**ORIGINAL ARTICLE**

# SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF PHENYLSELENYL TRIBROMIDE AND ITS FUSED THIENOPYRIMIDINE DERIVATIVES

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**Abstract**

Fused thienopyrimidine derivatives of phenylselenyl tribromide (compound III) were synthesized and characterized by spectral and elemental analyses. The parental compound and its derivatives were tested *in vitro* for their antimicrobial activity. The minimum inhibitory (bacteriostatic or fungistatic) concentrations and minimum bactericidal or fungicidal concentrations were estimated for the two most active compounds: 1-bromo-2-[(*E*)-1-bromomethyliden]-1,5-diphenyl-6-oxo-2,3,5,6,7,8,9,10-octahydrobenzo[*b*]thieno[3',2',5,6]pyrimido-[2,1-*b*][1,4,3] thiaselenazin-12-ium bromide (compound IVc), the derivative with the lowest toxicity, and phenylselenyl tribromide (compound III), the starting chemical with the highest toxicity. These two compounds yielded mainly bactericidal effects on the tested bacteria. In comparison with the minimum bactericidal concentrations of cefotaxime, this effect was significantly weaker. Of the two afore-mentioned compounds, only the derivative IVc demonstrated fungicidal action against yeasts. In comparison with the minimum fungistatic concentrations of fluconazole, the inhibitory potency of this compound against yeasts was two times stronger. The compound manifested fungicidal effects against yeasts at doses of 275 - 310 μg/mL, while fluconazole yielded only fungistatic actions. Among the studied compounds, III and IVc appear to be most active against yeasts and also possess poor to moderate activity against Gram-positive and Gram-negative bacteria. The antibacterial activity can be improved by introducing changes into the chemical structure of the compounds. Considering the obtained results, these products and their derivatives may be of practical benefit.

**Rezumat**

Derivații de condensare a tienopirimidinei cu tribromura fenilselenică au fost sintetizați și caracterizați prin analiză spectrală și elementală. Compușii parentali și derivații lor au fost testați *in vitro* pentru activitatea antimicrobiană. Concentrația minimă inhibitorie (bacteriostatică sau fungistatică) și concentrațiile minime bactericide sau concentrațiile fungicide au fost estimate pentru doi dintre cei mai activi compuși: 1-brom-2-[(*E*)-1-bromometiliden-1,5-difenil-6-oxo-2,3,5,6,7,8,9,10-octohidrobenzo [*b*] tieno [3', 2', 5, 6] pirimido-[2,1-*b*] [1,4,3] tiaselenazin-12-iu bromură (compusul IVc), un derivat cu toxicitate redusă și tribromura fenilselenică (compus III), compusul de bază cu cea mai mare toxicitate. Acești doi compuși au prezentat un efect bactericid. Comparativ cu concentrația minimă bactericidă a cefotaximului, aceste efecte bactericide au fost mult mai slabe. Dintre cei doi compuși, numai derivatul IVc a demonstrat activitate fungicidă asupra drojdiilor. Față de concentrațiile minime fungistatice ale fluconazolului, proprietățile inhibitoare ale acestui compus asupra drojdiilor au fost de două ori mai puternice. Acțiunea fungicidă a acestui compus asupra drojdiilor apare la doze 275 - 310 mg/mL, în timp ce fluconazolul a relevat doar o acțiune fungistatică. Printre compușii studiați, III și IVc au fost cei mai activi asupra drojdiei și au demonstrat o activitate slabă sau moderată împotriva bacteriilor gram-pozitive și gram-negative. Activitatea antibacteriană poate fi îmbunătățită prin modificarea structurii chimice a compușilor.

**Keywords**: antibacterial and antifungal activity, fused thienopyrimidines, phenylselenyl tribromide, synthesis

## Introduction

Despite toxicity found in many heterocycles containing selenium, these compounds are of great interest for many scientists [11, 14]. Researchers are particularly interested in new approaches for their syntheses and structures optimization [3-6, 8, 26, 29, 30] and, in a wide range, of the biological activities of these chemicals [15, 18]. Selenium containing heterocycles exhibit antiarrhythmic [5], antiprotozoal [28], antiviral [1, 6, 22, 23, 25], antibacterial [1, 6, 9, 14, 22, 23, 25, 35], antifungal and cytotoxic effects [8].

Fused thienopyrimidine derivatives of phenylselenyl tribromide were obtained. So far there have been no reports in literature regarding such syntheses or on the biological activity of these compounds.

The purpose of this study was the chemical synthesis and evaluation of the antimicrobial

activity of these compounds against some bacteria and fungi.

## Materials and Methods

*Chemistry*

IR-spectra were obtained on a Pye-Unicam SP3-300 spectrometer using the KBr waver technique. NMR spectra were recorded on a Varian Mercury-400 instrument. Chemical shift values (δ) are given in parts per million (ppm) downfield from tetramethylsilane (TMS) as internal standard and it refers to the residual peak of the deuterated solvent used ($CDCl_3$, $(CD_3)_2SO$). 2D-NOESY experiments have been carried out for the compound 1-bromo-2-[(*E*)-1-bromomethyliden]-1,5-diphenyl-6-oxo-2,3, 5,6,7,8,9,10-octahydrobenzo[*b*]thieno[3',2',5,6] pyrimido-[2,1-*b*][1,4,3] thiaselenazin-12-ium bromide (IVc) in $CDCl_3$ on a Varian Mercury-400 instrument. Elemental analyses were performed at the micro-analytical unit of the Institute of Organic Chemistry (NAS, Kiev). All compounds gave satisfactory elemental analyses within 0.4% of the theoretical values. All melting points were determined using Kofler block equipment. All reagents were obtained from commercial suppliers and used without any further purification. Dry solvents were prepared according to the standard methods.

*Toxicity of compounds and antimicrobial studies*

Compounds toxicities were studied using the method of Poroikov *et al*. [24].

The microbes used in our experiments were *Bacillus licheniformis* CSES C, *Klebsiella pneumoniae* CSESK-56 (Collection of Microorganisms at Central Station of Epidemiology and Sanitary of Ukraine, Kiev), *Bacillus stearothermophillus* BKM-B-718 (Russian Collection of Non-pathogenic Microorganisms, Pushchino), *Micrococcus luteus* ATCC 3941, *Enterococcus faecalis* ATCC 19433, *Escherichia coli* K-12, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Candida albicans* ATCC 885-653 (American Type Culture Collection Rockville, MD) and *Proteus mirabilis* RSES 3171 (Collection of Micro-organisms at Regional Station of Epidemiology and Sanitary, Uzhgorod, Ukraine). *C. albicans* BS1, BS2 and *Saccharomyces cerevisiae* BS1, BS2 were isolated by the authors.

Bacteria were grown in nutritive broth at 37°C on a shaker, at 100 rpm during the night. Then, they were diluted with the same volume of fresh medium and cultured again for 4 hours to provide young cell mass. This also allowed production of the bacilli cell mass with minimum content of spores. *C. albicans* and *S. cerevisiae* were grown in Sabouraud broth for 1 day at 37°C and 24°C, respectively. The cell mass of each culture was centrifuged and used for preparation of saline suspensions with concentrations $10^8$ colony forming units (CFU)/mL by use of an optical standard. The cell suspensions (2 mL) were applied to the surface of Muller or Sabouraud agar plates for preparation of lawns. Excess suspension was decanted and the plates were dried opened at 24°C for 30 min.

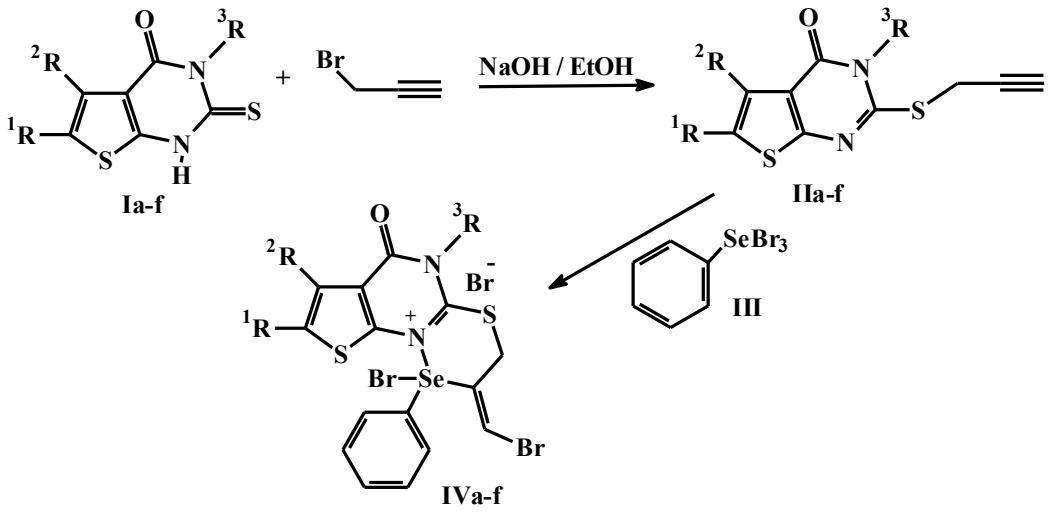
Compound IVc, which has the lowest toxicity, and III (the most toxic compound) and all its derivatives were first screened by the agar diffusion method for their antimicrobial activity by placing 500 μg portions of each compound onto just seeded "bacterial lawns" of *B. stearothermophillus* BKM-B-718. Inhibition zones (IZ) produced by the compounds were compared. This allowed us to select compound III and its derivative IVc as the most active for further antimicrobial studies. The action of compounds III and IVc on bacteria was compared with the activity of a standard, the cefotaxime sodium ("Kiyvmedpreparate", Kiyv, Ukraine), a third generation cephalosporine antibiotic. The antifungal activity of III and IVc was evaluated against yeasts, as was that of the fluconazole ("Technolog", Uman, Ukraine), a derivative of triazole, used as standard.

Paper disks containing 30 μg of cefotaxime were applied on microbial lawns, and saline solutions of cefotaxime were tested against microbes in saline suspensions. Fluconazole was placed onto lawns in 500 μg portions, and also applied in saline dilutions against yeasts. Serial dilutions of these 4 compounds in normal saline were prepared to provide the concentrations of 10, 15, 20, up to 2400 μg/mL in working solutions with test-microorganisms. To obtain working solutions, 10 μl drops of the compound solutions in normal saline were added to the same volume of bacterial and yeast saline suspensions. Then, they were incubated for 30 min at 24°C (*S. cerevisiae*) or 37°C (the rest of the microbes) and transferred to the numbered sectors on Sabouraud or Müller agar plates for absorption. After that, the microbial cells were dispersed by glass swab from the application site within the sector area and incubated at 24°C or 37°C to distinguish killing, inhibitive or no effects for each concentration [10]. We noted the lowest concentration required to stop the growth of microbes as the minimum inhibitory concentration (MIC) and the lowest concentration at which the inoculum was killed as the minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC). MBC/MIC and MFC/MIC ratios were calculated as in the previously published articles [2, 13, 20] to determine if the compound had bacteriostatic and fungistatic (MBC/MIC $\geq$ 4, MFC/MIC $\geq$ 4) or fungicidal and bactericidal effects (MFC/MIC $\leq$ 4, MBC/MIC $\leq$ 4). There were made 5 replicates of each experiment.

## Results and Discussion

*Chemistry of the compounds*

Figure 1 shows how the cyclization of 2-propargyl-thiothieno[2,3-*d*]pyrimidin-4-ones (IIa-f) was performed through the treatment with phenylselenyl tribromide. The required starting propynylthio derivatives IIa-f were conveniently obtained by reaction of the corresponding thieno[2,3-*d*]-pyrimidines Ia-f with propargyl bromide in alcohol media at equimolar presence of sodium hydroxide. The thioethers IIa-f reacted with III, both in glacial acetic acid and in chloroform. This procedure turned out to be a convenient access to the angular structure of polycyclic salts of (*E*)-halogen-methylidene substituted thieno[3',2':5,6]pyrimido-[2,1-b][1,4,3] thiaselenazinium (IVa-f).




**a:** $^1R = {}^2R = CH_3, {}^3R = C_6H_5$
**b:** $^1R + {}^2R = (CH_2)_3, {}^3R = C_6H_5$
**c:** $^1R + {}^2R = (CH_2)_4, {}^3R = C_6H_5$
**d:** $^1R = {}^2R = CH_3, {}^3R = H$
**e:** $^1R + {}^2R = (CH_2)_3, {}^3R = H$
**f:** $^1R + {}^2R = (CH_2)_4, {}^3R = H$

**Figure 1.**
The scheme for the synthesis of fused thienopyrimidine derivatives of phenylselenyl tribromide

In most of the transformations, each of the products was formed without significant impurities.

Parental compound III was produced using the method of Barnes *et al.* [19] from difenyldiselenide and bromine and has the following characteristics: Yield: 71%; mp: 107 - 108ºC ($CHCl_3$), (105 - 106ºC [4]); $^1$H NMR, 400 MHz, ($CDCl_3$): 7.41 m (3H, 3CH); 7.86 m (2H, 2CH); $^{13}$C NMR, 100 MHz ($CDCl_3$): 129.98, 131.05, 133.12, 133.63, 134.25, 135.19; $^{77}$Se NMR, 100 MHz ($CDCl_3$): 870.38.

*2-Prop-2-yn-1-ylthio-3-R-thieno[2,3-d]pyrimidin-4-ones (II): general procedure.* 20 mmol starting thieno[2,3-*d*]pyrimidines (I) [12] were dissolved by heating in 200 mL of ethanol (96 %) containing 20 mmol NaOH. 25 mmol propargyl bromide was added to the cooled solution and the reaction mixture was heated for 1 hour at 80ºC. The mixture was then cooled to room temperature. The solid product was filtered off and then washed with ethanol and warm water (20 and 50 mL, respectively). The pure product was obtained by crystallization from glacial acetic acid.

Colourless crystals of *5,6-dimethyl-3-phenyl-2-(prop-2-yn-1-ylsulfanyl)thieno[2,3-d]pyrimidin-4-one* (IIa). Yield: 71%; mp: 242ºC; IR (KBr): ν 1680 (C=O) sm$^{-1}$; $^1$H NMR, 400 MHz $(CD_3)_2SO$): δ 2.34, 2.36 (s, 3H each, $2CH_3$), 3.06 (bs, 1H, CH), 3.91 (d, 2H, *J* 2.4 Hz, $CH_2$), 7.37 (m, 2H, $C_6H_5$), 7.55 (m, 3H, $C_6H_5$) ppm.

Colourless crystals of *3-phenyl-2-(prop-2-yn-1-ylsulfanyl)-3,5,6,7-tetrahydro-cyclopenta[4,5]thieno[2,3-d]pyrimidin-4-one* (IIb). Yield: 62%; mp: 249ºC; IR (KBr): ν 1660 (C=O) sm$^{-1}$; $^1$H NMR, 400 MHz, $(CD_3)_2SO$): δ 2.39 (m, 2H, $CH_2$), 2.90 (m, 4H, $2CH_2$), 3.18 (bs, 1H, CH), 3.92 (d, 2H, *J* 2.4 Hz, $CH_2$), 7.43 - 7.57 (m, 5H, $C_6H_5$) ppm.

Colourless crystals of *3-phenyl-2-(prop-2-yn-1-ylsulfanyl)-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidin-4-one (IIc).* Yield: 66%; mp: 208ºC; IR (KBr): ν 1680 (C=O) sm$^{-1}$; $^1$H NMR, 400 MHz, $(CD_3)_2SO$: δ 1.77 (m, 4H, $2CH_2$), 2.77 (m, 4H, $2CH_2$), 3.17 (bs, 1H, CH), 3.96 (d, 2H, *J* 2.4 Hz, $CH_2$), 7.41 (m, 2H, $C_6H_5$), 7.56 (m, 3H, $C_6H_5$) ppm.

Syntheses and spectral characteristics of IId-f were described in previous works [31].

*1-bromo-2-(E)-Bromomethylidenethieno[3',2':5,6]pyrimido[2,1-b][1,4,3]-thiaselenazin-12-ium bromides* (IVa–f): *general procedure.* The corresponding propargyl-thioethers II (5 mmol) were dissolved in 50 mL dry chloroform. The mixture was cooled to 0ºC and stirred. A solution of III (5 mmol) in 20 mL glacial acetic acid (or in 20 mL chloroform - both variants yielded the same results) was added in drops, while stirring over 30 min. The temperature of the reaction mixture was kept below 15ºC. The stirring was then continued for 5 hours. The precipitate was collected by filtration after adding 70 mL ether. The pure targeted products Iva-f were obtained by washing the precipitate in 50 mL warm ether.

Yellow crystals of *1-bromo-2-[(E)-1-bromo-methyliden]-7,8-dimethyl-1,5-diphenyl-6-oxo-2,3,5,6-tetrahydrothieno[3',2':5,6]pyrimido[2,1-b][1,4,3]thiaselenazin-10-ium bromide* (IVa). Yield: 55%; mp: 139 - 141ºC; IR (KBr): ν 1725 (C=O), 1610 ($C=N^+$) $sm^{-1}$; $^1H$ NMR, 400 MHz ($CDCl_3$): δ 2.28, 2.30 (s, 3H each, $2CH_3$); 4.62 (bs, 2H, $CH_2$); 7.25 - 7.78 (m, 11H, =CHBr+10CH).

Yellow crystals of *1-bromo-2-[(E)-1-bromomethyl-iden]-1,5-diphenyl-6-oxo-2,3,5,7,8,9-hexahydro-cyclopenta[b]thieno[3',2':5,6]pyrimido[2,1-b][1,4,3]thiaselenazin-11-ium bromide* (IVb). Yield: 52%; mp: 135 - 137ºC; IR (KBr): ν 1725 (C=O), 1620 ($C=N^+$) $sm^{-1}$; $^1H$ NMR, 400 MHz ($CDCl_3$): δ 1.84 (m, 2H, $CH_2$); 2.76 (m, 2H, $CH_2$); 2.92 (m, 2H, $CH_2$); 4.81 (bs, 2H, $CH_2$); 7.48 (s, 1H, =CHBr); 7.30 - 7.74 (m, 10H, 10CH).

Yellow crystals of *1-bromo-2-[(E)-1-bromo-methyliden]-1,5-diphenyl-6-oxo-2,3,5,6,7,8,9,10-octahydrobenzo[b]thieno[3',2':5,6]pyrimido[2,1-b][1,4,3]thiaselenazin-12-ium bromide* (IVc). Yield: 61%; mp: 156-158ºC; IR (KBr): ν 1730 (C=O), 1620 ($C=N^+$) $sm^{-1}$; $^1H$ NMR, 400 MHz ($CDCl_3$): δ 1.86 (m, 4H, $CH_2$); 2.76 (m, 2H, $CH_2$); 2.91 (m, 2H, $CH_2$); 4.81 (bs, 2H, $CH_2$); 7.48 (s, 1H, =CHBr); 7.30-7.75 (m, 10H, 10CH).

Yellow crystals of *1-bromo-2-[(E)-1-bromo-methyliden]-7,8-dimethyl-6-oxo-1-phenyl-2,3,5,6-tetrahydrothieno[3',2':5,6]pyrimido[2,1-b][1,4,3]thiaselenazin-10-ium bromide* (IVd). Yield: 56%; mp: 154 - 156ºC; IR (KBr): ν 1720 (C=O), 1610 ($C=N^+$) $sm^{-1}$; $^1H$ NMR, 400 MHz ($CDCl_3$): δ 2.38, 2.40 (s, 3H each, $2CH_3$); 4.96 (bs, 2H, $CH_2$); 7.40 (s, 1H, =CHBr); 7.21 - 7.50 (m, 5H, 5CH).

Yellow crystals of *1-bromo-2-[(E)-1-bromo-methyliden]-6-oxo-1-phenyl-2,3,5,7,8,9-hexahydro-cyclopenta[b]thieno[3',2':5,6]pyrimido[2,1-b][1,4,3]thiaselenazin-11-ium bromide* (IVe). Yield: 53%; mp: 148 - 150ºC; IR (KBr): ν 1730 (C=O), 1610 ($C=N^+$) $sm^{-1}$; $^1H$ NMR, 400 MHz ($CDCl_3$): δ 1.82 (m, 2H, $CH_2$); 2.76 (m, 2H, $CH_2$); 2.95 (m, 2H, $CH_2$); 4.84 (bs, 2H, $CH_2$); 7.44 (s, 1H, =CHBr); 7.38 - 7.61 (m, 5H, 5CH).

Yellow crystals of *1-bromo-2-[(E)-1-bromo-methyliden]-6-oxo-1-phenyl-2,3,5, 6,7,8,9,10-octa-hydrobenzo[b]thieno[3',2':5,6]pyrimido[2,1-b][1,4, 3]thiaselenazin-12-ium bromide* (IVf). Yield: 68%; mp: 163 - 165ºC; IR (KBr): ν 1730 (C=O), 1610 ($C=N^+$) $sm^{-1}$; $^1H$ NMR, 400 MHz ($CDCl_3$): δ 1.86 (m, 4H, $CH_2$); 2.77 (m, 2H, $CH_2$); 2.92 (m, 2H, $CH_2$); 4.79 (bs, 2H, $CH_2$); 7.48 (s, 1H, =CHBr); 7.36 - 7.62 (m, 10H, 10CH).

The structural assignment of the products IVa-f was based on elemental microanalysis, as well as IR and NMR spectrometry. By reacting with III, the condensation of an additional (*E*)-halogenmethyl-idene substituted ring occurred. $^1H$ NMR spectra of fused products IVa-f lack the signals from the propargyl fragment of starting (thio-)ethers IIa-f, and the effects of a spin system resonance of the formed additional halogenmethylidene substituted ring appear for thieno[3',2',5,6]pyrimido[2,1-b] [1,4,3]thiaselenazinium bromide as follows: a broad singlet of the cyclic methylene protons at region 4.62 - 4.96 ppm; singlet of exocyclic methylydene proton at region 7.40 - 7.48 ppm. Such a shift to the weak region of *E*-methylidene proton signal in the $^1H$ NMR spectra can be explained as follows: the olefin proton of *E*-isomer has appeared in the zone of strong dezecranation by electronic sphere of a positively charged syn-periplanar fused nitrogen atom. The shift to the long wave region of absorbance bands from the C=o and $C=N^+$ moieties in the IR spectra of salts IVa-f confirms the presence of a positive charge on the nitrogen atom [11]. The ionic nature of fused cyclization products can also be corroborated by $AgBF_4$ equimolar titration of the halogenide ion in yielded salt IVc.

Also, in the formation of halogenmethylidene-substituted fused systems IVa-f, two possible stereo-isomeric products (*E*- or *Z*-isomer) had to be considered. However, treatment of thioethers IIa-f with III results in stereoselective formation of the *E*-isomeric products IVa-f, whose geometric elucidation was achieved by 2D-NOESY experiment [19] on compound IVc (Figure 2). The cross-peaks for the exocyclic methylydene protons and cyclic methylene protons are not displayed.

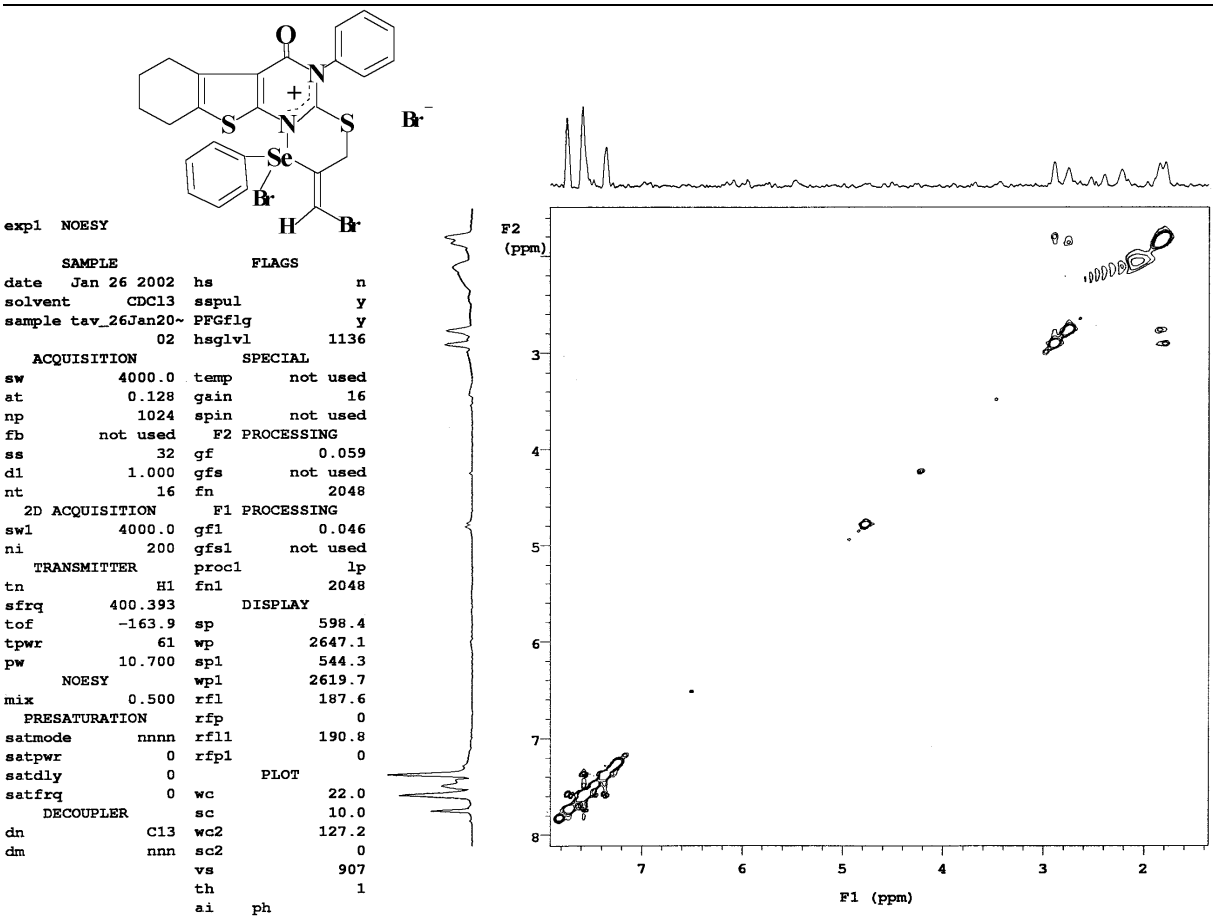



**Figure 2.**
NOESY spectra for compound IVc

*The toxicity and the antimicrobial activity of the tested compounds*

The results of the compounds toxicity evaluation by the method of Poroikov *et al.* [24] predicted that parental compound III is the most toxic among the compounds studied in this work. This is due to its low molecular weight and high bromine and selenium content. The lowest toxicity level was estimated for the derivative IVc (Table I) because of its significantly lower bromine and selenium contribution in molecular weight. The toxicity level does not correlate strictly with the antimicrobial activities of the componds.

**Table I**
The results of toxicity evaluation of compound III and its derivatives IVa-f

| Compounds | III | IVa | IVb | IVc | IVd | IVe | IVf |
|---|---|---|---|---|---|---|---|
| $LD_{50}$, mg/kg | 73 | 152 | 192 | 212 | 141 | 139 | 177 |

In a preliminary test, by comparing with other newly synthesized products which produced small IZ when placed as salts on *B. stearothermophillus* BKM-B-718 lawns, the antibacterial action of compounds III and IVc resulted in the formation of the largest IZ (> 34 mm in diameter), even when thick lawns of the culture were seeded onto Müller's agar medium by loop strokes.

Compounds III and IVc had similar suppressive effects on the growth of Gram-positive and Gram-negative bacteria, when these compounds were placed as salts onto just-seeded lawns. These compounds developed clear IZ ranging from about 5 to 40 mm in diameter in microbial lawns. Touch transfer by loop from zone peripheries to fresh medium indicated cell survival at the edge of each zone. A bacteriostatic effect was observed in all cultures and in all derivatives with ability to produce IZ, because concentrations of the studied compounds gradually decreased to bacteriostatic levels at zone edges due to radial diffusion into media. Compounds III and IVc were less active than cefotaxime against bacteria (Table II). However, they suppressed the growth of some cefotaxime-resistant microbes.

**Table II**

The activity of compounds III, IVc and cefotaxime in bacterial lawns

| Strains of microorganisms | Inhibition zone (d, mm)[a] around | | |
|---|---|---|---|
| | compounds | | cefotaxime Na disks, 30 μg |
| | III, 500 μg | IVc, 500 μg | |
| *B. licheniformis* CSES C | 36.3 ± 0.9 | 35.1 ± 0.5 | resistant |
| *B. stearothermophillus* BKM-B-718 | 34.2 ± 1.1 | 37.1 ± 0.7 | resistant |
| *M. luteus* ATCC 3941 | 40 ± 0.6 | 37.3 ± 0.5 | 40.3 ± 0.8 |
| *S. aureus* ATCC 25923 | 36.1 ± 0.7 | 37.2 ± 0.5 | 29.7 ± 0.9 |
| *E. faecalis* ATCC 19433 | 18.1 ± 0.3 | 17.4 ± 0.7 | 25.2 ± 0.6 |
| *E. coli* K-12 | 18.2 ± 0.3 | 13.3 ± 0.6 | 28.3 ± 0.9 |
| *K. pneumoniae* CSESK-56 | 21.4 ± 0.5 | 9.2 ± 0.4 | 30 ± 0.7 |
| *P. mirabilis* RSES 3171 | 5.2 ± 0.8 | 6.1 ± 0.3 | 37.3 ± 0.5 |
| *Ps. aeruginosa* ATCC 27853 | 6.3 ± 0.9 | 5.4 ± 0.2 | resistant |

[a]Size of IZ presented as mean ± standard error ($\bar{x}$ ± SE)

When the compounds were tested against bacteria in saline dilutions, they also demonstrated weaker antimicrobial activity in comparison to cefotaxime (Table III).

Compound III has MBC/MIC < 4 for *B. licheniformis* CSES C, *B. stearothermophillus* BKM-B-718, *M. luteus* ATCC 3941, *S. aureus* ATCC 25923, *E. faecalis* ATCC 19433, *P. mirabilis* RSES 3171 and *Ps. aeruginosa* ATCC 27853. This indicated a bactericidal action of these compounds in these cultures. MBC/MIC quotients were equal to 6.67 for *E. coli* K-12 and more than 4 for *K. pneumoniae* CSESK-56 suggesting bacteriostatic action. Compound III has only bacteriostatic effect on *K.pneumoniae* CSESK-56 even at as much as 2400 μg/mL. This was possibly due to the presence of this bacterium's capsule. The product IVc also yielded bactericidal action, as the MBC/MIC quotients were less than 4 for all bacteria, with the exception of *Ps. aeruginosa* ATCC 27853, for which it was equal to 4.05 (Table III). Thus, the action of cefotaxime is predominantly bacterio-static, with the exception of bactericidal activity against *E. coli* K-12. Cefotaxime is known to cause suppression of the cell wall assembly and an eventual autolysis of bacteria [33]. The light microscopy of bacteria from IZ and intact parts of the lawns demonstrated that compounds III and IVc had no effect on their shapes. This suggests that the cells were killed outright without profound alterations of their walls.

Compounds III and IVc were active against *C. albicans* ATCC 885 - 653, and antifungal action resulted in the culture growth inhibitions with clear IZ of approximately 8 and 20 mm in diameters, respectively. The *C. albicans* BS1 and BS2 demonstrated similar sensitivity to these compounds. As evidenced by IZ sizes, the antifungal activity of compound IVc was somewhat stronger than that of fluconazole. When applied on lawns of yeasts, compound III was less active than fluconazole against *C. albicans* (Table IV). The *S. cerevisiae* strains were insensitive to compound III in this test. Compound IVc did show activity against *S. cerevisiae* BS1 and BS2. Touch transfer by loop from IZ peripheries to fresh Sabourauds agar plates resulted in the growth of yeast colonies and this indicated fungistatic action at the edges of IZ of *C. albicans* and *S. cerevisiae* lawns.

**Table III**

Antibacterial doses of compounds III, IVc and cefotaxime in saline solution

| Microbial strains | Minimum effective concentrations, μg/mL | | | | | |
|---|---|---|---|---|---|---|
| | III | | IVc | | cefotaxime | |
| | MIC | MBC | MIC | MBC | MIC | MBC |
| *B. licheniformis* CSES C | 650 | 1250 | 300 | 605 | - | - |
| *B. stearothermophillus* BKM-B-718 | 625 | 1225 | 150 | 310 | - | - |
| *M. luteus* ATCC 3941 | 575 | 1200 | 305 | 610 | 5 | 105 |
| *S. aureus* ATCC25923 | 575 | 1175 | 600 | 1200 | 5 | 55 |
| *E. faecalis* ATCC19433 | 325 | 625 | 605 | 1210 | 5 | 100 |
| *E. coli* K-12 | 75 | 500 | 610 | 1215 | 105 | 125 |
| *K. pneumoniae* CSESK-56 | 600 | > 2400 | 610 | 1210 | 10 | 105 |
| *P. mirabilis* RSES 3171 | 225 | 450 | 150 | 300 | 5 | 50 |
| *Ps. aeruginosa* ATCC27853 | 350 | 625 | 295 | 1195 | - | - |

**Table IV**

The evaluation of compounds III and IVc for antifungal activity in yeast lawns

| Strains of yeasts | Inhibition zone around compounds (d, mm) | | |
|---|---|---|---|
| | III, 500 μg | IVc, 500 μg | fluconazole, 500 μg |
| *C. albicans* ATCC885-653 | 8.2 ± 0.4 | 23 ± 0.2 | 19.3 ± 0.3 |
| *C. albicans* BS1 | 10.1 ± 0.1 | 24 ± 0.4 | 20.4 ± 0.5 |
| *C. albicans* BS2 | 8 ± 0.2 | 22.3 ± 0.6 | 18.2 ± 0.4 |
| *S. cerevisiae* BS1 | - | 25.2 ± 0.1 | 20.1 ± 0.5 |
| *S. cerevisiae* BS2 | - | 25.1 ± 0.1 | 20.6 ± 0.5 |

The antifungal action of IVc was fungicidal as MFC/MIC quotients were less than 4 for all cultures of yeasts (Table V). This product had stronger antifungal activity than fluconazole, which had only fungistatic action on *C. albicans* and *S. cerevisiae* strains. The MICs were, on average, about 2 times lower in IVc than in fluconazole. The quantity of MFCs in IVc was close to the quantity of MICs in fluconazole. The latter compound required more than 4 times higher concentration than the former one to kill *S. cerevisiae* (Table V).

Fluconazole, in all concentrations caused only fungistatic effect on *C. albicans* cultures. Cultures of all yeast strains resisted even a 2400 μg/mL concentration of compound III (not included in Table V). It has been proven that a switch of *C. albicans* to hyphal growth is responsible for the cell change to its infective form [16, 17].

The light microscopy of yeasts taken from the edge of the IZ produced by IVc in the *C. albicans* ATCC 885-653 lawn and from intact parts thereof showed that the cells tend not to have either hyphal growth or formation of chlamydospores in the presence of the compound (Figure 3, left). Hyphal growth was observed in approximately 4% of the cells in intact lawns incubated at 37ºC (Figure 3, right).

This characteristic of our compound is promising when regarding it as an ingredient in antifungal formulations.

**Table V**

Antifungal doses of compound IVc and fluconazole in saline solution

| Microbial strains | Minimum effective concentrations, μg/mL | | | |
|---|---|---|---|---|
| | IVc | | fluconazole | |
| | MIC | MFC | MIC | MFC |
| *C. albicans* ATCC885-653 | 155 | 305 | 300 | fungistatic action |
| *C. albicans* BS1 | 160 | 310 | 305 | fungistatic action |
| *C. albicans* BS2 | 155 | 310 | 305 | fungistatic action |
| *S. cerevisiae* BS1 | 150 | 300 | 300 | 1250 |
| *S. cerevisiae* BS2 | 125 | 275 | 275 | 1200 |

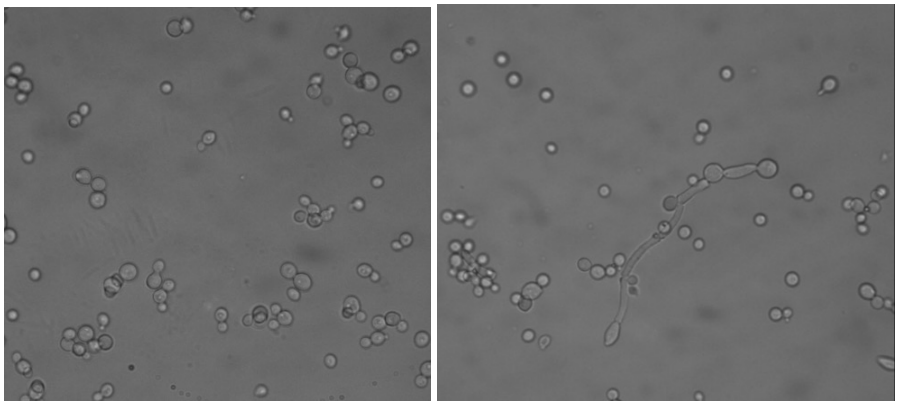


**Figure 3.**

The suppression of *C. albicans* ATCC 885-653 hyphae and chlamydospores formation by IVc: cells from the edge of IZ (left) and from the sites beyond IZ (right). Magnification ×400

Several anti-*Candida* drugs are available commercially. Most of them, however, are toxic (eg. nephrotoxic amphotericin B) or have only fungistatic activity (eg. fluconazole). Furthermore, *Candida* spp. strains resistant to particular antifungal agents or cross-resistant to some antibiotics and azoles were isolated [7, 21, 28, 32]. Even the new drug, echinocandin, has a problem of resistance to it in different *Candida* species [27]. This makes the acute need for the discovery of new remedies.

The superior antifungal activity of IVc over fluconazole could possibly be utilized in antifungal

formulations (at least against some *Candida* infections), if the product may be proven to be less toxic or to have the same toxicity as products already on the market.

Groups with significant effects on toxicity in molecules of our compounds were selenium and/or bromine fragments and tienopyrimidine fragment. The former, present in both III and IVc, enhanced the antimicrobial activity while the latter, present only in IVc, decreased the activity. This is supported by the calculation of the toxicity levels according to the method of Poroikov *et al.* [24].

## Conclusions

Among the studied compounds, III and IVc appear to be most active against yeasts and also to possess poor to moderate activity against bacteria. Antibacterial activity can be improved by introducing changes into the chemical structure of the compounds. Considering all the obtained results, these compounds and their derivatives may be of practical benefit.

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