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Synthesis and antimicrobial activity of 3,5-R-4-(3-(5-nitrofuran-2-yl)-allylidenamino) -1-R_1-4*H*-1,2,4-triazole halides derivatives

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Key words: *Triazoles, Anti-infective Agents, Analysis.*

Aim. A number of new 3,5-R-4-(3-(5-nitrofuran-2-yl)allylidenami-no)-1-R_1-4*H*-1,2,4-triazole halides have been synthesized, the structure of synthesized compounds has been proved by modern physical-chemical methods.

Methods and results. Experimental substantiation of the antimicrobial activity of newly synthesized derivatives of 3,5-R-4-(3-(5-nitrofuran-2-yl)allylidenamino)-1-R_1-4*H*-1,2,4-triazole halides using disco-diffusion method on Mueller Hinton medium has been performed.

Conclusion. Analysis of the results of microbiological research allowed to determine the substituent's influence on the antimicrobial activity of 3,5-R-4-(3-(5-nitrofurans-2-yl)allylidenamino)-1-R_1-4H-1,2,4-triazolo halides derivatives. The results indicate the prospects for further study.

Синтез та антимікробна активність серед похідних 3,5-R-4-(3-(5-нітро-фуран-2-іл)аліліденаміно) -1-R_1-4*H*-1,2,4-тріазолію галогенідів

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Уперше синтезували ряд нових похідних 3,5-R-4-(3-(5-нітрофуран-2-іл)аліліденаміно)-1-R_1-4*H*-1,2,4-тріазолію галогенідів, структура яких підтверджена за допомогою сучасних фізико-хімічних методів. Експериментально обґрунтували антимікробну дію отриманих похідних 3,5-R-4-(3-(5-нітрофуран-2-іл)аліліденаміно)-1-R_1-4*H*-1,2,4-тріазолію галогенідів з використанням диско-дифузійного методу на середовищі Мюллера – Хінтона. Аналіз результатів мікробіологічного дослідження дав можливість визначити вплив замісників у структурі тріазолового циклу на антимікробну активність похідних 3,5-R-4-(3-(5-нітрофуран-2-іл)аліліденаміно)-1-R_1-4H-1,2,4-тріазолію галогенідів. Результати свідчать про перспективність наступних досліджень.

Ключові слова: *тріазоли, антимікробні агенти, аналіз.*

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Синтез и противомикробная активность среди производных 3,5-R-4-(3-(5-нитрофуран-2-ил)аллилиденамино)-1-R_1-4*H*-1,2,4-триазолия галогенидов

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Впервые синтезирован ряд новых производных 3,5-R-4-(3-(5-нитрофуран-2-ил)аллилиденамино)-1-R_1-4*H*-1,2,4-триазолия галогенидов, структура которых подтверждена с помощью современных физико-химических методов. Экспериментально обоснована противомикробная активность полученных производных 3,5-R-4-(3-(5-нитрофуран-2-ил)аллилиденамино)-1-R_1-4*H*-1,2,4-триазолия галогенидов с использованием диско-дифузионного метода в среде Мюллера – Хинтона. Анализ результатов микробиологического исследования позволил определить влияние заместителей в структуре триазолового цикла на антимикробную активность производных 3,5-R-4-(3-(5-нитрофуран-2-ил)аллилиденамино)-1-R_1-4H-1,2,4-триазолия галогенидов. Полученные данные свидетельствуют о перспективности дальнейшего исследования.

Ключевые слова: *триазолы, антимикробные агенты, анализ.*

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The study of antimicrobial activity remains important step in finding more safe and effective drugs that would be able to satisfy the requirements of physicians [6]. Our attention was caught by 1,2,4-triazoles, which are known to be structural fragments of biologically active compounds with different types of activity [3]. The investigation of scientific literature showed that considerable interest is devoted to antimicrobial activity of 1,2,4-triazoles derivatives [1,2,5,7,8]. Thus, we believe, that introduction of nitrofuran cycle and lengthening of alkyl chain within furan cycle can change the types of activities with primary manifestation of antimicrobial activity.

The aim of the work was the synthesis and identification of potential compounds with antimicrobial activity among 3,5-R-4-(3-(5-nitrofuran-2-yl)allylidenamino)-1-R_1-4*H*-1,2,4-triazole halides.

Materials and methods of research

The research of some physical-chemical properties of the synthesized compounds was carried out according to the methods described in the State Pharmacopoeia of Ukraine (SPU, Vol. 1). The melting point was determined by capillary method on the TAP-M device (a device for determining the melting point of crystalline materials, Russia). The structure of the compounds was confirmed by elemental analysis instrument on Elemental Vario EL cube (Elementar Analysensysteme, Germany), IR spectra (4000 – 400 cm^{-1}) were taken from modules ALPHA-T spectrometer Bruker ALPHA FT-IR (Bruker optics, Germany). Determination of elemental analysis of the compounds was performed with the help of elemental analyzer Elemental Vario EL cube (Elementar Analysensysteme, Germany), IR spectra (4000 – 400 cm^{-1}) were taken from modules ALPHA-T spectrometer Bruker ALPHA FT-IR (Bruker optics, Germany). The identity of the synthesized compounds was confirmed by thin layer chromatography plates «Sorbfil» produced by JSC "Sorbpolimer" in the solvent system acetone:hexane. Investigation

of antimicrobial activity of the compounds was performed in bacteriological laboratories of Zaporizhzhya Regional Hospital by disco-diffusion method [4] on Mueller-Hinton medium using the following test strains of microorganisms: gram-positive cocci (*Staphylococcus aureus ATCC 25923, Enterobacter aerugenes, Enterococcus faecalis ATCC 29212*), gram-negative bacilli (*Pseudomonas aeruginosa PSS27853, Escherichia coli ATCC 25922*), facultative anaerobic gram-negative rods (*Klebsiella pneumonia*). In determining the sensitivity a standard inoculum was used, which corresponds to 0,5 standard Mack Farlanda, that contains about $1,5 \cdot 10^8$ the numbers of colony forming units (CFU) per cm^3. The standard inoculum was applied to the surface of the Petri dish by pipette with nutrient medium in a volume of 1,2 cm^3. It was steadily distributed on the surface by shaking; excess of inoculum was removed by pipette. On the agar surface were deposited standard sterilized paper discs (6 mm diameter), which were impregnated with a solution of synthesized compounds of dimethyl sulfoxide (DMSO) (100 mg/disk). The duration of incubation of dishes with bacteria was 24 h at 35 °C, while with fungi it was from 48 to 72 h at 28 – 30 °C. The diameter of the zones of growth retardation was measured with the accuracy within 1 mm. Only disks with DMSO did not cause inhibition growth of specified microorganisms.

3,5-R-4-(3-(5-nitrofuran-2-yl)allyliden)-4H-1,2,4-triazoles-4-amine (compound 1 and 5, table 1)

To a solution of 0,01 mole of 4*H*-1,2,4-triazole-4-amine or 3,5-dimethyl-4*H*-1,2,4-triazole-4-amine in 30 ml of ethanol and a catalytic amount of hydrochloric acid is added 0,01 mole of 5-nitro-2-furyl and leave at room temperature for 12-24 hours. The formed precipitates are yellow crystalline substances, slightly soluble in water and soluble in organic solvents.

3,5-R-4-(3-(5-nitrofuran-2-yl)allylidenamino)-1-alkyl-4H-1,2,4-triazole halo-genides (compound 2, 3, 6 and 7, table 1)

To a solution of 0,01 mole of 4-amino-1-alkyl-4*H*-1,2,4-triazole halogenides or 3,5-dimethyl-4-amino-1-alkyl-4*H*-1,2,4-triazole halides in 30 ml of ethanol was added 0,01 mole of 5-nitro-2-furyl. The mixture was left at room temperature for 10-12 hours. The solvent was evaporated, the precipitates of the compounds were washed with ether and dried.

3,5-R-4-(3-(5-nitrofuran-2-yl)allylidenamino)-1-(carboxymethyl)-4H-1,2,4-triazole chlorides (compound 4 and 9, table 1)

To a solution of 0,01 mole of 4-amino-1-(carboxymethyl)-4*H*-1,2,4-triazole chloride or 3,5-dimethyl-4-amino-1-(carboxymethyl)-4*H*-1,2,4-triazole chloride in 30 ml of ethanol was added 0,01 mole of 5-nitro-2-furyl and left at room temperature for 10-12 hours. The solvent was evaporated, the precipitates of compounds were washed with ether and dried.

3,5-dimethyl-4-(3-(5-nitrofuran-2-yl)allylidenamino)-1-(2-oxophenylethyl)-4H-1,2,4-triazole bromide (compound 8, table 1)

To a solution of 0,01 mole of 3,5-dimethyl-4-amino-1-(2-oxophenylethyl)-4*H*-1,2,4-triazole bromide in 30 ml of ethanol was added 0,01 mole of 5-nitro-2-furyl and left at room temperature for 10–12 hours. The solvent was evaporated, the precipitate was washed with ether and dried.

For the analysis 1–9 compounds were purified with ethanol.

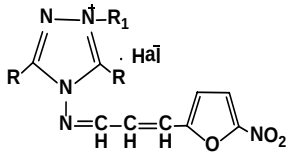
The results and their discussion

As the result of interaction of 3,5-R-4*H*-1,2,4-triazole-4-amine, 4-amino-1-alkyl-4*H*-1,2,4-triazole halides, amino-1-(carboxymethyl)-4*H*-1,2,4-triazole chloride 4-amino-1-(2-oxophenylethyl)-4*H*-1,2,4-triazole bromide with an equivalent amount of 5-nitro-2-furyl were derived Schiff bases (compounds 1-9, Tab. 1). The result of chromatographic investigation proved that compounds 1-9 are individual matters.

IR spectrum of these compounds are characterized by signals of C=N-groups of triazole cycle in the range of 1631-1618 cm^{-1} ($\nu_{C=N}$) while nitro group manifested in the form of absorption bands in the range of 1351-1346 cm^{-1} ($\nu_{NO_2}^{as}$), 851-845 cm^{-1} and

Table 1

Physico-chemical properties derivatives 3,5-R-4-(3-(5-nitrofuran-2-yl)allylidenamino)-1-R_1-4*H*-1,2,4- triazole halides



№ compound	R	R_1	Hal$^-$	m.p.,°C	Formula	Yield, %	Found, %:			Calculated, %		
							C	H	N	C	H	N
1	H	H	-	> 230	$C_8H_5N_5O_3$	65	43,73	2,30	32,02	43,84	2,30	31,96
2	CH_3	H	-	178-180	$C_{10}H_9N_5O_3$	70	48,44	3,68	28,39	48,58	3,67	28,33
3	H	C_8H_{17}	Br	175-177	$C_{17}H_{24}BrN_5O_3$	65	47,78	5,66	16,45	47,90	5,67	16,43
4	H	$C_{10}H_{21}$	Cl	123-125	$C_{19}H_{28}BrN_5O_3$	67	55,51	6,83	17,15	55,67	6,88	17,09
5	H	CH_2COOH	Cl	170-175	$C_{11}H_{10}ClN_5O_5$	65	40,21	3,08	21,41	40,32	3,08	21,37
6	CH_3	C_8H_{17}	Br	>230	$C_{19}H_{28}BrN_5O_3$	70	50,10	6,20	15,43	50,23	6,21	15,41
7	CH_3	$C_{10}H_{21}$	Cl	180-182	$C_{21}H_{32}ClN_5O_3$	70	57,42	7,36	16,01	57,59	7,36	15,99
8	CH_3	$CH_2CO\text{-}C_6H_5$	Br	108-110	$C_{19}H_{18}BrN_5O_4$	69	49,43	3,93	15,26	49,58	3,94	15,22
9	CH_3	CH_2COOH	Cl	218-220	$C_{13}H_{14}ClN_5O_5$	70	43,78	3,94	19,73	43,89	3,97	19,69

Table 2

Antimicrobial activity derivatives 3,5-R-4-(3-(5-nitrofuran-2-yl)allylidenamino)-1-R_1-4*H*-1,2,4- triazole halides

№ compound	Conc., mcg	The diameter of the zones of growth inhibition of the test cultures synthesized compounds, mm					
		Escherichia coli	Pseudomonas aeruginosa	Klebsiella pneumonia	Enterobacter aerugenes	Enterococcus faecalis	Staphylococcus aureus
1	100	38	6	25	22	28	28
2	100	32	6	23	22	28	30
3	100	31	6	20	15	26	29
4	100	28	6	16	14	21	21
5	100	34	6	22	23	27	28
6	100	14	6	8	6	15	14
7	100	26	6	15	12	22	24
8	100	19	6	12	13	16	16
9	100	17	6	8	8	14	16
Furazolidone	100	25	-	-	-	-	22
Furadonin	50	22	-	-	-	-	22
Fluconazole	25	-	-	-	-	-	-

1345-1335 cm^{-1} ($\nu_{NO_2}^{s}$) 3105 ($\nu_{=CH}$), 2973 (ν_{CH}), 2932 (ν_{CH}), 1450 ($\nu_{C=C}$), 1270 ($\nu_{N-N=C}$).

^{1}H NMR (DMSO-d_6), δ, ppm: 10,35-8,92 (s, 1H, -N=CH); 7,75 (d, 1H, furan H-4); 7,60 (d, 1H, furan H-3); 3,35-2,40 (s, 3H, CH_3).

The results obtained from initial microbiological screening (Tab. 2) showed that the antimicrobial effect of derivatives 3,5-R-4-(3-(5-nitrofuran-2-yl)allylidenamino)-1-R_1-4*H*-1,2,4-triazole halides is common to all the compounds.

During the comparative characteristics of compounds 1-9 with previously described derivatives 3,5-R-1-alkyl-4-(5-nitrofuran-2-yl)methylenamino-4*H*-1,2,4- triazole halides [2] different structure-dependent effect was observed.

So replacing of 5-nitrofuryl group by 5-nitro-2-furyl group significantly increased the activity of the compounds relatively to *E. coli, Kl. pneumonia, E. aerugenes, E. faecalis* and *S. aureus*. It should be noted that, both above mentioned compounds and the derivatives of 3,5-R-4-(3-(5-nitrofuran-2-yl)allylidenamino)-1-R_1-4*H*-1,2,4-triazole halides have lack of antimicrobial activity for *P. aeroginosa*.

The attention should be paid to the introduction of methyl group into the structure of triazole cycle (compound 5), which resulted the increase of antimicrobial activity for *E. coli, Kl. pneumonia, E. aerugenes, E. faecalis and S. aureus*. The replacement of a hydrogen atom at N_1-atom of triazole cycle by octyl (compound 6) leads to the reduction of the activity of radical for the strains of these microorganisms. When the alkyl radical is extended (compound 7) susceptibility to *E. coli, Kl. pneumonia, E. aerugenes, E. faecalis and S. aureus.* Thus compounds that do not contain methyl substituents at the 3 and 5 positions of triazole cycle (compound 2 and 3) on the contrary reduced the sensitivity to the above mentioned strains. So, it must be emphasized that the most active are the compounds with less substituents that confirms the importance of nitrofuran group in the structure of triazole cycle.

Conclusions

1. The synthesis of 9 new compounds among derivatives 3,5-R-4-(3-(5-nitro- furan-2-yl)allylidenamino)-1-R_1-4*H*-1,2,4-triazole halides has been done. Their structure has been confirmed by physical and chemical methods.

2. The analysis of antimicrobial activity of the synthesized compounds has been done and the influence of substituents in the structure of triazole cycle at the antimicrobial activity of derivatives 3,5-R-4-(3-(5-nitrofuran-2-yl)allylidenamino)-1-R_1-4*H*-1,2,4- triazole halides has been defined.

3. Substance which have an increased sensitivity relatively *E. coli, Kl. pneumonia, E. faecalis* and *St. aureus.* was found among the synthesized compounds.

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