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STUDY OF NEW ORGANIC COMPOUNDS, THE DERIVATES OF THIENO[2,3-D]PYRIMIDIN-4-ONES AND 1,3-THIAZOLIN-4-ONE FOR ANTIMICROBIAL ACTIVITY AGAINST MICROBES ISOLATED FROM MAN, ANIMALS AND PLANTS AND AGAINST HALOBACTERIA

Sharga B.M.[1,3], Chapesh A.V.[2], Tindik L.M. [2], Slivka M.V.[1], Plesha M.V. [1], Nikolaychuk V.I. [1], Maga I.M.[1,3], Chripak S.M. [1]

Study of new organic compounds, the derivates of thieno[2,3-d]pyrimidin-4-ones and 1,3-thiazolin-4-one for antimicrobial activity against microbes isolated from man, animals and plants and against halobacteria.1,3-Sharga B.M., 2-Chapesh A.V.,2- Tindik L.M.,1- Slivka M.V.,1- Plesha M.V.,1- Nikolaychuk V.I., 1,3-Maga I.M., 1-Chripak S.M.*– Ten newly synthesized organic compounds, the derivates of thieno[2,3-d]pyrimidin-4-ones and 1,3-thiazolin-4-one, were studied for their activity against microorganisms from different species (bacilli, pseudomonads, enterobacteria, halobacteria, coccae, yeasts). Only some of these compounds (3-phenylureido-4,5,6,7-tetrahydrobenzo[b]thiophene, 2-hydroxy-5,6-dimethyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine) showed neither antibacterial nor antifungal action in Petri dish experiments. Groups with possible antimicrobial action were estimated in molecules of the compounds.*

Key words*: derivates of thieno[2,3-d]pyrimidin-4-ones, antimicrobial action*

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Дослідження на антимікробну активність нових органічних речовин – похідних тієно[2,3-d]піримідин-4-онів і 1,3-тіазолін-4-онів до мікробів, ізольованих від людини, тварин і рослин, та до галобактерій.–1,3-Шарга Б.М., 2-Чейпеш А.В., 2-Тиндик Л.М., 1-Сливка М.В., 1-Плеша М.В., 1-Ніколайчук В.І., 1,3-Мага І.М., 1-Хрипак С.М.– *Десять нових синтезованих органічних сполук, похідних тієно[2,3-d]піримідин-4-онів та 1,3-тіазолін-4-ону, досліджено на активність проти мікроорганізмів різних видів (бацили, псевдомонаси, ентеробактерії, галобактерії, кокки, дріжджі). Тільки окремі з цих речовин (3-фенілуреїдо-4,5,6,7-тетрагідробензо[b]тіофен, 2-гідрокси-5,6-диметил-4-оксо-3-феніл-3,4-дигідротієно[2,3-d]піримідин) в експериментах у чашках Петрі не виявили ні антибактеріальної, ні антифунгальної дії. Визначено групи в молекулах речовин, які, можливо, спричинюють антимікробну дію.*

Ключові слова*: похідні тієно[2,3-d]піримідин-4-онів та 1,3-тіазолін-4-ону, антимікробна дія*

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Introduction

Modern medicine, veterinary and plant protection have strong demand on new antimicrobial compounds. Most agents in man and animal medicine are sulfonamides and antibiotics with broad spectrum of antimicrobial activity. Although nontoxic, they could have bad side effects on health in man and animals chiefly in the form of allergic responses. Other concern is development of microbial strains with resistance to sulfonamides and antibiotics [9]. Intensive agriculture became more and more dependent on agrochemicals which provide economic stability of the production. However, increase of pesticides inputs causes several negative effects, i.e., development of pathogen resistance to the applied agents and their non-target environmental impacts [3]. Many pathogens already developed resistance to the fungicides and antibiotics used in crop protection [7, 8].

Thousands of different chemicals are synthesized in laboratories around the world every year and evaluated for biological activity against microorganisms pathogenic to man, animals and plants. However, usually only some of these compounds are effective in curing of diseases of man, ani-

mals or plants or in use as sterilizing agents and disinfectants.

This work was aimed to evaluate ten compounds, the derivates of thieno[2,3-d]pyrimidin-4-ones and 1,3-thiazolin-4-one for antimicrobial activity against variety of microbes (bacilli, pseudomonads, enterobacteria, halobacteria, coccae, yeasts) isolated from man, animals, plants and other natural objects.

Materials and Methods

The potato agar and sucrose agar used for cultivation of Erwinia carotovora 8982 and Erwinia amylovora 9057, respectively. The Sabouraud agar plates were used for cultivation of yeasts. The solid medium with 25% NaCl content [2] was utilized for cultivation of halobacteria. Oxoid N3 nutritive agar plates were used for cultivation of the rest of microbes.

Small (4 mm in diameter) wells were made in each plate in Petri dishes. The plates were inoculated by microbial cultures listed in Table 1. Suspensions (0.2 ml, 108cfu/ml) of each culture in isotonic NaCl solution in distilled water were dispersed by swab over the plates. Then Petri dishes were dried to remove excess of humidity from the media. Ten newly synthesized organic compounds (Fig.1-Fig.10) were placed onto just inoculated microbial lawns in amounts of 3 mg in numbered wells. The presence (or absence) of growth inhibition zones was observed in lawns after incubation of inoculations of bacteria at 37ºC for 24 h, halobacteria at 37ºC for 48 h and yeasts at 24ºC for 48 h. The mean zone size (r, mm) was estimated among five replicates for each of compounds.

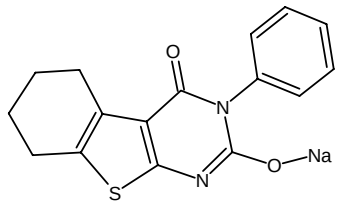

Fig.1. Compound № 1: Sodium salt of 2-hydroxy-3-phenyl-3,4,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-one

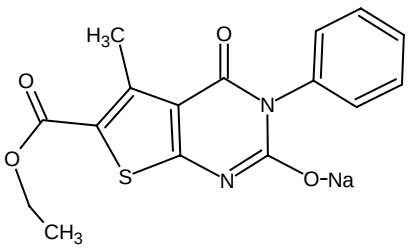

Fig.2. Compound № 2: Sodium salt of 2-hydroxy-6-carbetoxy-5-methyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine

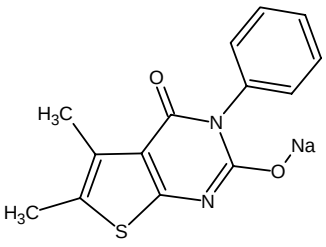

Fig.3. Compound № 3: Sodium salt of 2-hydroxy-5,6-dimethyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine

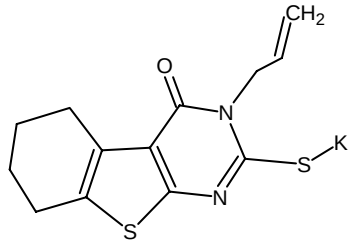

Fig.4. Compound № 4: Potassium salt of 3-allyl-2-sulfanyl-3,4,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-one

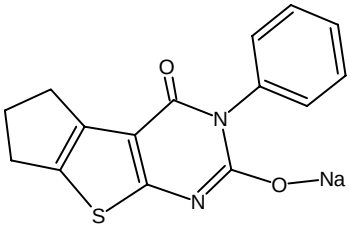

Fig.5. Compound № 5: Sodium salt of 2-hydroxy-3-phenyl-3,5,6,7-tetrahydro-4H-cyclopenta[4,5]-thieno[2,3-d]pyrimidin-4-one

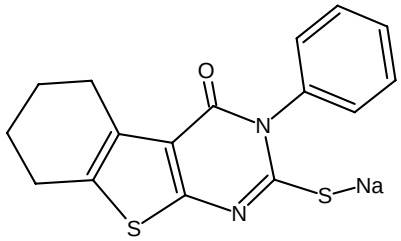

Fig.6. Sodium salt of 2-hydroxy-6-carbetoxy-5-methyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine

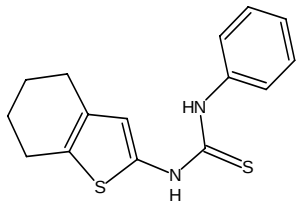

Fig.7. Compound № 7: 3-Phenylthioureido-4,5,6,7-tetrahydrobenzo[b]thiophene

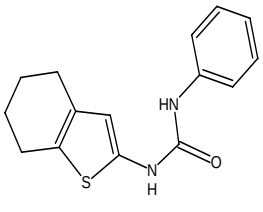

Fig.8. Compound № 8: 3-Phenylureido-4,5,6,7-tetrahydrobenzo[b]thiophene

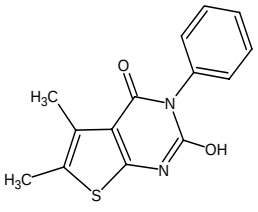

Fig.9. Compound № 9: 2-Hydroxy-5,6-dimethyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine

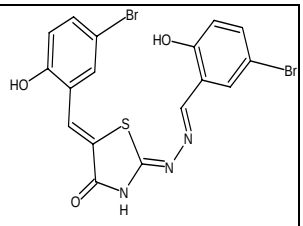

Fig.10. Compound № 10: 2-(2- Hydroxy-5-bromotoluidendiazo)-5-(2-hydroxy-5-bromotoluideno)thiazol-4-one

Results and Discussion

The antimicrobial activity was observed in sodium salt of 2-hydroxy-3-phenyl-3,4,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-one, sodium salt of 2-hydroxy-6-carbetoxy-5-methyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine, sodium salt of 2-hydroxy-5,6-dimethyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine, potassium salt of 3-allyl-2-sulfanyl-3,4,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-one, sodium salt of 2-hydroxy-3-phenyl-3,5,6,7-tetrahydro-4H-cyclopenta[4,5]-thieno[2,3-d]pyrimidin-4-one, sodium salt of 3-phenyl-2-sulfanyl-3,4,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-one, 3-Phenylthioureido-4,5,6,7-tetrahydrobenzo[b]thiophene, 2-(2-hydroxy-5-bromotoluidendiazo) -5-(2-hydroxy-5-bromotoluideno)thiazol-4-one (compounds №1, 2, 3, 4, 5, 6, 7 and 10).

The 3-phenylureido-4,5,6,7-tetrahydrobenzo [b]thiophene (compound №8) and 2-Hydroxy-5,6-dimethyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d] pyrimidine (compound №9) were inactive against tested microbes.

The sodium salt of 3-phenyl-2-sulfanyl-3,4,5, 6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-one (compound № 6) caused largest zones of inhibition in lawns of sensitive microbes. The bacilli were most sensitive bacteria to this compound. Their lawns developed zones 7-20 mm in radius. The sodium salt of 3-phenyl-2-sulfanyl-3,4,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-one caused production of double zones of inhibition in bacterial lawns of Bacillus subtilis BS 924, 934, 1109, Bacillus thuringiensis subsp. sotto ATCC 19270, but not in the lawns of Bacillus subtilis BS 953, Bacillus cereus, Bacillus stearothermophillus BKM B-718, Bacillus licheniformis CSES C.

All 8 strains of Bacillus were sensitive to potassium salt of 3-allyl-2-sulfanyl-3,4,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-one (compound № 4) and sodium salt of 3-phenyl-2-sulfanyl-3,4,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-one (compound № 6) and 7 strains of bacilli were inhibited by sodium salt of 2-hydroxy-6-carbetoxy-5-methyl-4-oxo-3-phenyl-3,4-dihydrothieno [2, 3-d]pyrimidine (chemical № 2).

Only compound № 6 was active against Candida albicans and Saccharomyces vini BS1. In recent years, the C. albicans caused serious infections in human [1, 10] and Saccharomyces can also cause health problems in patients with immunodeficiency [4, 10, 11]. Candida spp. with medical importance tends to be more resistant to antifungal agents [5, 6, 10] and practice has strong demand for new more effective anti-Candida preparations. It will be interesting to look in further studies if 3-phenyl-2-sulfanyl-3,4,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-one is active against other strains of these yeasts.

This compound demonstrated activity against Enterococcus faecalis ATCC 19433, Micrococcus luteus ATCC 3941, Staphylococcus aureus ATCC 25923, Staphylococcus epidermitidis ATCC 14990 and archaea Halobacterium cutirubrum 353П, Halobacterium cutirubrum ET1001 also, however, failed to inhibit the cells of Escherichia, Erwinia, Shigella, Salmonella, Klebsiella, Proteus, Serratia, Pseudomonas strains used in this study.

The compound №6 has almost the same structure as the compound №1 has. The difference is a presence of second sulphur atom in compound №6 instead of oxygen near the sodium atom. This, obviously, increases the antimicrobial effectiveness and makes wider the spectrum of action of the sodium salt of 3-phenyl-2-sulfanyl-3,4,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-one, in comparison to the sodium salt of 2-hydroxy-3-phenyl-3,4,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-one.

The sodium salt of 2-hydroxy-5,6-dimethyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine, sodium salt of 2-hydroxy-3-phenyl-3,5,6,7-tetrahydro-4H-cyclopenta[4,5]-thieno[2,3-d]pyrimidin-4-one and 2-(2-hydroxy-5-bromotoluidendiazo)-5-(2-hydroxy-5-bromotoluideno)thiazol-4-one (compounds № 3, 5 and 10) were inactive against bacilli.

With exception of Shigella sonnei CSES S1 demonstrating sensitivity to sodium salt of 2-hydroxy-3-phenyl-3,4,5,6,7,8-hexahydrobenzo[4,5] thieno[2,3-d]pyrimidin-4-one, sodium salt of 2-hydroxy-6-carbetoxy-5-methyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine, sodium salt of 2-hydroxy-5,6-dimethyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine (compounds №1, 2, 3), all strains of enterobacteria were resistant to the chemicals tested in this experiment.

The bacteria Staphylococcus aureus ATCC 25923, Staphylococcus epidermitidis ATCC 14990 and Micrococcus luteus ATCC 3941 were sensitive to 7, 3 and 6 of used compounds, respectively. The Halobacterium cutirubrum 353П Halobacterium cutirubrum ET1001 and Halobacterium cutirubrum S were sensitive to 3, 4 and 3 compounds, respectively (Table 1). These indicates a certain level of the specificity of antimicrobial action between the species and strains. The zones of inhibition in sensitive cultures remained constant in size during week of observation.

The thiophene and benzene rings present in molecules of studied compounds, possibly, not associated with antimicrobial activity, as such rings present in non-active compounds № 8 and 9. The carbetoxyl fragment of the compound № 2 may have antimicrobial activity as a source of ethanol in watered medium.

The compound № 7 is active due to sulfur in thiourea component of the molecule. The sulfur substitution to oxygen (in urea group of compound № 9) rendered the compound to be inactive.

The compound № 3 could be obtained from compound № 8 only by substitution of hydrogen in OH group to Na. This change gives antimicrobial properties to the molecule of sodium salt of 2-

hydroxy-5,6-dimethyl-4-oxo-3-phenyl-3,4-dihydro-thieno[2,3-d]pyrimidine. The 2-hydroxy-5,6-dimethyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d] pyrimidine itself remains inactive agains microbes, regardless presence of methyl groups.

The activity of the compound № 10 is due to the presence of two groups of para-bromphenol which dissociate from the molecule in watered medium and, possibly, due to action of thiasoline and diazyne group.

According to our experiments, the wide spectra of action have compounds № 6, 2 and 4 suppressed 18, 15 and 13 strains, respectively, out of 33 used in the study. The chemical №1 was active against 4 strains and other substances inhibited 3 strains each.

Table 1. Antimicrobial action of the derivates of thieno[2,3-d]pyrimidin-4-ones and 1,3-thiazolin-4-one

Microorganisms	Size (r, mm) of inhibition zone around compound							
	№1	№2	№3	№4	№5	№6	№7	№10
Bacillus licheniformis CSES C	-	-	-	4.1±0.1	-	7.2±0.2	3±0.4	-
Bacillus stearothermophillus BKM-B-718	-	1.2±0.2	-	3.1±0.2	-	8.3±0.3	-	-
Bacillus cereus	-	1.2±0.1	-	4.1±0.3	-	8.2±0.1	-	-
Bacillus subtilis BS 924	-	4.1±0.5	-	6±0.2	-	15±0.2	-	-
Bacillus subtilis BS 934	-	4±0.3	-	8.2±0.2	-	14±0.5	-	-
Bacillus subtilis BS 953	1.1±0.2	1.3±0.1	-	6.2±0.4	-	15.1±0.3	-	-
Bacillus subtilis BS 1109	-	5.2±0.5	-	5±0.4	-	20.1±0.4	-	-
Bacillus thuringiensis subsp. sotto ATCC 19270	-	5.4±0.3	-	4.2±0.1	-	14.2±0.2	-	-
Candida albicans ATCC 885-653	-	-	-	-	-	6.3±0.4	-	-
Candida albicans 1283	-	-	-	-	-	5.5±0.4	-	-
Candida albicans 1286	-	-	-	-	-	2±0.5	-	-
Enterococcus faecalis ATCC 19433	8.2±0.6	-	-	-	-	3.2±0.2	-	-
aEscherichia coli 25922	-	-	-	-	-	-	-	-
Escherichia coli 055	-	-	-	-	-	-	-	-
Erwinia amylovora 9057	-	-	-	-	-	-	-	-
Erwinia carotovora 8982	-	-	-	-	-	-	-	-
Klebsiella pneumoniae CSESK-56	-	-	-	-	-	-	-	-
Micrococcus luteus ATCC 3941	3.1±0.2	6.2±0.1	5.4±0.1	6.6±0.2	4.5±0.1	8.3±0.6	-	-
Proteus mirabilis RSES 3171	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa ATCC 27853	-	-	-	-	-	-	-	-
Pseudomonas fluorescens	-	-	-	-	-	-	-	-
Saccharomyces vini BS1	-	-	-	-	-	10.2±0.1	-	-
Salmonella enteritidis RSES 25/13	-	-	-	-	-	-	-	-
Salmonella typhimurium RSES 9/474	-	-	-	-	-	-	-	-
Salmonella reading RSES 1	-	-	-	-	-	-	-	-
Serratia marcescens CSES 1	-	6.4±0.3	-	-	-	-	-	-
Shigella flexneri CSES 1a8516	-	-	-	-	-	-	-	-
Shigella sonnei CSES S1	6.4±0.3	4.3±0.2	5.5±0.3	-	-	-	-	-
Staphylococcus aureus ATCC 25923	-	4.3±0.2	3.2±0.3	6.3±0.1	2±0.4	9.4±0.3	2±0.1	3.2±0.3
Staphylococcus epidermitidis ATCC 14990	-	15.1±0.5	-	6±0.3	-	13.3±0.4	-	-
Halobacterium cutirubrum 353П	-	10.3±0.4	-	5.2±0.2	-	10.1±0.5	-	-
Halobacterium cutirubrum ET1001	-	10.2±0.3	-	5.2±0.2	6.3±0.1	8.3±0.3	-	-
Halobacterium cutirubrum S	-	5±0.1	-	-	-	-	6.2±0.2	5.1±0.2

Notes:
ATCC, American Type Cultures Collection, Rockville, MD, USA; BKM, Russian Collection of Non- pathogenic Microorganisms, Pushchino, Russia; CSES, Collection of Microorganisms at Central Station of Epidemiology and Sanitary of Ukraine, Kiev; RSES, Collection of Microorganisms at Regional Station of Epidemiology and Sanitary, Uzhgorod, Ukraine. A Culture provided by L. Tarasevich Institute, Moscow, Russia. Size of inhibition zones presented as mean with standard error ($\bar{x} \pm$ SE). Resistant cultures indicated by symbol of minus.

Fig.11. Zones of growth inhibition into the loan of Staphylococcus aureus ATCC 25923 caused by compounds:
Left –3-phenyl-2-sulfanyl-3,4,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-one;
right - sodium salt of 2-hydroxy-6-carbetoxy-5-methyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine;
center - 2-(2- Hydroxy-5-bromotoluidendiazo)-5-(2-hydroxy-5-bromotoluideno)thiazol-4-one;
right bottom – potassium salt of 3-allyl-2-sulfanyl-3,4,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-one; left bottom - sodium salt of 2-hydroxy-5,6-dimethyl-4-oxo-3-phenyl-3,4-dihydrothieno[2,3-d]pyrimidine.

All things considered, the experiment has yielded three compounds with broad spectrum of action and good inhibitive effect in Petri dish experiment. The presumable active groups were estimated and their antimicrobial action should be proved in future experiments. It will be interesting to compare the effectiveness of antimicrobial action of our compounds and effectiveness of antimicrobial action of chemicals, which are in current use as antimicrobial agents against microbes of the same species and strains as we tested. We like to look in further studies if our chemicals are active against cells of eukaryotes other than yeasts and against prokaryotes of other species.

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