UDC 615.31:547.792.1]:615.282.015.4 DOI: http://dx.doi.org/10.14739/2310-1210.2015.4.50302

A. S. Gotsulya, O. M. Kamyshnyi, N. M. Polishchuk, O. I. Panasenko, Ye. G. Knysh

Research of the antimicrobial and antifungal activity of 7-((3-thio-4-R-4*H*-1,2,4-triazoles-3-yl) methyl)theophylline S-derivatives

Zaporizhzhia State Medical University

Key words: *1,2,4-triazoles, Theophylline, Antimicrobial, Antifungal Activity.*

Aim. The research of antimicrobial and antifungal activity of 7-((3-thio-4-R-4*H*-1,2,4-triazoles-3-yl)methyl)theophylline S-derivatives has been erformed.

Methods and results. *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 885-653 have been taken as a set of standard test-strains. It has been proved that the most active alkyl-derivatives are 7-((3-thio-4-R-4H-1,2,4-triazole-3-yl)methyl)theophylline. Among the synthesized salts, which turned to be active against *Staphylococcus aureus*, the 2-(5-((theophylline-7-yl)methyl)-4-phenyl-4*H*-1,2,4-triazole-3-ylthio)acetate ammonium has attracted our attention.

Conclusion. The perspective class of compounds for further research on this type of biological activity has been defined.

Дослідження протимікробної та протигрибкової активності S-похідних 7-((3-тіо-4-R-4*H*-1,2,4-тріазол-3-іл)метил)теофіліну

А. С. Гоцуля, О. М. Камишний, Н. М. Поліщук, О. І. Панасенко, Є. Г. Книш

Здійснили дослідження протимікробної та протигрибкової активності серед S-похідних 7-((3-тіо-4-R-4*H*-1,2,4-тріазол-3-іл)метил) теофіліну. Як набір стандартних тест-штамів взято *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 885-653. Встановили, що найбільш активними серед сполук, які одержали, є алкілпохідні 7-((3-тіо-4-R-4*H*-1,2,4-тріазол-3-іл)метил)теофіліну. Серед синтезованих солей привернув увагу амоній 2-(5-((теофілін-7-іл)метил)-4-феніл-4*H*-1,2,4-тріазол-3-ілтіо)ацетат, який виявився активним стосовно *Staphylococcus aureus.* Визначили перспективний клас сполук для наступних досліджень за цим видом біологічної активності.

Ключові слова: *1,2,4-тріазол, теофілін, антимікробна, протигрибкова активність.*

Запорізький медичний журнал. – 2015. – №4 (91). – С. 95–99

Исследование противомикробной и противогрибковой активности S-производных 7-((3-тио-4-R-4*H*-1,2,4-триазол-3-ил)метил)теофиллина

А. С. Гоцуля, А. М. Камышный, Н. Н. Полищук, А. И. Панасенко, Е. Г. Кныш

Проведены исследования противомикробной и противогрибковой активности среди S-производных 7-((3-тио-4-R-4*H*-1,2,4-триазол-3-ил)метил)теофиллина. В качестве набора стандартных тест-штаммов взяты *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 885-653. Установлено, что наиболее активными среди полученных соединений являются алкилпроизводные 7-((3-тио-4-R-4*H*-1,2,4-триазол-3-ил)метил)теофиллина. Среди синтезированных солей наибольшее внимание привлёк аммоний 2-(5-((теофиллин-7-ил)метил)-4-фенил-4*H*-1,2,4-триазол-3-илтио)ацетат, который оказался активным по отношению к *Staphylococcus aureus*. Определён перспективный класс соединений для дальнейших исследований по данному виду биологической активности.

Ключевые слова: *1,2,4-триазол, теофиллин, антимикробная, противогрибковая активность.*

Запорожский медицинский журнал. – 2015. – №4 (91). – С.95–99

Introduction. Infectious diseases caused by bacteria are becoming more and more dangerous, despite many important advances in antibacterial therapy. The explanation lies in the broad and not always reasonable use of antibiotics, which in turn leads to the appearance of resistant to antibiotics strains of bacteria [2, 4, 7, 10].

In particular, the appearance of multidrug-resistant gram-positive bacteria such as methicillin-resistant strains of the genus *Staphylococcus*, vancomycin-resistant representatives of the genera *Streptococcus* and *Enterococcus*, has become a serious problem in the treatment of bacterial diseases [8]. Obvious for the discussion remain the questions regarding the toxicity of antibiotics (tetracyclines, aminoglycosides, cephalosporins, etc.), their effect on the immune system (allergic reactions, immunosuppression), dysbiosis.

Thus, obtaining new compounds that would have shown minimal negative impact on the human body has become one of the most important areas of chemical and biological research today [7].

The aim of the work was the research of the antimicrobial and antifungal activity of 7-((3-thio-4-R-4H-1,2,4-triazole-3-yl) methyl)theophylline S-derivatives.

Objects and methods of research

The sensitivity of microorganisms to newly synthesized compounds was determined in accordance with the guidelines "Determination of the sensitivity of microorganisms to antibiotics" (approved by the order №167 from 05.04.2007) [3] and methodical recommendations "Study of the specific activity of antimicrobial drugs" [1]. During the research, from the initial drug concentration of 1mg/ml, there was prepared a series of twofold serial dilutions of the drug in the broth Mueller-Hinton in the volume of 1 ml, and then added to each tube 0,1 ml of microbial veil (10^6 microbial cells / ml).

The minimum inhibitory concentration (MIC) was determined by the lack of visible growth in vitro with the minimal drug concentration, the minimum bactericide / fungicide concentration (MBtsC / MFtsC) – in the absence of growth on agar after seeding with transparent tubes. Dimethyl sulfoxide was used as a solvent of the compounds in the research.

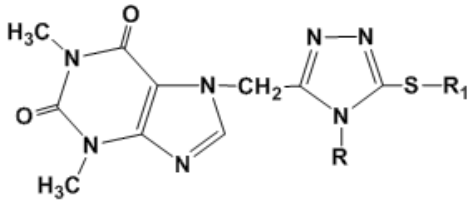
For primary screening study of synthesized substances we used standard test – cultures of both gram-positive and gram-negative bacteria belonging to different morphological properties of clinically significant groups of pathogens of infectious diseases. As a set of standard test-strains *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 885–653 were taken. All test-strains were obtained from the bacteriological laboratory of the State institution "Zaporizhzhia Regional Laboratory Center of the state sanitary-epidemiological service of Ukraine". Antibacterial drug trimethoprim was used for the qualitative control of antimicrobial activity of compounds with respect to the studied strains. Additionally, a control over nutrient media and solvent using conventional techniques was performed.

As the table shows, the MIC for *E. coli* averaged 100.52 μg/ml MBtsC – 325.4 μg/ml for *S. aureus* MIC – 78.4 μg/ml MBtsC – 380.3 μg/ml for *P. aeruginosa* MIC – 64.6 μg/ml MBtsK – 279.2 μg/ml. Minimum fungal activity averaged 75.4 μg/ml, MFtsC – 114.6 μg/ml.

Studies have shown that the greatest antimicrobial activity have alkyl derivatives 7-((3-thio-4-R-4*H*-1,2,4-triazole-3-yl) methyl)theophylline, namely 7-((3-(heptylthio)-4-phenyl-4*H*-1,2,4-triazole-5-yl)methyl)theophylline, 7-((3-(decylthio)-4-ethyl-4*H*-1,2,4-triazole-5-yl)methyl)theophylline, 7-((3-(octylthio)-4-ethyl-4*H*-1,2,4-triazole-5-yl)methyl)theophylline, 7-((3-(nonylthio)-4-ethyl-4*H*-1,2,4-triazole-5-yl)-methyl) theophylline. Changing the length of the alkyl fragment with sulfur atom surely affects the activity. Thus, compounds with an impair number of Carbon atoms turned to be more active than binate one. The lengthening of the carbon chain first causes a slight increase in activity (the transition to nonyl radical), and then it decreases (moving from nonyl fragment to decyl). Compounds with ethyl radical position 4 of 1,2,4-triazole fragment were more active than with a phenyl radical.

Table

Antimicrobial and antifungal activity of 7-((3-thio-4-R-4H-1,2,4-triazole-3-yl)methyl)theophylline S-derivatives



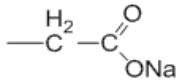
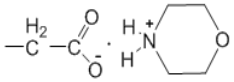
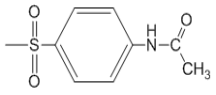
№	R	R_1	The strains that were used in the research	The result of the research	
				MIC, μg/ml	MBtsC (MFtsC for C. albscans), μg/ml
1	2	3	4	5	6
1	CH_3		E. coli	62,5	125
			S. aureus	125	500
			P. aeruginosa	62,5	500
			C. albicans	125	125
2	CH_3		E. coli	62,5	125
			S. aureus	62,5	250
			P. aeruginosa	62,5	250
			C. albicans	62,5	125
3	CH_3		E. coli	62,5	250
			S. aureus	15,6	500
			P. aeruginosa	62,5	125
			C. albicans	62,5	62,5
4	C_2H_5	H	E. coli	250	500
			S. aureus	31,25	250
			P. aeruginosa	62,5	250
			C. albicans	62,5	62,5
5	C_2H_5	$-C_8H_{17}$	E. coli	125	250
			S. aureus	7,8	31,25
			P. aeruginosa	62,5	250
			C. albicans	31,25	31,25
6	C_2H_5	$-C_9H_{19}$	E. coli	62,5	125
			S. aureus	3,9	7,8
			P. aeruginosa	62,5	125
			C. albicans	62,5	125

Table Continuation

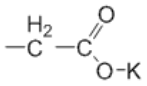
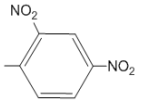
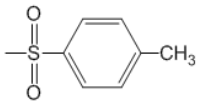
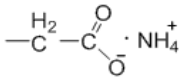
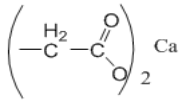
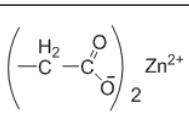
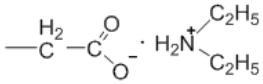
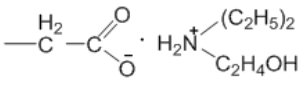
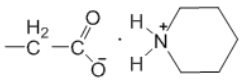
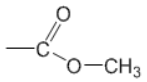
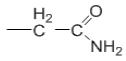
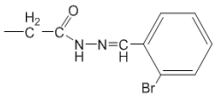
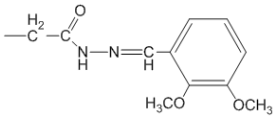
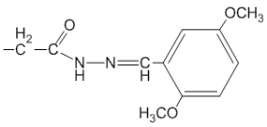
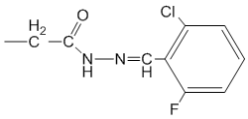
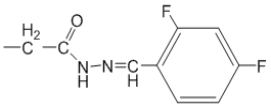
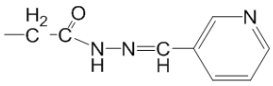
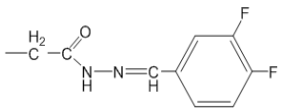
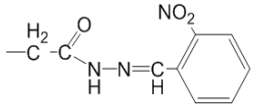
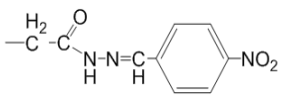
1	2	3	4	5	6
7	C_2H_5	$-C_{10}H_{21}$	E. coli	62,5	125
			S. aureus	31,25	500
			P. aeruginosa	62,5	250
			C. albicans	62,5	125
8	C_2H_5		E. coli	62,5	125
			S. aureus	125	500
			P. aeruginosa	62,5	125
			C. albicans	62,5	62,5
9	C_6H_5	H	E. coli	125	125
			S. aureus	62,5	125
			P. aeruginosa	62,5	125
			C. albicans	31,25	250
10	C_6H_5	$-C_4H_9$	E. coli	125	250
			S. aureus	62,5	500
			P. aeruginosa	62,5	125
			C. albicans	62,5	125
11	C_6H_5	$-C_7H_{15}$	E. coli	125	125
			S. aureus	31,25	250
			P. aeruginosa	62,5	250
			C. albicans	12,5	125
12	C_6H_5		E. coli,	125	500
			S. aureus,	7,8	250
			P. aeruginosa,	62,5	250
			C. albicans	31,25	31,25
13	C_6H_5		E. coli	62,5	250
			S. aureus	31,25	500
			P. aeruginosa	62,5	500
			C. albicans	62,5	125
14	C_6H_5		E. coli	62,5	125
			S. aureus	31,25	250
			P. aeruginosa	62,5	500
			C. albicans	125	125
15	C_6H_5		E. coli	125	125
			S. aureus	125	500
			P. aeruginosa	62,5	500
			C. albicans	125	250
16	C_6H_5		E. coli	62,5	250
			S. aureus	125	500
			P. aeruginosa	62,5	125
			C. albicans	62,5	250
17	C_6H_5		E. coli	62,5	250
			S. aureus	62,5	500
			P. aeruginosa	62,5	500
			C. albicans	62,5	125
18	C_6H_5		E. coli,	62,5	250
			S. aureus,	62,5	500
			P. aeruginosa,	62,5	250
			C. albicans	62,5	125
19	C_6H_5		E. coli	125	250
			S. aureus	62,5	500
			P. aeruginosa	62,5	125
			C. albicans	62,5	125
20	C_6H_5		E. coli	125	250
			S. aureus	125	125
			P. aeruginosa	125	125
			C. albicans	125	125

Table Continuation

1	2	3	4	5	6
21	C_6H_5		E. coli	62,5	250
			S. aureus	7,8	125
			P. aeruginosa	62,5	250
			C. albicans	62,5	62,5
22	C_6H_5		E. coli	125	250
			S. aureus	125	500
			P. aeruginosa	62,5	250
			C. albicans	62,5	125
23	C_6H_5		E. coli	125	250
			S. aureus	125	500
			P. aeruginosa	62,5	250
			C. albicans	125	125
24	C_6H_5		E. coli	125	250
			S. aureus	125	500
			P. aeruginosa	62,5	500
			C. albicans	62,5	125
25	C_6H_5		E. coli	125	500
			S. aureus	7,8	500
			P. aeruginosa	62,5	125
			C. albicans	62,5	125
26	C_6H_5		E. coli	125	250
			S. aureus	125	500
			P. aeruginosa	62,5	125
			C. albicans	125	125
27	C_6H_5		E. coli	125	250
			S. aureus	125	500
			P. aeruginosa	62,5	125
			C. albicans	62,5	125
28	C_6H_5		E. coli	125	250
			S. aureus	125	500
			P. aeruginosa	62,5	125
			C. albicans	125	125
29	C_6H_5		E. coli	125	250
			S. aureus	125	500
			P. aeruginosa	62,5	500
			C. albicans	62,5	62,5
30	C_6H_5		E. coli	125	250
			S. aureus	125	500
			P. aeruginosa	62,5	500
			C. albicans	62,5	125

Attention is also attracted by 7-((3-thio-4-R-4*H*-1,2,4-triazole-3-yl)-methyl)theophylline S-sulfone derivatives. Thus, 3-(4-methylbenzene)thiosulfonyl-4-phenyl-5-((theophylline)-methyl)-4*H*-1,2,4-triazole and 3-(4-acetamidobenzen)thio-sulfonyl-4-methyl-5-((theophylline)methyl)-4*H*-1,2,4-triazole showed bacteriostatic activity against *S. aureus*.

Obtaining salts also affected the activity. Comparing salts with organic and inorganic bases, organic proved to be more active. Among the synthesized salts of 2-(5-((theophylline-7-yl)methyl)-4-phenyl-4*H*-1,2,4-triazole-3-ylthio)acetate ammonium, which turned to be active against *S. aureus* has attracted the attention.

Among the active compounds should also be noted 7-((5-(2,4-dinitro-phenylthio)-4-phenyl-4*H*-1,2,4-triazole-3-yl) methyl)theophylline, 2-(5-((theophylline)methyl)-4-phenyl-4*H*-1,2,4-triazole-3-ylthio)acetamid, piperidine 2-(5-((theophylline) methyl)-4-phenyl-4*H*-1,2,4-triazole-3-ylthio)acetate, S-5-((theophylline)methyl)-4-phenyl-4*H*-1,2,4-triazole-3-yl-2-(2-chloro-6-fluorobenzyliden)-hidrazincarbothiomide.

The research of fungistatic and fungicidal activity of the synthesized compounds against *C. albicans* showed that the most active were alkyl derivatives 7-((3-thio-4-R-4*H*-1,2,4-triazole-3-yl)methyl)theophylline. Among these compounds the particular interest is caused by 7-((3-(heptylthio)-4-phenyl-4*H*-

1,2,4-triazole-5-yl)methyl)theophylline (MFtsC – 125 μg/ml) and 7-((3-(octylthio)-4-ethyl-4*H*-1,2,4-triazole-5-yl)methyl)-theophylline (MFtsC – of 31,25 μg/ml). Active has also been 7-((3-(2,4-dinitrophenylthio)-4-phenyl-4*H*-1,2,4-triazole-5-yl)-methyl)theophylline (MFtsC – of 31,25 μg/ml).

Conclusions

1. The research of bacteriostatic and bacterial / fungal activity of 7-((3-thio-4-R-4*H*-1,2,4-triazoles-3-yl)methyl)theophylline S-derivatives has been conducted.

2. Some regularities of correlation between «structure-activity» have been established.

3. It has been proved that the most active alkyl-derivatives are 7-((3-thio-4-R-4*H*-1,2,4-triazole-3-yl)methyl)theophylline.

References

1. Volianskyi, Yu. L., Hrytsenko, I. S., Shyrobokov, V. P., et al. (2004) *Vyvchennia spetsyfichnoi aktyvnosti protymikrobnykh likarskykh zasobiv [Studying of the specific activity of antimicrobial drugs]*. Kyiv. [in Ukrainian].
2. Kyreev, I. V. (2009) Issledovanie antibakterialnoj I fungistaticheskoj aktivnosti zameshchennykh i annelirovannykh proizvodnykh ksantina [Research of the antibacterial and fungystatic activity of substituted and anyllate ksantine derivatives]. *Visnyk Sumskoho derzhavnoho universytetu,* 1, 22–29. [in Ukrainian].
3. Nakaz Ministerstva okhorony zorovia Ukrainy Pro zatverdzhennia metodychnykh vkazivok «Vyznachennia chutlyvosti mikroorhanizmiv do antybakterialnykh preparativ 5 kvitnia 2007 roku №167 [Order of the Ministry of Health of Ukraine "On Approval of guidelines" Determining the sensitivity of microorganisms to antibiotics" from April 5 2007, №167] [in Ukrainian].
4. Bilyi, A. K., Kovalenko, S. I., Antypenko, L. M., Kamyshnyi, O. M., Polishchuk, N. M. (2013) Protyhrybkova ta protymikrobna aktyvnist {2-[3-heteryl-1N-1,2,4-triazol-5-il]fenil}aminiv i produktiv yikh heterotsyklizatsii [Antifungal and antimicrobial activity of {2-[3-heteryl-1H-1,2,4-triazoles-5-yl]phenyl}amine and products of their heterocyclization]. *Aktualni pytannia farmatsevtychnoi i medychnoi nauky ta praktyky,* 2(12), 80–82. [in Ukrainian].
5. Ruping, T., Linhong, J., Chengli, M., Juan, Y., Song, B., Deyu, H., et al. (2013) Synthesis, antifungal and antibacterial activity for novel amide derivatives containing a triazole moiety. *Chemistry Central Journal*, 7(30), 1–7. doi:10.1186/1752-153X-7-30.
6. Shobha, D., Umes, L., Rajani, B., & Shankar, B. (2013) Synthesis and antimicrobial activities of some new 1,2,4-triazole derivatives. *Indian Journal of Chemistry*, 52B(08), 1176–1181.
7. Kumari, S., Nitin, K., Sushma, D., & Pramod, K. S. (2011) Advances in synthetic approach to and antifungal activity of triazoles. *J. Org. Chem.,* 7, 668–677. doi: 10.3762/bjoc.7.79.
8. Jacob, H. J., Fawzi, I. I., & Yaseen, A. A. (2013) Antibacterial activity of some selected 1,2,4-triazole derivatives against standard, environmental, and medical bacterial strains. *Advanced Studies in Biology,* 5(6), 291–301. http://dx.doi.org/10.12988/asb.2013.3418.
9. Sumitra, C., Yogesh, B., & Shipra, B. (2010) Synthesis and antibacterial activity of some new triazole derivatives. *Archives of Applied Science Research*, 2(3), 117–126.
10. Waled, A. E., Eman, M. F., & Eman, M. H. (2012) Anticancer and antimicrobial activities of some synthesized pyrazole and triazole derivatives. *Der Pharma Chemica*, 4(1), 23–32.

Information about authors:

Gotsulya A. S., Ph.D. Senior Lecturer of the Department of Toxicology and Inorganic Chemistry Zaporizhzhia State Medical University,
E-mail: andrey_goculya@mail.ru.
Kamyshny O. M., Dr.hab., Head of the Department of Microbiology, Virology and Immunology, Zaporizhzhia state medical university.
Polishchuk N. M., Ph.D., Senior Lecturer of the Department of Microbiology, Virology and Immunology, Zaporizhzhia state medical university.
Panasenko O. I., Dr.hab., Professor, Head of the Department of Toxicology and Inorganic Chemistry, Zaporizhzhia State Medical University.
Knysh Ye. G., Dr.hab., Professor, Head of the Department of Management and Pharmacy Economics, Medical and Pharmaceutical Commodity Research, Zaporizhzhia State Medical University.

Відомості про авторів:

Гоцуля А. С., к. фарм. н., старший викладач каф. токсикологічної та неорганічної хімії, Запорізький державний медичний університет,
E-mail: andrey_goculya@mail.ru.
Камишний О. М., д. мед. н., професор, зав. каф. мікробіології, вірусології та імунології, Запорізький державний медичний університет.
Поліщук Н. М., к. фарм. н., старший викладач каф. мікробіології, вірусології та імунології, Запорізький державний медичний університет.
Панасенко О. І., д. фарм. н., професор, зав. каф. токсикологічної та неорганічної хімії, Запорізький державний медичний університет.
Книш Є. Г., д. фарм. н., професор, зав. каф. управління та економіки фармації, медичного та фармацевтичного правознавства, Запорізький державний медичний університет.

Сведения об авторах:

Gotsulya A. S., MD., Ph.D. Senior Lecturer of the Department of Toxicology and Inorganic Chemistry Zaporizhzhia State Medical University,
E-mail: andrey_goculya@mail.ru.
Kamyshny O. M., MD., Ph.D. DSci., Head of the Department of Microbiology, Virology and Immunology, Zaporizhzhia State Medical University.
Polishchuk N. M., MD., Ph.D., Senior Lecturer of the Department of Microbiology, Virology and Immunology, Zaporizhzhia State Medical University.
Panasenko O. I., MD., PhD., DSci., Professor, Head of the Department of Toxicology and Inorganic Chemistry, Zaporizhzhia State Medical University.
Knysh Ye. G., MD., PhD., DSci., Professor, Head of the Department of Management and Pharmacy Economics, Medical and Pharmaceutical Commodity Research, Zaporizhzhia State Medical University.

Поступила в редакцию 10.02.2015 г.