concentration Study of Compounds Synthesized from Substituted 2*H*-pyrano[2,3-*c*]-pyridines with Regard to Strains *Y. enterocolitica*, n=25

The concentration of the drug, μg / ml	Substance code / Number of sensitive strains, %							
	1{1}	2{1}	2{3}	3{5}	4{1}	4{2}	4{3}	4{4}
200,0	-	-	-	-	-	-	33,3 %	-
100,0	100 %	-	80,9 %	80,9 %	-	19,1 %	47,6 %	9,5 %
50,0	-	80,9 %	90,5 %	95,2 %	100 %	80,9 %	19,1 %	90,5 %
25,0	-	-	90,5 %	95,2 %	-	-	-	-
M ±m	125,0±0	56,6±2,7	68,4±18,3	56,5±2,7	62,5±0	74,4±5,4	154,8±15,7	68,5±4,0

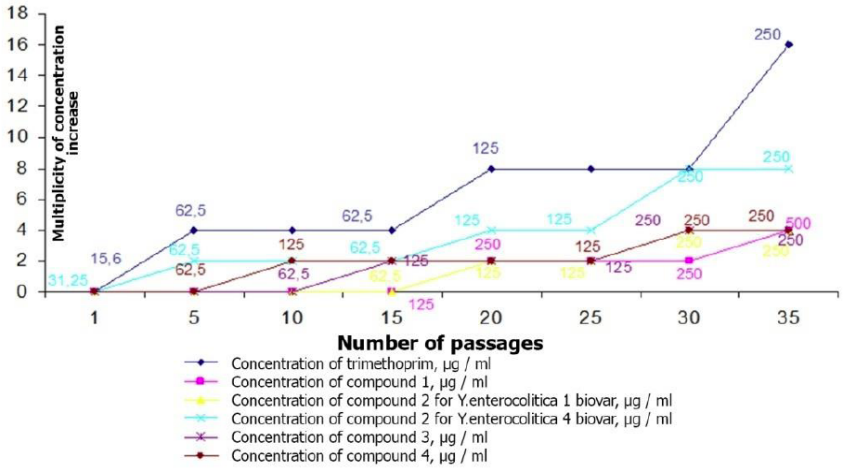

Figure 1: Influence of 2*H*-pyrano[2,3-*c*]-pyridines derivatives on the formation of resistance to them for *Y. enterocolitica.*

According to the research results on the anti-herrine action of new promising antimicrobial compounds which have been synthesized on the basis of derivatives of 2*H*-pyrano[2,3-*c*]pyridines, in order to substantiate the feasibility of a new antimicrobial agent creation on their basis, it has been established that MIC compounds of Group I for all strains *Y. enterocolitica* were 100.0 μg/ml. MIC for the majority of Yersinia cultures (72.3%) was 200.0 μg / ml. The average inhibitory concentration of this compound was 100.0 ± 0 μg/ml, bactericidal 200.0 ± 0 μg / ml.

Among the compounds derived from 2-N_2-arylimino-5-hydroxy-methyl-8-methyl-2*H*-pyrano[2,3-*c*]pyridine-3-N_1-aricarbosamides (Group II), the compound anti-worry activity was compounded 2{3}. The inhibition effect on Yersinia was detected at a concentration of 25.0 μg/ml, while the MIC of other compounds in this group ranged from 50.0 to 100.0 μg/ml. As a result, the average inhibitory concentration of the chemical compound 2{3} (25.0 ± 0 μg/ml) significantly exceeded the mean inhibitory concentration of compounds 2{1} (50.0 ± 0 μg/ml) and 2{2} (100.0 ± 0 μg/ml). MIC for both of these compounds was 100.0-200.0 μg/ml, respectively.

The chemical compound related to 2-N_2-arylimino-3-N_1-arylcarboxamido-8-methyl-2*H*-pyrano-[2,3-*c*] pyridin-5-yl)-methylacetates (Group III, compound 3) had a high enough antimicrobial activity, delaying the growth of the majority of Yersinia strains (95.3%) with MIC 50.0 μg/ml. In this case, the average inhibitory concentration was 25.0 ± 0 μg/ml. At MBC 50.0 μg/ml, growth stopped completely in 65.4% of cultures, whereas for 34.6% MBC it was 100.0 μg/ml (average bactericidal concentration - 100.0 ± 0 μg / ml).

Compounds with moderate antimicrobial activity were found in the 2-*N*-arylimino-5-hydroxymethyl-8-methyl-2*H*-pyrano[2,3-*c*]pyridine-3-carboxamide group (Group IV). Thus, the MIC compounds 4{1} were 50.0 μg/ml, 4{2} and 4{4} 50.0-100.0 μg/ml compounds 4{3} 200.0 μg/ml. In this case, the lowest average inhibitory concentration of this group of derivatives was 50.0 ± 0 μg / ml (compound 4{1}), the highest was (200.0 ± 0) μg / ml (compound 4 {3}). MIC compounds 4{1}, 4{2} and 4{4} were 100.0-200.0 μg / ml, while 4{3} - 200.0 μg / ml. However, the average bactericidal concentration of compounds 4{4}, 4{3} was equal to (100.0 ± 0) and (200.0 ± 0) μg / ml, whereas in compounds 4{1} and 4{2} - (400.0 ± 0) μg/ml, respectively.

DISCUSSION

Environmental pollution [22, 23], infectious diseases [24] creation of new medications are becoming a challenge for modern society, which is reflected in the development of medicine in which sometimes unexpected ways are used for problem solutions [25, 26] for microbial contamination [27,28].

Yersinosis is one of the common infectious diseases known since the last century. At present, the pathogen of yersiniosis is a sufficiently studied microorganism, both in the medical and in the general biological aspect [29]. But despite the predictable sensitivity to many antibiotics, the issues of yersiniosis and pseudotuberculosis antibiotic therapy need further study. The whole world is involved in research on the problem of combating yersiniosis: the countries of North and South America, the United Kingdom, the countries of Central Europe, Scandinavia, Africa, and

also Russia, China, South Korea, Japan, Israel [1, 10]. In recent years, cases of yersinosis have become more frequent in many countries around the world.

According to WHO, there has been an increase in diseases caused by pathogens of the genus *Yersinia Enterobacteriactae* (pseudotuberculosis and intestinal yersiniosis). Cases and epidemic outbreaks of the disease have also been reported in Ukraine. In the acute intestinal infection group, between 6 and 10.8% of patients with yersiniosis occur. Different clinical forms of yersinosis differ in severity and duration of course, but many clinical cases are mistakenly registered under other diagnoses, as the disease is characterized by polymorphism of clinical manifestations with involvement of different organs and systems in the pathological process, relapsing course, formation of secondary focal forms [11].

The relevance of the problem of yersiniosis is also due to the adverse outcomes of acute forms. The problem of rational therapy of yersiniosis infection in children is still topical. The treatment of patients with yersiniosis depends on the clinical variant of the disease. Along with the positive evaluation of antibiotic therapy, works have appeared in which a decrease in the effect due to increased drug resistance of pathogens; an increase in the number of adverse reactions, the appearance of a large number of patients with intestinal microflora disorder and, as a result, long-term intestinal disorders have been noted [30]. So, despite the predictable sensitivity to many antibiotics, the antibiotic therapy of yersinosis and pseudotuberculosis requires further research.

As a result of our work after statistical data processing, pyridine derivatives with the most effective bacteriostatic and bactericidal action on strains *Y. enterocolitica* were identified. For this group, compounds 2{3} and 3{5} are included. Thus, compound 2{3}, which is a derivative of 2-N_2-arylimino-5-hydroxy-methyl-8-methyl-2*H*-pyrano[2,3-*c*]pyridine-3-N_1-aricarbosamides, inhibited the growth of *Yersinia* in a concentration of 25.0 ± 0 μg/ml and showed the bactericidal activity in a concentration of 100.0-200.0 μg of the substance in 1 ml of medium. At the same time, compound 3{5}, which is a derivative of 2-N_2-arylimino-3-N_1-arylcarboxamido-8-methyl-2*H*-pyrano-[2,3-*c*]pyridin-5-yl)-methylacetates exhibited bacteriostatic action in a concentration of 25.0 ± 0 μg/ml and bactericidal action of 100.0 ± 0 μg / ml. The results indicate that these compounds exhibit high anti-*Yersinia* activity *in vitro*, which allows us to consider the promising development of antibacterial drugs for the treatment of yersiniosis.

The results obtained open the prospect of a further search for substances from the group of 7-azacoumarins and indicate that these compounds exhibit high antiyersinia activity *in vitro*, which makes the development of antibacterial agents for the treatment of yersinosis promising, which may contribute to the optimization of therapeutic tactics that could improve the life of patients as we observed for other infectious pathology [31, 32, 33] due to more often pathogenic Y. enterocolitica and the significance of this untypical strain in human and animal infections.

CONCLUSIONS

1. The obtained results of the research showed a high activity of 2*H*-pyrano[2,3-*c*]pyridine derivatives.

2. From the group of 2-N2-arylimino-5-hydroxymethyl-8-methyl-2H-pyrano[2,3-c]pyridine-3-N_1-aricarbosamide derivatives, compound 2{3} showed the greatest anti-yersinios activity and inhibited the growth of both museum and clinical strains of *Yersinia*.

3. The highest antibacterial activity was found in the Group 3 of substances, namely, in the group of derivatives of 2-N_2-arylimino-5-hydroxy-methyl-8-methyl-2*H*-pyrano [2,3-*c*]pyridine-3-N_1-aricarbosamides.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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