Romanian Biotechnological Letters *Vol. 15, No.5, 2010*

ORIGINAL PAPER

Antimicrobial activity of some new 2-thiophene carboxylic acid thioureides

Received for publication, December 10, 2008
Accepted, September 8, 2010

CARMELLINA DANIELA BĂDICEANU[1], ALEXANDRU-VASILE MISSIR[1], MARIANA CARMEN CHIFIRIUC[2], OLGUTA DRACEA[2], IULIANA RAUT[2], CRISTINA LARION[2], LIA MARA DITU[2], GRIGORE MIHAESCU[2]
[1]Pharmaceutical Chemistry Department, Faculty of Farmacy, „Carol Davila" University of Medicine and Pharmacy , Traian Vuia 6, sect. 2, 020956, Bucharest, Romania
[2]Microbiology-Immunology Department, Faculty of Biology, University of Bucharest, Ale. Portocalelor 1-3, sect. 5, Bucharest, Romania
Corresponding author email address: <carmen_balotescu@yahoo.com>

Abstract

The aim of this study was to evaluate the in vitro antimicrobial activity of some newly synthesized 2-thiophene carboxylic acid thioureides.

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, the disposal of tested solutions in agar wells and the spotting of tested solutions on solid medium seeded with microbial inoculums.

The in vitro antimicrobial testing was performed by binary microdilution method, in 96 multi-well plates, in order to establish the minimal inhibitory concentration (MIC) against Gram-positive: Staphylococcus aureus, Bacillus subtilis, Gram-negative: Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, as well as Candida albicans and Aspergillus niger, using both reference and clinical, multidrug resistant strains.

All the tested compounds were active against reference strains, but also against Gram-negative clinical strains MIC, ranging from 250 to 31.25 μg/mL. All tested compounds exhibited antifungal activity MIC ranging from 62.5 to 31.25 μg/mL. As concerning the Gram positive strains, all tested compounds presented antimicrobial activity against Bacillus subtilis (MIC from 125 to 7.8 μg/mL), but for multi-drug resistant Staphylococcus aureus the MIC values were higher (MIC from 500 to 125 μg/mL).

Keywords: thioureides, qualitative screening, minimal inhibitory concentration

Introduction

Antibiotics were treated as miracle drugs when they first became available about 60 years ago, proving to be a major asset in the fight against infectious bacteria. Over the second half of the century, it has become well-known that antibiotics are loosing their effectiveness as bacteria evolve resistance against them and new drugs only rarely reach the market. Antimicrobial resistance threatens the effectiveness of successful treatment of infections and is a public health issue with local, national, and global dimensions. Antimicrobial resistance can result in increased morbidity, disease burden, and mortality. Surveillance of antimicrobial resistance proportions provides data that are needed to raise the awareness to the problem and instigate necessary interventions.

Since 2001, Romania participates in the European Antimicrobial Resistance Surveillance System (EARSS) which collects comparable and validated antimicrobial susceptibility data for public health action. EARSS performs on-going surveillance of antimicrobial susceptibility for *Streptococcus pneumoniae, Staphylococcus aureus, Escherichia coli, Enterococcus faecalis/faecium, Klebsiella pneumoniae* and *Pseudomonas aeruginosa,* causing invasive infections and monitors variations of antimicrobial resistance over time and place. The data included in the interactive website available at www.rivm.nl/earss, show that our country is in the top of the resistance levels for the most important groups of antibiotics (1).

Antibiotics have traditionally been plucked from nature's battleground. For billions of years, tiny organisms have engaged in an arms race, hurling toxic molecules at each other in the struggle to prosper. Nearly all of today's antibiotics are versions of weapons long wielded by microbes and fungi. Chemical synthesis of entirely human-created antibiotics has so far yielded only fluoroquinolones and linezolid (15).

The specialized literature mentions a series of thioureides tested for a large spectrum for biological activities, such as: antidepressant, anticonvulsant, antihelmintic, vermicides, diuretics, platelet aggregation inhibitors, antispasmodic, antitusive, analgesic, antihistaminic, anaesthetic (local), insecticides or herbicides and also antibacterial activity (2, 3). In previous papers we presented the synthesis and the structural proofs of some thioureides of 2-thiophene carboxylic acid [4, 5, 6] and 3-thiophene carboxylic acid (5).

The preliminary positive results determined us to continue this research which resulted in the obtaining of 2-thiophene carboxylic acid thioureides. The chemical structures of the new compounds were confirmed by ^{1}H-NMR and ^{13}C-NMR spectral analysis.

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, the disposal of tested solutions in agar wells and the spotting of tested solutions on culture media previously seeded. 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 antimicrobial properties of the new thiouiredes were tested against bacterial and fungal strains recently isolated from clinical specimens belonging to the following genera and species: *Staphylococcus (S.) aureus, Bacillus (B.) subtilis, Pseudomonas (P.) aeruginosa, Escherichia (E.) coli, Klebsiella (K.) pneumoniae, Candida (C.) albicans* and *Aspergillus (A.) niger*, cultivated on liquid/solidified Mueller Hinton (for bacterial strains) and YPG (Yeast Peptone Glucose) medium (for yeast strains). The microbial inoculums were prepared in sterile saline from 15-18 hours microbial cultures (4-5 isolated colonies) developed on solid medium and adjusted by nephelometry to a standard density of $1.5x10^{8}$ CFU/ mL (corresponding to 0.5 MacFarland).

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 dimethylphormamide (DMF) (1 mg/mL), using adapted variants of the disk 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 solidified 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 three variants, the Petri dishes were left at room temperature for 30 minutes to ensure the equal diffusion of the compound in the medium or to allow the drop of the solution to be adsorbed in the medium and afterwards the dishes were incubated at 37^{O}C for 24 hours. The DMF solvent was also tested to evaluate its potential antimicrobial activity (9- 14, 16).

If in a plate containing a compound the inoculated strain did not grow, then it was considered that the respective compound exhibited a bactericidal effect. If the bacterial

growth could be observed, the culture density was compared with that of the positive control plate. In the case of a bacterial growth less abundant then that of the standard culture, we appreciated that the substance exhibited a bacteriostatic effect.

If the growth intensity was comparable for the tested plate and for the standard culture, then the substance does not influence notably the growth and the development of the tested bacterial stain.

For the quantitative (MIC) assay of the antimicrobial activity of the new compounds 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 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^{O}C. The minimal inhibitory concentration 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 with a microbial growth, Gram stained smears were performed for the results confirmation (7-14).

Results and discussion

For the qualitative methods of paper filter disks impregnated with the tested compounds solution and disposal of the respective solutions in agar wells the reading of the results was done 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 spotting of the tested solutions on the seeded medium, the results being very well correlated with the results of the MIC quantitative assay (Fig.1-3).

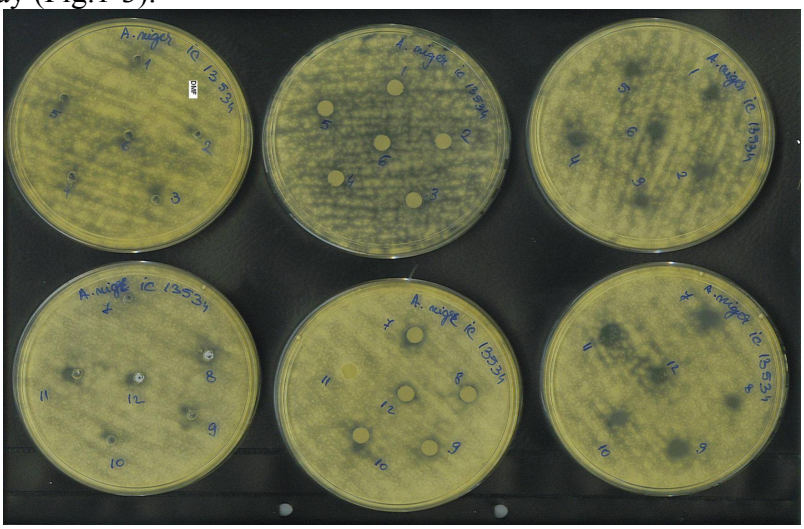


Figure 1. The appearance of antimicrobial activity of the tested compounds against *A. niger* IC 13534 by the three qualitative screening methods (from left to right: agar well diffusion (left); disk diffusion (middle); and spotting the tested compounds solutions on the agar surface (right); all the assays were performed in duplicates- the results of individual assays on each row).

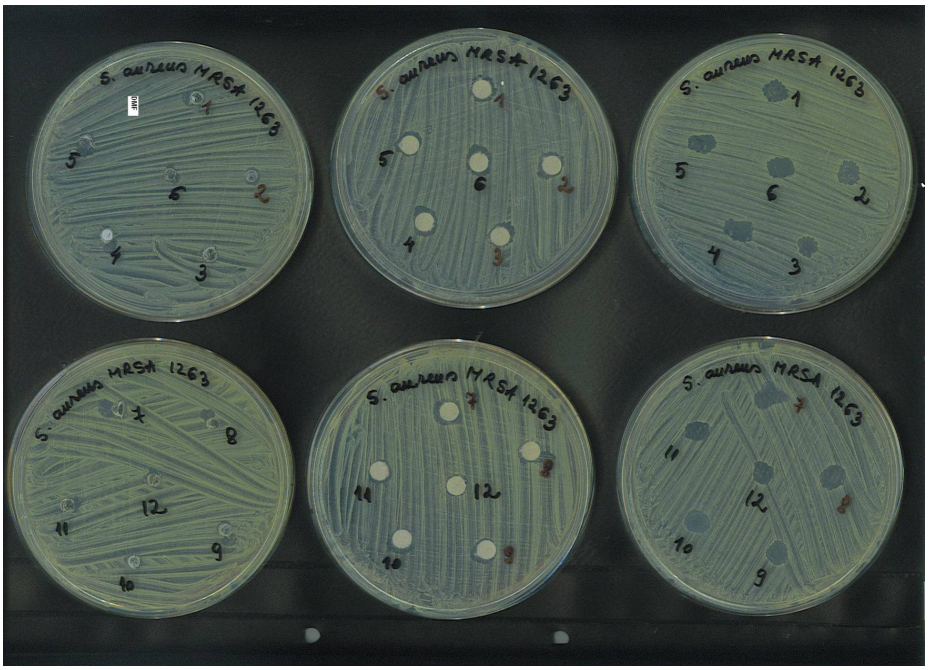


Figure 2. The appearance of antimicrobial activity of the tested compounds against *K. pneumoniae* 1204 by the three qualitative screening methods by the three qualitative screening methods (from left to right: agar well diffusion (left); disk diffusion (middle); and spotting the tested compounds solutions on the agar surface (right); all the assays were performed in duplicates- the results of individual assays on each row).

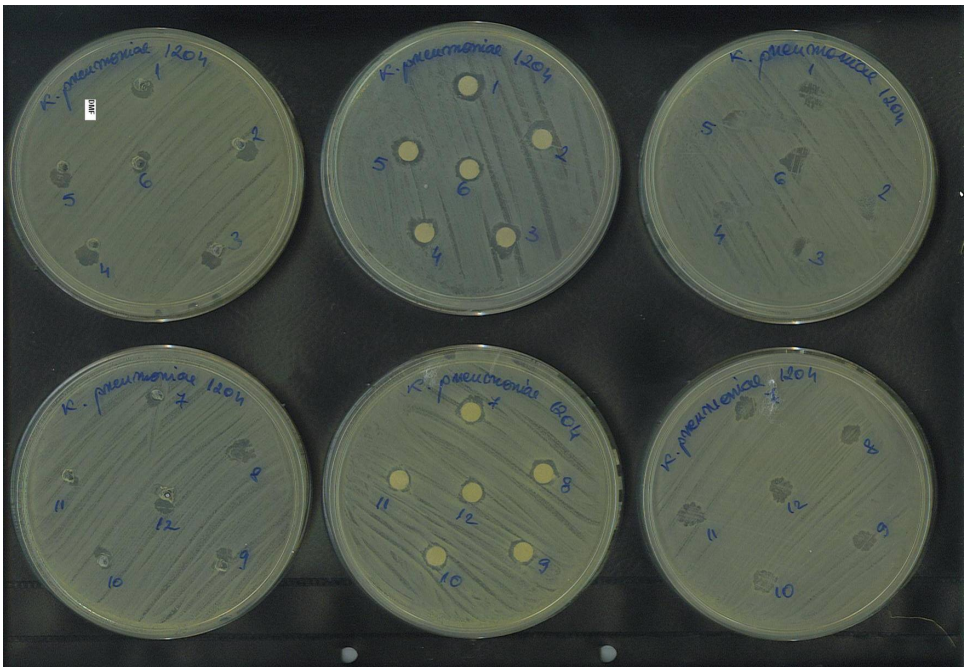
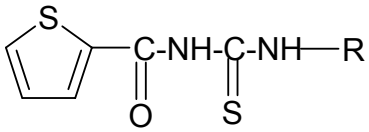
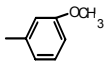
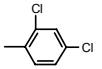
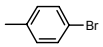
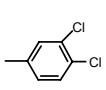
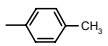
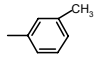
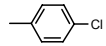
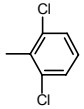
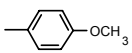


Figure 3. The appearance of antimicrobial activity of the tested compounds against *S. aureus* 1263 by the three qualitative screening methods (from left to right: agar well diffusion (left); disk diffusion (middle); and spotting the tested compounds solutions on the agar surface (right); all the assays were performed in duplicates- the results of individual assays on each row).

In table 1 there are presented the results of the quantitative assay of the antimicrobial and antifungal activities of the new compounds, being known that a concentration of 32 μg/mL represents a very strong effect and a 256 μg/mL concentration represents a moderate effect. The tested compounds presented an antimicrobial activity at concentrations between 500 and 7.8 μg/ mL.

Table 1. The results of the antimicrobial activity of the new thioureides



Microbial strain / Tested compound	 1.	 2.	 3.	 4.	 5.	6.	 7.	 8.	 9.	 10.	 11.	 12.
K. pneumoniae 1204	**31.25**	62.5	62.5	125	62.5	62.5	62.5	250	62.5	125	62.5	62.5
K. pneumoniae IC 13420	62.5	62.5	62.5	62.5	62.5	62.5	62.5	250	125	125	62.5	62.5
E.coli 13147	**31.25**	**31.25**	62.5	250	250	**31.25**	125	250	125	125	**31.25**	**31.25**
E.coli IC 13529	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	125	62.5	62.5
S.aureus 1263	250	500	500	250	125	500	125	250	125	125	250	250
S.aureus IC 13204	125	62.5	125	125	62.5	62.5	62.5	250	**31.25**	**31.25**	**31.25**	**31.25**
P.aeruginosa 1246	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	**31.25**	62.5	**31.25**	**31.25**
P.aeruginosa IC 13202	62.5	62.5	62.5	125	62.5	62.5	62.5	62.5	125	125	125	125
B. subtilis IC 12488	62.5	62.5	62.5	62.5	62.5	62.5	62.5	**7.8**	125	125	125	62.5
C.albicans 26/2007	**31.25**	62.5	**31.25**	**31.25**	**31.25**	**31.25**	62.5	**31.25**	**31.25**	**31.25**	**31.25**	**31.25**
C.albicans IC 249	**31.25**	62.5	**31.25**	**31.25**	**31.25**	**31.25**	**31.25**	62.5	**31.25**	62.5	**31.25**	62.5
A.niger IC 13534	**31.25**	**31.25**	**31.25**	**31.25**	**31.25**	**31.25**	**31.25**	**31.25**	**31.25**	**31.25**	**31.25**	**31.25**

Many of the tested compounds exhibited a broad spectrum of antimicrobial activity, being active at low concentrations both on Gram-positive, Gram-negative bacteria and fungal strains. It is worth to be noticed the good antimicrobial activity of most of the tested compounds against *B. subtilis* (MIC = 125 to 7.8 µg/mL), *A. niger and C. albicans* (MIC= 62.5 to 31.25 µg/mL), which can represent new therapeutical options in the treatment of fungal infections, which are difficult to treat and eradicate, because of the very high levels of natural and acquired resistance of these microorganisms. The activity on *S. aureus* was moderate, the tested compounds exhibiting MIC values ranging from 500 to 125 µg/ mL.

The synthesis reaction scheme is presented in the Fig. 4.

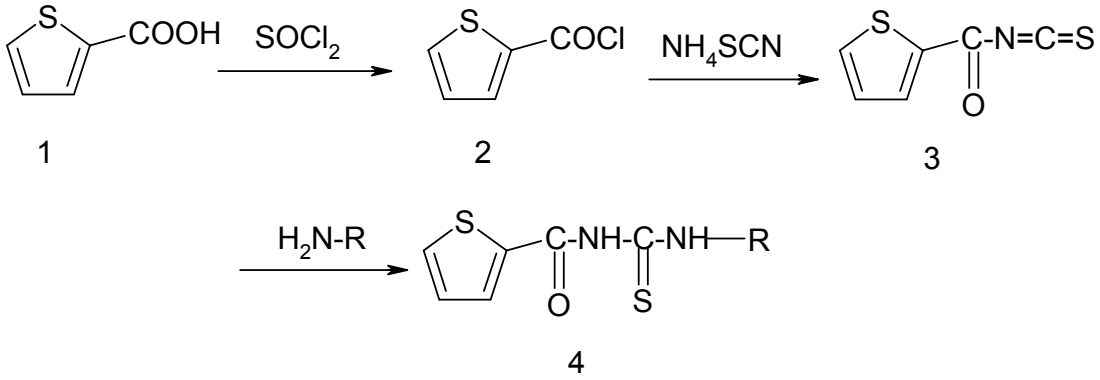


Figure 4. The synthesis of the new thioureides (6)

Conclusions

This paper presents some biological activities (the antimicrobial and antifungal activity) of some new thioureides of 2-thiophene carboxilic acid. The in vitro qualitative and quantitative antimicrobial activity assay showed that the new thioureides exhibited significant antimicrobial activity with MICs ranging from 7.8 µg/mL to 500 µg/mL. All tested compounds exihibited very low MIC on *A. niger* strains, and 9 of 12 compounds on *C. albicans* strains, demonstrating their antifungal properties. The most effective antimicrobial activity has been exhibited by the compounds no. 11 and 12 with low MIC values on the majority of the tested microbial strains. Our studies demonstrated that among other biological activities of thioureides of 2-thiophene carboxylic acid, some of these compounds exhibit also selective and effective antimicrobial properties that could lead to the selection and use of these compounds as effcient antimicrobial agents, especially for the treatment of multidrug resistant infections.

References

1. *** *EARSS Annual Report*. 2006. www.earss.rivm.nl
2. *** Merck Index, 13th Edition, Merck&Co, Inc., Whitehouse Station, New Jersey, 2001
3. *** Pharmazeutische Stoffliste– List of Pharmaceutical Substances 10th edition, Ed. ABDATA, Eschborn/Taunus, 10, 85 (1997).
4. BADICEANU, D.C. *New thioureides of 2-thiophenecarboxylic acid with potential pharmacological activity*. European Journal of Drug Metabolism and Pharmacokinetics, 32, 6-7 (2007).
5. BADICEANU, D.C., MISSIR, A. *Experimental researches concerning the synthesis and physico-chemical characterization of some new thioureides of 2-thiophene carboxylic acid,* Farmacia, vol. LV, 4, 416-421 (2007).
6. BADICEANU, D.C., MISSIR, A. *Synthesis of new thioureides compounds with potential pharmacological activity from thiophene-3-carboxylic acid*. Farmacia, LV, 6, 710-716 (2007).

7. CLINICAL AND LABORATORY STANDARDS INSTITUTE. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard—Third Edition* M27-A3 ,Vol. 0 No. 0, Replaces M27-A2, 22, 15 (2007).
8. CLINICAL AND LABORATORY STANDARDS INSTITUTE. *Performance Standards for Antimicrobial Susceptibility Tests; Eighteen informational supplement M100-S18* (2008).
9. LAZAR V., BALOTESCU M.C., MOLDOVAN L., ALEXANDRU V., DITU L.M., BULAI D., CERNAT R. *Comparative evaluation of qualitative and quantitative methods used in the study of antifungal and antibacterial activity of hydroalcoholic vegetal extracts*. Romanian Biotechnological Letters. 9 (6), 2225 – 2232 (2005).
10. LIMBAN C., BALOTESCU CHIFIRIUC M.C., MISSIR A.V., CHIRIȚĂ I.C., BLEOTU C. *Antimicrobial activity of some new thioureides derived from 2-(4-chlorophenoxymethyl) benzoic acid.* Molecules. 13, 3, 567-80 (2008).
11. OLAR R., BADEA M., CARP O., MARINESCU D., LAZAR V., BALOTESCU C., DUMBRAVA A. *Synthesis, characterisation and thermal behaviour of some thiosulfato- and sulfato copper (II) complexes. Antibacterial activity*. J. THERM. ANAL. CALOR. 92, 245-251 (2008).
12. OLAR R., BADEA M., CRISTUREAN E., GRECU N., MARINESCU D., LAZAR V., BALOTESCU C. *Thermal behavior, spectroscopic and biological characterization of copper(II) complexes with N,N-dimethylbiguanide*. J. Therm. Anal. Calor. 92, 1, 239-243 (2008).
13. OLAR R., BADEA M., CRISTUREAN E., LAZAR V., BALOTESCU C. CRISTUREAN E., MARINESCU D. *Thermal behavior of some N,N-dimethylbiguanide derivatives displaying antimicrobial activity*. J. Therm. Anal. Calor. 88, 2, 323-327 (2007).
14. OLAR R., BADEA M., CRISTUREAN E., LAZAR V., CERNAT R., BALOTESCU C. *Thermal behavior, spectroscopic and biological characterization of Co (II), Zn (II) and Pt (II) complexes with N,N-dimethylbiguanide*. J. Thermal Analysis and Calorimetry, 80, 451 – 455 (2005).
15. POWLEDGE T. M. *New Antibiotics—Resistance Is Futile.* PLoS Biol. 2(2), 53 (2004).
16. ROSU T., PASCULESCU S., LAZAR V., CHIFIRIUC C., CERNAT R. *Copper(II) Complexes with Ligands Derived from 4-Amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one: Synthesis and Biological Activity*. Molecules. 113, 11, 904-914 (2006).