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ANTIMICROBIAL ACTIVITY OF [1,2,4]TRIAZOLO[4,3-*a*]PYRAZIN-8(7*H*)-ONE DERIVATIVES

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The search for new and effective antimicrobial and antifungal agents is an important task of medical chemistry. In this paper is set out the results of microbiological screening of new derivatives of [1,2,4]triazolo[4,3-*a*]pyrazine. Investigations have shown that these compounds show moderate antimicrobial activity against standard test systems, and some of them can be used for further modification in order to enhance their influence on the microbial cell.

Key words: [1,2,4]triazolo[4,3-*a*]pyrazin-8(7*H*)-one derivatives, antimicrobial activity, antifungal activity.

The massive and uncontrolled use of antimicrobial and antifungal agents leads to appear microbial resistance to popular drugs [1-3] and in turn it limit the antibiotic use, which is one of the important causes of treatment failure [4]. In the latest report by the World Health Organization (WHO) [5], the problem of resistance to antimicrobial agents, including antibiotics, is considered globally. This means that the threat is not just a prediction for the future, but it's the real situation in many countries today. The main solution to this problem is prevention of bacterial infection (personal hygiene, access to clean drinking water, etc.) in order to reduce the need for antibiotics and the search for new effective drugs with a new formula for the treatment of infectious diseases.

From the literature it is known that derivatives of [1,2,4]triazolo[4,3-*a*]pyrazine testify have a wide range of biological actions [6-12]. Our attention was attracted by works, which had studied the antimicrobial activity some of compounds from this row [12] and their antifungal action [7].

Materials and methods

We made a library of these compounds for virtual screening using the PASS C&T (Prediction Activity Spectra for Substances: Complex & Training) program [13-20] lean on the published data of the fragment of structures [1,2,4]triazolo[4,3-*a*]pyrazine activity. According to the results we have chosen [1,2,4]triazolo[4,3-*a*]pyrazin-8(7*H*)-one derivatives as the researching objects that had the most likelihood of manifestation of these pharmacological effects (P_a) is within $0.5 \leq P_a \leq 0.8$. These substances were synthesized by the authors according to the previously developed scheme at the National University of Pharmacy [21].

As a result, we have received 35 synthetic compounds of 7 series, namely: 3-alkyl-N^7-aryl/benzyl-[1,2,4]triazolo[4,3-*a*]perazin-8(7*H*)-ones **1***{1-5}*, N^7-aryl-3-(8-oxo-7*H*-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)carboxylic acids **2***{1-5}*, 3-aryl-N^7-aryl/benzyl-[1,2,4]triazolo[4,3-*a*]pyrazin-8(7*H*)-ones **3***{1-5}* (Fig. 1), N^7-aryl/benzyl-3-thioxo-[1,2,4]triazolo[4,3-*a*]pyrazin-8(7*H*)-ones **4***{1-5}*, N^7-aryl-[1,2,4]triazolo[4,3-*a*]pyrazin-3,8(2*H*,7*H*)-diones **5***{1-5}*, N^2-arylacetamide/benzyl-N^7-aryl-[1,2,4]triazolo[4,3-*a*]pyrazin-3,8(2*H*,7*H*)-diones **6***{1-5}* (Fig. 2), 3-(*S*-*N*-arylacetamide/benzyl)thio-N^7-aryl-[1,2,4]triazolo[4,3-*a*]pyrazin-8(7*H*)-ones **7***{1-5}* (Fig. 3, Tab. 1).

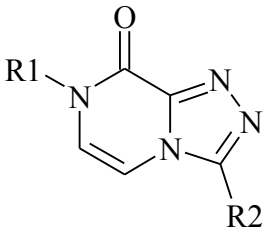

Figure 1. The structural formula of the compounds of series **1**, **2** and **3**

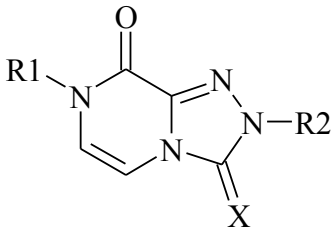

Figure 2. The structural formula of the compounds of series **4**, **5** and **6**

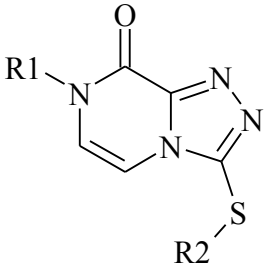

Figure 3. The structural formula of the compounds of series **7**

Table 1. Objects of research

Number	R1	R2	X
1*{1}*	4-FPh	H	-
1*{2}*	4-ClPhCH_2	Me	-
1*{3}*	4-OMePh	Et	-
1*{4}*	Ph	Pr	-
1*{5}*	4-ClPhCH_2	*i*-Pr	-
2*{1}*	3,4-diMePh	$(CH_2)_2$COOH	-
2*{2}*	4-OMePh	$(CH_2)_2$COOH	-
2*{3}*	4-ClPh	$(CH_2)_3$COOH	-
2*{4}*	3-FPh	$(CH_2)_3$COOH	-
2*{5}*	4-OMePh	$CH_2CH(CH_3)CH_2$COOH	-
3*{1}*	Ph	4-ClPh	-
3*{2}*	4-OMePh	3,4-diOMePh	-
3*{3}*	4-FPh	4-ClPh	-
3*{4}*	4-FPh	3,4-diOEtPh	-
3*{5}*	4-MePhCH_2	3,4-diOMePh	-
4*{1}*	4-iPrPh	-	S
4*{2}*	4-BrPh	-	S
4*{3}*	4-FPh	-	S
4*{4}*	3-Cl-2-MePh	-	S
4*{5}*	4-ClPhCH_2	-	S
5*{1}*	2-OMePh	-	O
5*{2}*	4-OEtPh	-	O
5*{3}*	4-BrPh	-	O
5*{4}*	3,4-diFPh	-	O
3*{5}*	3-Cl-4-OMePh	-	O
6*{1}*	Ph	4-MePhCH_2	O
6*{2}*	Ph	4-ClPhCH_2	O
6*{3}*	4-OMePh	4-ClPhCH_2	O
6*{4}*	4-FPh	4-MePhCH_2	O
6*{5}*	4-OMePh	4-ClPhNHCOCH_2	O
7*{1}*	Ph	4-ClPhCH_2	-
7*{2}*	4-OMePh	4-MePhCH_2	-
7*{3}*	4-FPh	4-MePhCH_2	-
7*{4}*	Ph	2-CF_3PhNHCOCH_2	-
7*{5}*	4-BrPh	4-OMePhNHCOCH_2	-

* Me – methyl, Et – ethyl, Pr – propyl, Ph – phenyl.

The research of antimicrobial and fungicidal activity of the synthesized compounds was carried out in the laboratory of antimicrobial agents State Institution «I. Mechnikov Institute of Microbiology and Immunology National Academy of Medical Sciences of Ukraine» under the leadership of PhD, senior scientist V.V.Kazmirchuka.

The activity of the synthesized compounds were studied by conventional method of the two-fold serial dilutions in liquid and solid nutrient medium [22-23]. For primary screening we have used a set of clinical and reference strains of microorganism: *Escherichia coli* ATCC 25922 (F-50), *Staphylococcus aureus* ATCC 25923 (F-49), *Bacillus anthracoides* ATCC 1312, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 885-653, as bacterial cultures, recommended by the State Pharmacopoeia of Ukraine to determine antimicrobial drugs [24]. As the reference preparations were chosen pipemidic acid (Palin, «Lek» Pharmaceutical Company d.d., Slovenia) - modern

antimicrobial agent of class of fluoroquinolones, nalidixic acid (Nevigramon, «CHINOIN», Pharmaceutical and Chemical Works Co.Ltd., Hungary) - nalidixic acid derivative and fluconazole (Fluconazole, Open FC «Zdorovye», Ukraine) - modern antifungal agent. Conversion of IBC was held on the content of the active substance.

The sensitivity of bacteria to the synthesized compounds were determined in Hottinger's meat infusion broth (135 mg% amine nitrogen, pH 7,2-7,4). For cultivation of fungi of the genus *Candida* was used Sabouraud medium.

Activity of substances determined by the minimum bacteriostatic ($MB_{st}K$) and minimal bactericidal (MB_cK) concentrations. The minimum bacteriostatic concentration ($MB_{st}K$) was determined by the absence of visible growth of microorganisms in a liquid nutrient medium. The medium remained transparent in those tubes where the concentration of the substance was sufficient for complete inhibition of growth of the test microorganism. The minimum bactericidal concentration (MB_cK) was determined by the absence of growth colonies after seeding of microorganisms from tubes on solid culture medium.

All experiments were accompanied by appropriate controls (of the environmental control, bacterial cultures) and repeated three times. For the statistical data was used standard statistical software package Statistica 6.0 [25-29].

Results and Discussion

As a result of microbiological screening were identified that antimicrobial and antifungal activity of some compounds of series [1,2,4]triazolo[4,3-*a*]pyrazin-8-ones are equal or higher than the reference preparation (Table 2, 3).

Table 2. Antimicrobial activity of [1,2,4]triazolo[4,3-*a*]pyrazines to reference strains of *St.aureus, E.coli, Ps.aeruginosa*

Number	*Staphylococcus aureus* ATCC 25923		*Escherichia coli* ATCC 25922		*Pseudomonas aeruginosa* ATCC 27853	
	$MB_{st}K$	MB_cK	$MB_{st}K$	MB_cK	$MB_{st}K$	MB_cK
Palinum	6.25		25.0		12.5	
Nevigramon	50.0		50.0		50.0	
Fluconazolum						
1*{1}*	50.0	100.0	50.0	100.0	100.0	200.0
1*{2}*	25.0	100.0	50.0	200.0	50.0	100.0
1*{3}*	100.0	200.0	50.0	100.0	50.0	200.0
1*{4}*	50.0	100.0	100.0	200.0	100.0	200.0
1*{5}*	50.0	100.0	50.0	100.0	50.0	100.0
2*{1}*	50.0	100.0	50.0	100.0	100.0	200.0
2*{2}*	50.0	200.0	25.0	50.0	100.0	200.0
2*{3}*	25.0	50.0	50.0	100.0	50.0	100.0
2*{4}*	50.0	100.0	25.0	100.0	25.0	50.0
2*{5}*	50.0	100.0	50.0	200.0	100.0	200.0
3*{1}*	50.0	100.0	25.0	50.0	25.0	100.0
3*{2}*	100.0	200.0	100.0	200.0	50.0	100.0
3*{3}*	50.0	100.0	50.0	100.0	25.0	50.0
3*{4}*	50.0	100.0	50.0	100.0	25.0	50.0
3*{5}*	100.0	200.0	50.0	200.0	50.0	100.0
4*{1}*	50.0	100.0	50.0	100.0	50.0	100.0
4*{2}*	25.0	50.0	50.0	100.0	12.5	25.0
4*{3}*	25.0	50.0	50.0	100.0	25.0	50.0
4*{4}*	25.0	50.0	25.0	50.0	12.5	25.0
4*{5}*	25.0	100.0	50.0	100.0	50.0	100.0
3*{1}*	100.0	200.0	50.0	100.0	100.0	200.0
5*{2}*	50.0	100.0	50.0	100.0	100.0	200.0
5*{3}*	25.0	100.0	25.0	50.0	50.0	100.0
5*{4}*	50.0	100.0	25.0	50.0	50.0	100.0
5*{5}*	25.0	100.0	25.0	50.0	25.0	50.0
6*{1}*	100.0	200.0	50.0	200.0	50.0	100.0
6*{2}*	50.0	100.0	25.0	50.0	50.0	100.0
6*{3}*	50.0	100.0	50.0	200.0	100.0	200.0
6*{4}*	25.0	50.0	50.0	100.0	50.0	100.0
6*{5}*	25.0	50.0	12.5	25.0	50.0	100.0
7*{1}*	25.0	50.0	50.0	100.0	50.0	100.0
7*{2}*	50.0	100.0	100.0	200.0	50.0	100.0

7*{3}*	25.0	50.0	50.0	100.0	50.0	100.0
7*{4}*	12.5	50.0	25.0	50.0	50.0	100.0
7*{5}*	12.5	25.0	25.0	50.0	12.5	25.0

Table 3. Antimicrobial activity of [1,2,4]triazolo[4,3-*a*]pyrazines to reference strains of *Pr.vulgaris, B.anthracoides, C.albicans*

Number	*Proteus vulgaris* ATCC 4636		*Bacillus anthracoides* ATCC 1312		*Candida albicans* ATCC 885-653	
	$MB_{st}K$	MB_cK	$MB_{st}K$	MB_cK	$MF_{st}K$	MF_cK
Palinum	12.5		12.5		12.5	
Nevigramon			6.25			
Fluconazolum					50.0	
1*{1}*	50.0	200.0	100.0	200.0	25.0	50.0
1*{2}*	25.0	50.0	100.0	200.0	25.0	100.0
1*{3}*	50.0	100.0	100.0	200.0	50.0	100.0
1*{4}*	100.0	200.0	50.0	100.0	100.0	200.0
1*{5}*	25.0	50.0	50.0	100.0	50.0	100.0
2*{1}*	100.0	200.0	50.0	100.0	50.0	100.0
2*{2}*	100.0	200.0	100.0	200.0	50.0	100.0
2*{3}*	50.0	100.0	25.0	50.0	25.0	50.0
2*{4}*	50.0	100.0	50.0	200.0	25.0	50.0
2*{5}*	100.0	200.0	50.0	100.0	25.0	50.0
3*{1}*	50.0	100.0	50.0	100.0	50.0	100.0
3*{2}*	50.0	100.0	100.0	200.0	50.0	100.0
3*{3}*	25.0	50.0	50.0	200.0	25.0	50.0
3*{4}*	100.0	200.0	50.0	100.0	25.0	50.0
3*{5}*	50.0	100.0	50.0	200.0	50.0	100.0
4*{1}*	25.0	50.0	50.0	100.0	25.0	50.0
4*{2}*	50.0	100.0	25.0	50.0	25.0	50.0
4*{3}*	25.0	50.0	25.0	50.0	25.0	50.0
4*{4}*	25.0	100.0	12.5	25.0	12.5	25.0
4*{5}*	25.0	50.0	50.0	100.0	50.0	100.0
3*{1}*	100.0	200.0	100.0	200.0	50.0	100.0
5*{2}*	100.0	200.0	100.0	200.0	100.0	200.0
5*{3}*	100.0	200.0	50.0	100.0	25.0	50.0
5*{4}*	50.0	100.0	50.0	100.0	25.0	50.0
5*{5}*	50.0	200.0	25.0	50.0	50.0	100.0
6*{1}*	100.0	200.0	50.0	100.0	100.0	200.0
6*{2}*	50.0	100.0	100.0	200.0	50.0	100.0
6*{3}*	50.0	100.0	50.0	100.0	25.0	50.0
6*{4}*	50.0	100.0	50.0	100.0	50.0	100.0
6*{5}*	25.0	100.0	25.0	50.0	12.5	25.0
7*{1}*	50.0	200.0	25.0	50.0	25.0	50.0
7*{2}*	25.0	50.0	50.0	100.0	50.0	200.0
7*{3}*	100.0	200.0	25.0	50.0	25.0	50.0
7*{4}*	25.0	50.0	12.5	50.0	25.0	50.0
7*{5}*	12.5	50.0	25.0	50.0	12.5	25.0

Among of the analyzing compounds of the first row – 3-alkyl-N^7-aryl/benzyl-[1,2,4]triazolo[4,3-*a*]pyrazin-8(7*H*)-ones – the most active was compound **1***{2}*. It showed antimicrobial activity for all reference test strains and was more active than control *Nevigramon* and *Flutsonazolum*. The most sensitive strains for this substance were *S.aureus*, *Pr.vulgaris*, *C.albicans*.

Compound **2***{4}* has attracted our attention during the research of N^7-aryl-3-(8-oxo-7*H*-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)carboxylic acids. Sensitive to it were *E.coli*, *P.aeruginosa*, *C.albicans.* Generally, this group showed low antimicrobial activity, especially to gram-negative bacteria.

The third group of these substances showed moderate antimicrobial and antifungal activity. There is 3-(4-chloro-phenyl)-7-(4-fluoro-phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazin-8(7*H*)-one **3***{3},* which has the best indicators of activity of the group.

The substance of the fourth series - N^7-aryl/benzyl-3-thioxo-[1,2,4]triazolo[4,3-*a*]pyrazin-8(7*H*)-ones - showed the best antimicrobial and antifungal activity of all synthesized compounds. There is also

regularity of increasing activity, if the structure of molecules containing halogen atom. Compound **4***{4}* showed high values of antimicrobial activity against gram-negative microorganisms ($MB_{st}K$ – 12.5-25.0 mkg/ml, MB_cK – 25.0-50.0 mkg/ml) and was the most promising for further development.

Compounds of the series *N*[7]-aryl-2*H*,7*H*-[1,2,4]triazolo[4,3-*a*]pyrazine-3,8-diones showed sufficiently high results of antimicrobial activity concerning to *E. coli* ($MB_{st}K$ – 25.0-50.0 mkg/ml, MB_cK – 50.0-100.0 mkg/ml). The most active was compound **5***{3}*, which was more active than control strains to *S.aureus* and *C.albicans.*

Alkylated derivatives of [1,2,4]triazolo[4,3-*a*]pyrazines showed concerning high rates of activity. The substances which deserve attention are compounds that contain an acetamide fragment as in position number 2 (compound **6***{5}*), and in position number 3 of heterocycle (compounds **7***{4}* i **7***{5}*). They have shown highest values among all of the studied compounds, which correlates well with the literature data [7, 12].

Experimental

The weight of the substance (5 mg) was dissolved in 5 ml of dimethylformamide (DMF). The 2 ml of nutrient medium was poured in every of 10 sterile tubes. Then in the each tubes of the first row we brought to 2 ml of the substance, carefully mixed and than 2 ml was transferred to the next tube. The process was performed in sterile conditions with the full range of tubes. From the last tube 2 ml of the fluid was poured. In each row one tube with 2 ml of nutrient medium was left as a control. In this way we have received serial dilutions of the test substances in a liquid medium in concentrations ranging from 400 to 0.02 mg/ml. Next to the test tubes brought culture in an amount of $2 \cdot 10^5$ CFU/ml and incubated them for 18-24 h and temperature (37±0.5)°C. Microbial load for *Candida albicans* is $5 \cdot 10^5$ CFU/ml, tubes with this culture were incubated 48 h at temperature (30±0.5)°C.

Conclusions

Design of molecular structure as the results of virtual screening is a convenient and effective tool to intensify the search for antimicrobial agents based on the new base structures. During the screening research we identified series of [1,2,4]triazolo[4,3-*a*]pyrazine-8-one derivatives with antimicrobial and antifungal activity.

In the experiments was confirmed the presence of these types of activities for all of the synthesized compounds. The most pronounced effect showed compounds that contains in their structure aryl moiety with halogen atom, or N-arylatsetamide group in position 3 or 2 of the heterocycle. Principal condition for the demonstration of antifungal activity is presence of Sulfur atom in the triazole cycle.

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ANTIMICROBIAL ACTIVITY OF [1,2,4]TRIAZOLO[4,3-*a*]PYRAZIN-8(7*H*)-ONE DERIVATIVES

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Today the problem of microbial resistance to antibacterial agents becomes the global one. Antimicrobial drugs that are in the pharmaceutical market do not satisfy the needs of modern treatment regimens, particularly Hospital-acquired infections. Therefore, the search for new and effective means of this pharmacological group is an important task of medical chemistry. From the literature it is known that derivatives of [1,2,4]triazolo[4,3-*a*]pyrazine show a wide range of biological actions, including antimicrobial and fungicidal. This makes it relevant microbiological study of primary derivatives of [1,2,4]triazolo[4,3-*a*]pyrazine for identifying promising compounds of the series and then study it in biological experiment.Using the PASS C&T (Prediction Activity Spectra for Substances: Complex & Training) program and based on published data, we have generated virtual library of derivatives of [1,2,4]triazolo[4,3-*a*]pyrazine. As a result, we have received 35 new synthetic compounds of 7 series that were not previously described in the literature.

Materials and methods

The research of antimicrobial and fungicidal activity of the synthesized compounds was carried out in the laboratory of antimicrobial agents GA "Mechnikov Institute of microbiology and immunology" under the leadership of PhD, senior scientist V.V.Kazmirchuka. The activity of the synthesized compounds were studied by conventional method of the two-fold serial dilutions in liquid and solid nutrient medium. For primary screening we have used a set of clinical and reference strains of microorganism: *Escherichia coli* ATCC 25922 (F-50), *Staphylococcus aureus* ATCC 25923 (F-49), *Bacillus anthracoides* ATCC 1312, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 885-653. As the reference preparations were chosen Palin - modern antimicrobial agent of class of fluoroquinolones, Nevigramon - nalidixic acid derivative and Fluconazole -

modern antifungal agent. Activity of substances determined by the minimum bacteriostatic ($MB_{st}K$) and minimal bactericidal (MB_cK) concentrations. All experiments were accompanied by appropriate controls.

Results and Discussion

As a result of microbiological screening of 35 compounds we have allowed to identify a number of derivatives of [1,2,4]triazolo[4,3-*a*]pyrazine-8(7H)-one with antimicrobial and antifungal activity. The most pronounced effect showed compounds that contains in their structure aryl moiety with halogen atom, or N-arylatsetamide group in position 3 or 2 of the heterocycle. Principal condition for the demonstration of antifungal activity is presence of Sulfur atom in the triazole cycle.

Conclusions

The substance of the series N^7-aryl/benzyl-3-thioxo-[1,2,4]triazolo[4,3-*a*]pyrazin-8(7*H*)-ones showed the best antimicrobial and antifungal activity of all synthesized compounds. Compound 7-(3-chloro-2-methyl-phenyl)-3-thioxo-[1,2,4]triazolo[4,3-a]pyrazin-8(7*H*)-one **4***{4}* showed high values of antimicrobial activity against gram-negative microorganisms ($MB_{st}K$ – 12.5-25.0 mkg/ml, MB_cK – 25.0-50.0 mkg/ml) and was the most promising for further development.

Key words: [1,2,4]triazolo[4,3-*a*]pyrazin-8(7*H*)-one derivatives, antimicrobial activity, antifungal activity.