ANTIMICROBIAL ACTIVITY OF SOME NEW THIOUREIDES FROM 2-THIOPHENEACETIC ACID

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Abstract

The aim of this study was to evaluate the „*in vitro*" antimicrobial activity of nine new compounds derived from 2-thiopheneacetic acid using qualitative and quantitative methods. These compounds have been synthesized and characterized in a previous paper [7].

The qualitative screening of the susceptibility spectra of different microbial strains to these compounds was performed by three adapted diffusion methods: paper filter disk impregnation with the tested substances solutions in DMSO (dimethyl sulfoxide), the disposal of the tested solutions in agar wells and the spotting of the tested solutions on solid medium seeded with microbial inoculums. The quantitative assay of the antimicrobial activity was performed by broth microdilution method in 96-well microplates in order to establish the minimal inhibitory concentration (MIC).The antimicrobial activity was tested against Gram-positive strains (*Staphylococcus (S.) aureus, Bacillus (B.) subtilis*), Gram-negative (*Escherichia (E.) coli, Pseudomonas (P.) aeruginosa, Klebsiella (K.) pneumoniae*) and fungal strains (*Candida (C.) albicans*).

As concerning the Gram positive strains, the tested compounds exhibited no significant antimicrobial activity against S. *aureus,* but two of these compounds were active on *B. subtilis*. All tested compounds exhibited antifungal activity against *C. albicans* and four of them against other Gram-negative clinical strains, especially *K. pneumoniae.*

Rezumat

Scopul acestei lucrări a fost evaluarea „*in vitro*" a activității antimicrobiene a nouă noi tioureide ale acidului 2-tiofenacetic, utilizând metode calitative şi cantitative de determinare. Aceşti compuşi au fost sintetizați şi caracterizați într-o lucrare anterioară.[7]

Screening-ului calitativ al spectrului de sensibilitate al diferitelor tulpini microbiene pe aceşti compuşi a fost realizat utilizând variante adaptate ale metodei difuzimetrice: impregnarea discurilor de hârtie de filtru cu soluțiile în DMSO (dimetilsulfoxid) ale substanțelor de testat, repartizarea soluțiilor testate în godeuri decupate în agar şi repartizarea soluțiilor în spot pe mediul însămânțat cu cultura microbiană.

Evaluarea cantitativă a activității antimicrobiane a fost realizată prin metoda microdiluțiilor în mediu lichid, în plăci cu 96 de godeuri, cu scopul de a stabili concentrația minimă inhibitorie (CMI). Activitatea antimicrobiană a fost testată împotriva tulpinilor Gram-pozitive (*Staphylococcus (S.) aureus, Bacillus (B.) subtilis*), Gram-negative (*Escherichia (E.) coli, Pseudomonas (P.) aeruginosa, Klebsiella (K.) pneumoniae*) şi pe tulpini fungice (*Candida (C.) albicans*).

În ceea ce priveşte tulpinile Gram pozitive, compuşii testaţi nu au prezentat o activitate antimicrobiană semnificativă împotriva *S. aureus,* dar doi dintre aceştia au fost activi împotriva *B. subtilis*. Toţi compuşii testaţi au prezentat activitate antifungică împotriva *C. albicans* şi patru dintre ei împotriva tulpinilor Gram-negative, în special împotriva *K. pneumoniae.*

Key words: thioureides, antimicrobial activity

Introduction

According to the literature data [1, 2], thioureea derivatives can be highly active as: analgesic, antidepressant, anticonvulsant, anesthetic (local), antihelmintic (nematode), diuretics, platelet aggregation inhibitors, spasmolytic or antihistaminic agents.

In our previous papers we presented the synthesis, the structural characterisation and sometimes the antimicrobial activity of some thioureides from 2-thiophene carboxylic acid [3, 4, 6-8] and 3-thiophene carboxylic acid [5].

The preliminary positive results determined us to continue this research and to obtain new 2-thiopheneacetic acid thioureides. In a previous paper [7] we present the synthesis, the physical and NMR spectra characterization of the new nine compounds presented in this paper.

The *in vitro* antimicrobial activity was evaluated using qualitative screening of the susceptibility spectra of different microbial strains to these compounds using adapted diffusion methods: paper filter disk impregnation with the tested substances solutions in DMSO, the disposal of the tested solutions in agar wells and the spotting of the tested solutions on microbial inoculums seeded medium.

The quantitative assay of the antimicrobial activity was performed by nutrient broth microdilution method in order to establish the minimal inhibitory concentration (MIC).

Materials and methods

The new thioureides of the 2-thiopheneacetic acid, with the structure presented in Table I, were tested for their antimicrobial activity using a qualitative screening assay of the antimicrobial properties by the adapted disk diffusion method (Kirby- Bauer method). We also performed the quantitative assay of the antimicrobial activity by binary microdilution method, in order to establish the minimal inhibitory concentration (MIC).

The antimicrobial activity of the investigated compounds was tested against bacterial and fungal strains: Gram positive (*Staphylococcus (S.) aureus, Bacillus (B.) subtilis*), Gram-negative (*Escherichia (E.) coli,*

Pseudomonas (P.) aeruginosa, Klebsiella (K.) pneumoniae) and fungal strains (*Candida (C.) albicans*).

The microbial strains were identified using a VITEK I automatic system. VITEK cards for the identification and the susceptibility testing (GNS-522) were inoculated and incubated according to the manufacturer's recommendations. The results were interpreted using the software version AMS R09.1.

In our experiments there were used bacterial suspensions of $1.5x10^8$ UFC/ mL or 0.5 McFarland density obtained from 15- 18 h bacterial cultures developed on solid media.

The antimicrobial activity was tested on Mueller- Hinton medium recommended for the bacterial strains and Yeast Peptone Glucose (YPG) medium for *Candida albicans*.

We used solutions of the new compounds in DMSO (dimethyl sulfoxide) having 2048 μg/ mL concentration.

Qualitative screening of the antimicrobial properties of the tested compounds

The testing of the antimicrobial and antifungal activity of the new thioureides was investigated by qualitative screening of the susceptibility spectrum of different microbial strains to the tested compounds solubilised in DMSO (1 mg/mL) using adapted variants of the diffusion method.

In the 1st variant, 10 μL of the compound solution were equally distributed on the paper filter disks placed on Petri dishes previously seeded "in layer" with the tested bacterial strain inoculums. In the 2nd variant, 10 μL of the tested compounds solutions were placed in the agar wells cut in the solid culture medium seeded with the microbial inoculum.

In the 3rd variant of the qualitative antimicrobial activity assay, 10 μL of the compounds solutions were spotted on Petri dishes seeded with bacterial/yeast inoculum. In all the three variants, the Petri dishes were left at room temperature to ensure the equal diffusion of the compound in the medium or to allow the drop of solution to be adsorbed in the medium and afterwards the dishes were incubated at 37^OC for 24 hours. The solvent used was also tested in order to evaluate a potential antimicrobial activity [10, 12].

Quantitative assay of the antimicrobial activity

For the quantitative assay of the antimicrobial activity of the new compounds (noted 1-9) by the microdilution method in liquid medium distributed in 96-well plates, binary serial dilutions of the tested compounds solutions were performed. There were obtained concentrations from 1000 μg/mL to 0.97 μg/mL in a 200 μL culture medium final volume, afterwards each well was seeded with a 50 μL microbial suspension of 0.5 MacFarland

density. In each test a microbial culture control (a series of wells containing exclusively culture medium with the microbial suspension) and a sterility control (a series of wells containing exclusively culture medium) were performed. The plates were incubated for 24 hours at 37^0C [9, 11, 12].

Results and discussion

For the qualitative methods, i.e. paper filter disks impregnated with the tested compounds solution and disposal of the respective solutions in agar wells, the reading of the results was performed by measuring the microbial growth inhibition zones around the filter disks impregnated with the testing compounds and around the wells, respectively.

The most efficient qualitative method proved to be the direct spotting of the tested solutions on the seeded medium, the results being very well correlated with the results of the MIC quantitative assay (Fig. 1).

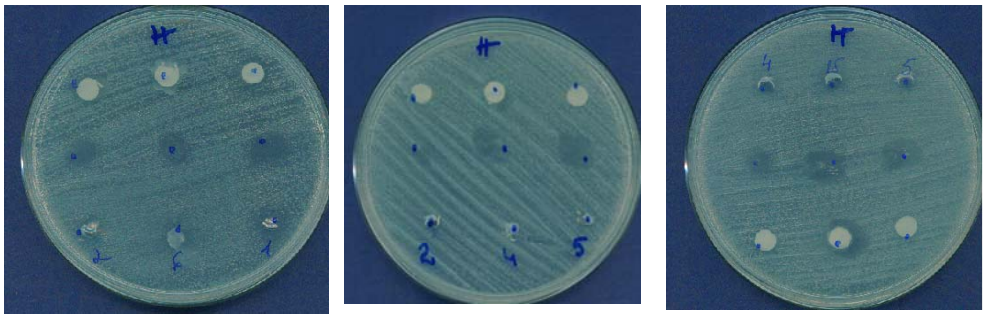


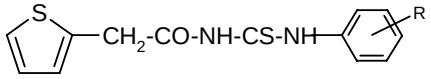
Figure 1
The qualitative screening results of the antimicrobial activity on the investigated compounds exemplified for the *C. albicans* strain

For the quantitative methods of the antimicrobial activity of the tested compounds by the microdilution method in liquid medium, the MIC was read by wells observation: in the first wells containing high concentrations of compounds, the culture growth was not visible, the microbial cells being killed or inhibited by the tested compound. At lower concentrations of the tested compounds, the microbial culture become visible. The lowest concentration which inhibited the visible microbial growth was considered the MIC (μg/mL) value for the tested compound. In the next wells, including the standard culture growth control wells, the medium become muddy as a result of the microbial growth. In the sterility control wells series, the medium had to remain clear. From the last well without any visible microbial growth and from the first one that presented microbial growth, Gram stained smears were performed for the results confirmation.

In table I there are presented the results of the quantitative assay of the antimicrobial and antifungal activities of the new nine thioureides of 2-thiopheneacetic acid.

Table I

The results of the antimicrobial activity for the new thioureides, expressed in μg/ mL (MIC)*



N-aryl-N'-(2-thienylacetyl)-thioureea

No.	R	*K. pneumoniae* IC 13420	*E. coli* IC 13529	*S. aureus* IC 13204	*P. aeruginosa* ATCC 27853	*B. subtilis* ATCC 6633	*C. albicans* IC 249
1	2-Cl	62.5	125	250	125	62.5	7.8
2	3-Cl	125	125	250	125	125	15.6
3	4-Cl	62.5	125	250	125	31	7.8
4	2-Br	125	125	250	125	125	15.6
5	3-Br	125	125	250	125	125	7.8
6	4-Br	125	125	250	125	125	7.8
7	2-I	125	125	250	125	250	31
8	3-I	62.5	125	250	125	125	15.6
9	4-I	62.5	125	250	125	125	62.5
Blank DMSO		125	125	250	125	125	125

* MIC =minimal inhibitory concentration

Our results have shown that the tested compounds were not active on S. *aureus* and two of them (compounds no. 1 and 3) exhibited antimicrobial activity on *B. subtilis.* All tested compounds were highly active against C. *albicans*, suggesting their possible use in the treatment of fungal infections. Four of these compounds (compounds no. 1, 3, 8 and 9) have shown moderate antimicrobial activity against *K. pneumoniae.*

Conclusions

This paper presents the biological activities (the antimicrobial and antifungal activity) of nine new thioureides derived from 2-thiopheneacetic acid. The *in vitro* qualitative and quantitative antimicrobial activity assay has shown that the new thioureides exhibited significant antimicrobial activity on *C. albicans* with MICs ranging from 7.8 μg/mL to 62.5 μg/mL. Our studies demonstrated that among other biological activities of the

thioureides, the 2-thiopheneacetic acid thioureides exhibit antimicrobial properties that could lead to the selection and use of these compounds as effcient antimicrobial agents, especially for the treatment of C. *albicans* infections.

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