ANTIMICROBIAL ACTIVITY INVESTIGATION OF SOME THIOSEMICARBAZIDES AND THEIR CYCLIZATION PRODUCTS

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Abstract

The aim of the present study was the investigation of the potential antimicrobial activity of some thiosemicarbazides (C1 and C2) and their cyclization products from 1,2,4-triazole (C3 and C4) and 1,3,4-thiadiazole (C5 and C6) class. All these compounds contain in their molecule X-$C_6H_4SO_2C_6H_4$- (X=H, Br) and 2-F-C_6H_4- radicals and their synthesis was recently reported. The compounds C1-C6 have been tested against 10 reference microbial strains and 30 clinical fungal isolates using the broth microdilution method, in order to determine their minimum inhibitory concentrations (MIC) and minimum bactericidal/fungicidal concentrations (MBC/MFC), respectively. The MIC values ranged between: 32-256 μg/mL for thiosemicarbazide C1 and triazole C3, 128->512 μg/mL for thiosemicarbazide C2 and triazole C4, and 256->512 μg/mL for thiadiazoles C5 and C6. In all cases, the MBC/MIC or MFC/MIC ratio was less or equal to 4.

As thiosemicarbazide C1 and triazole C3 presented the best antibacterial and antifungal activity from all studied compounds, it was considered that further investigations for these two compounds must be performed against more microbial strains.

Rezumat

Scopul studiului de faţă l-a reprezentat investigarea prezumtivă a activităţii antimicrobiene a unor tiosemicarbazide (C1 şi C2) şi a produşilor de ciclizare ai acestora din clasa 1,2,4-triazolilor (C3 şi C4) şi a 1,3,4-tiadiazolilor (C5 şi C6). Toţi aceşti compuşi conţin în molecula lor fragmentele X-$C_6H_4SO_2C_6H_4$- (X=H, Br) şi 2-F-C_6H_4-, sinteza acestora fiind raportată recent. Compuşii C1-C6 au fost testaţi faţă de 10 tulpini microbiene de referinţă şi 30 de tulpini fungice recent izolate, prin metoda microdiluţiilor în bulion, urmărindu-se determinarea concentraţiilor minime inhibitorii (CMI) şi a concentraţiilor minime bactericide (CMB) sau respectiv, fungicide (CMF) ale acestora. Valorile CMI au variat între: 32-256 μg/mL pentru tiosemicarbazida C1 şi triazolul C3, 128- >512 μg/mL atât pentru tiosemicarbazida C2, cât şi pentru triazolul C4, şi 256- >512 μg/mL pentru

tiadiazolii C5 şi C6. În toate cazurile, valoarea raportului CMB/CMI sau CMF/CMI a fost mai mică sau egală cu 4.

Deoarece dintre toţi compuşii studiaţi, tiosemicarbazida C1 şi triazolul C3 au prezentat cea mai bună activitate antibacteriană şi antifungică, s-a considerat ca testările ulterioare pentru aceşti doi compuşi să fie efectuate pe un număr sporit de tulpini microbiene.

Keywords: thiosemicarbazide, triazole, thiadiazole, antimicrobial activity.

Introduction

The extensive use of antibiotics has led to the widespread emergence of the resistant microorganisms. It is well-known that *Staphylococcus aureus* and especially the meticillin-resistant strains are frequently involved in human pathology and are responsible for high morbidity and mortality, as well as for high healthcare costs [1]. *Escherichia coli* is the most common facultative-anaerobic microorganism colonizing the normal gastrointestinal tract, but a lot of antibiotic resistant strains are often isolated, especially in hospital setting [2, 3]. The use of beta-lactam antibiotics in hospitalized patients explains the selection of extended spectrum beta-lactamase producing isolates belonging to the *Enterobacteriaceae* and their involvement in hospital-associated infections, especially, *E. coli* and *Klebsiella pneumoniae*, but also *Enterobacter cloacae* [4] and other bacteria belonging to this family. *Acinetobacter baumannii* is well-known as an important infectious agent in hospitals, showing in the past decade an increased antibiotic resistance [5, 6, 7]. This species possesses a high potential in acquiring extensive drug resistance [8]. *Pseudomonas aeruginosa* is mostly responsible for nosocomial infections, often very difficult to treat, due in part to its high level of intrinsic resistance to beta-lactam and other antibiotics [9]. Intrinsically resistance to most penicillins and cephalosporins of second and third generation has been proven also among some Gram-positive bacilli, like *Bacillus cereus* [10]. Adding to these aspects also the increasing prevalence of the serious fungal infections and the difficulty of their treatment [11], it could be concluded that there is an urgent need for new antimicrobial agents with clinical efficacy and low resistance potential, and a renewed interest in searching for alternative compounds for treating the infectious diseases caused by pathogens resistant to the current antibiotics and antifungals.

It has been reported in the literature that certain five membered heterocyclic compounds possess various biological properties. Among pentaatomic heterocyclic compounds, numerous 1,2,4-triazole and 1,3,4-

thiadiazole derivatives exhibit a broad range of biological activity including antimicrobial activity [12-18]. Furthermore, thiosemicarbazides, compounds that can be used as raw materials for the synthesis of these heterocyclic compounds, have also antimicrobial properties [19, 20].

As a follow-up of the studies of testing the biological action of some heterocyclic derivatives containing diphenylsulfone and fluorophenyl radical [21-23], the authors of the present paper have intended to extend their investigation on other derivatives, in order to obtain new antimicrobial agents.

The present research dealt with the investigation of the antimicrobial activity of 6 organic compounds, previously synthesized [24], with the following structures:

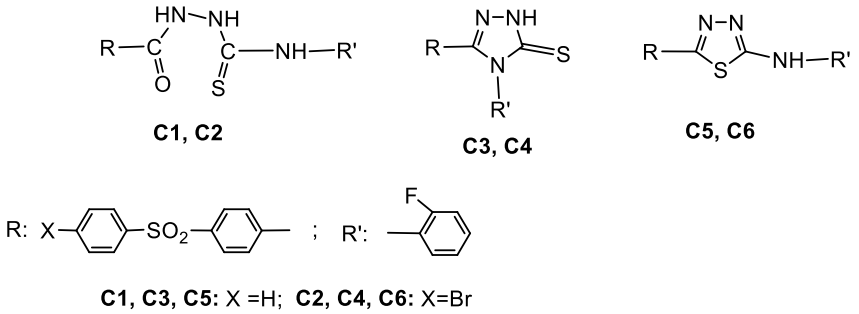

Figure 1
The chemical structures of the studied compounds

Materials and Methods

The following compounds were tested for their potential antimicrobial activity: *N^1-[4-(phenylsulfonyl)benzoyl]-N^4-(2-fluorophenyl)-thiosemicar-bazide* (C1), *N^1-[4-(4-bromophenylsulfonyl)benzoyl]-N^4-(2-fluorophenyl)-thiosemicarbazide* (C2), *5-(4-(phenylsulfonyl)phenyl)-4-(2-fluorophenyl)-2H-1,2,4-triazole-3(4H)-thione* (C3), *5-(4-(4-bromophenyl sulfonyl)phenyl)-4-(2-fluorophenyl)-2H-1,2,4-triazole-3(4H)-thione* (C4), *5-(4-(phenylsulfo-nyl)phenyl)-N-(2-fluorophenyl)-1,3,4-thiadiazol-2-amine* (C5) and *5-(4-(4-bromophenylsulfonyl)phenyl)-N-(2-fluorophenyl)-1,3,4-thiadiazol-2-amine* (C6). Briefly, the synthesis of these compounds [24] was performed in the following way: 1,2,4-triazoles C3 and C4 were synthesised by reaction of the coresponding acylthiosemicarbazides (C1 and C2) in basic medium, while the 1,3,4-thiadiazoles C5 and C6 were synthesised by reaction of the same acylthiosemicarbazides (C1 and C2) in acidic medium.

The acylthiosemicarbazide intermediates C1 and C2 were obtained by treating 4-(4-X-phenylsulfonyl)-benzoic acid hydrazides (X =H or Br) with 2-fluorophenyl isothiocyanate. The antibacterial and antifungal activity of the compounds was investigated by the broth microdilution method, in 96 flat-bottomed wells microplates (Nunc, Denmark). The dimethyl sulfoxide was used as solvent for preparation of the stock solution of the compounds, which yielded a concentration of 2048 μg/mL.

The antimicrobial action of the newly-synthesized compounds was tested against 6 reference bacterial strains: *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *E. cloacae* ATCC 49141, *A. baumannii* ATCC 19606, *P. aeruginosa* ATCC 27853, *B. cereus* ATCC 13061, and 4 reference yeast strains: *C. albicans* ATCC 90028, *C. glabrata* ATCC 15126, *C. parapsilosis* ATCC 22019 and *C. tropicalis* ATCC 13803. In addition, the compounds were tested against 30 clinical yeast isolates belonging to an in-house strains collection, at the laboratory of the Microbiology Department of the Faculty of Dental Medicine, the University of Medicine "Carol Davila", Bucharest. Previously to this study, these clinical strains had been isolated on Sabouraud Gentamicin Chloramphenicol Agar (BioMérieux, France) from tongue swabs collected from healthy young subjects, and stored on cryo-bead tubes (AES Laboratoire, France), at -70^0C. During the present research work, these isolates were identified at species level by conventional methods (including the germ tube test) [25, 26] and ID 32 C system (BioMerieux, France).

All the 96 wells of the microplate were filled in with 50 μL of Müller-Hinton broth when testing compounds against bacteria and Sabouraud broth when testing against the yeasts. Series of two-fold dilutions of the 6 compounds were performed in the Műller-Hinton or Sabouraud broth, from 1:2 to 1:1024.

In the case of the reference bacterial strains, the *inoculum* was prepared by suspending 5 distinct colonies from a 24h culture obtained on Columbia Blood Agar (BioMérieux, France), in a tube with 5mL of Müller-Hinton broth. After vortex-mixing and adjusting the density to the turbidity of the 0.5 McFarland standard, the bacterial suspension was diluted 1:100 in Müller-Hinton broth, in order to obtain the working *inoculum*. Afterwards, each well of the microdilution plates containing 50 μL of Müller-Hinton broth with compound was inoculated within 15' with 50 μL of the bacterial *inoculum*, including the growth control wells and excepting the sterility control wells, filled in only with 100 μL compound-free Müller-Hinton broth.

In the case of the reference yeast strains and the yeast clinical isolates, the *inoculum* was prepared by suspending 5 distinct colonies from a 24h culture obtained on Sabouraud Dextrose Agar, in a tube with 5mL of sterile distilled water. After vortex-mixing and adjusting the density to the turbidity of the 0.5 McFarland standard, the fungal suspension was diluted in sterile distilled water in order to obtain a working *inoculum* of 1-5 x 10^5 CFU/mL. Each well of the microdilution plates containing 50 µL of Sabouraud broth with compound was inoculated with 50 µl yeast inoculum within 15', including the growth control wells, but not the sterility control wells, which were filled in only with 100 µL compound-free Sabouraud broth.

After performing the *inoculum* controls from the growth control wells, the microplates were incubated at 37°C for 24 h. The lowest concentration of each compound able to inhibit the visible microbial growth was considered the MIC value (minimum inhibitiry concentration). Afterwards, 10 µL from the wells without visible microbial growth were applied in spot with an electronic pipette on Columbia Blood Agar in case of bacteria, and on Sabouraud Agar in the case of yeasts. After the incubation of the plates at 37°C for 48h, the MBC (minimum bactericidal concentration) and MFC (minimum fungicidal concentration) were considered the lowest concentration of the respective compound able to kill 99.9% of the bacterial or fungal amount, respectively, indicated previously by the *inoculum* control.

Results and Discussion

The suitable methods for establishing the activity of new antimicrobial agents are considered to be the dilution methods, and this was the reason for choosing the broth microdilution method for the present study, and for determining the MIC and MBC or MFC of the respective antimicrobial agents.

Beside the reference strains of Gram-positive cocci, Gram-positive bacilli, Gram-negative bacilli and different species of *Candida*, the 6 compounds have been tested against a number of 30 yeast clinical isolates, which were identified at species level during the present study. All these fungal strains were identified by the conventional methods (including the positive germ tube test) and the ID 32 C system as *C. albicans*.

The dimethyl sulfoxide showed no antimicrobial activity against the tested strains. In the case of the reference bacterial strains, the MIC values of the compounds ranged between: 32-256 µg/mL for thiosemicarbazide C1 and triazole C3, 128-512 µg/mL for thiosemicarbazide C2, 256-512 µg/mL

for triazole C4, while the values of the MBC ranged between: 128- >512 μg/mL for C1 and C3, and 256- >512 μg/mL for C2 and C4 (Table I). The MIC and MBC values for both thiadiazoles C5 and C6 were >512 μg/mL against all the reference bacterial strains. Among the 6 bacterial strains, the highest degree of growth inhibition was noticed in the case of *A. baumannii* when tested against C1 and C3, while the growth of the Gram-positive bacteria (*S. aureus* and *B. cereus*) was inhibited by a higher concentration of the compounds.

Table I

The MIC and MBC values of the compounds tested against the reference bacterial strains

Compound	Value of MIC (μg/mL) / value of MBC (μg/mL)					
	S. aureus **ATCC** 25923	***E. coli*** **ATCC** 25922	***E. cloacae*** **ATCC** 49141	***A. baumannii*** **ATCC** 19606	***P. aeruginosa*** **ATCC** 27853	***B. cereus*** **ATCC** 13061
C1	128/512	64/256	64/256	32/128	64/128	256/>512
C2	256/512	128/512	512/512	256/256	256/256	512/>512
C3	128/512	128/512	128/512	32/128	64/256	256/>512
C4	512/512	512/512	512/512	256/256	256/256	512/>512
C5	>512/>512	>512/>512	>512/>512	>512/>512	>512/>512	>512/>512
C6	>512/>512	>512/>512	>512/>512	>512/>512	>512/>512	>512/>512

In the case of the reference yeast strains, the MIC values of the compounds ranged between: 32-64 μg/mL for thiosemicarbazide C1, 32-128 μg/mL for triazole C3 and 256-512 μg/mL for thiosemicarbazide C2, while those of MFC were between: 64-256 μg/mL for C1 and 128-256 μg/mL for C3. The derivatives from thiadiazole class (C5 and C6) showed both MIC and MFC >512 μg/mL, while the MIC values did not exceed 512 μg/mL in the case of C2 and C4, in contrast to the MFC values, which were >512 μg/mL against all the tested reference yeast strains (Table II).

Table II

The MIC and MFC values of the compounds tested against the reference yeast strains

Compound	Value of MIC (μg/mL) / value of MFC (μg/mL)			
	C. albicans **ATCC 90028**	***C. glabrata*** **ATCC 15126**	***C. parapsilosis*** **ATCC 22019**	***C. tropicalis*** **ATCC 13803**
C1	64/128	64/128	32/64	64/256
C2	256/512	512/512	512/512	512/512
C3	64/128	128/128	32/128	64/256
C4	512/512	512/512	512/512	512/512
C5	>512/>512	>512/>512	>512/>512	>512/>512
C6	>512/>512	>512/>512	>512/>512	>512/>512

The values of MIC of the compounds tested against the clinical isolates of *C. albicans* are shown in table III. It can be noticed that both thiosemicarbazide C1 and triazole C3 presented the lowest values of MIC_{min} (the lowest value of MIC among the tested isolates) and MIC_{50} (the MIC that inhibited the growth of 50% of the tested isolates) of 32 μg/mL and 64 μg/mL, respectively, while the thiadiazoles C5 and C6 showed the highest values of MIC_{min}, of more than 512 μg/mL. The lowest value of MIC_{90} (the MIC that inhibited the growth of 90% of the tested isolates) was expressed by thiosemicarbazide C1, followed by triazole C3, of 128 μg/mL and 256 μg/mL, respectively, while the rest of the compounds exhibited a value of 512 μg/mL or more (Table III).

The results obtained by testing the compounds against the reference and clinical strains indicated that the compounds C1 and C3 exhibited the highest degree of microbial growth inhibition, while the derivatives C5 and C6, belonging to the class of thiadiazole, showed the lowest antimicrobial activity. Compared to thiosemicarbazide C1 and triazole C3, the antimicrobial action decreesed in both acylthiosemicarbazide C2 and triazole C4 containing a bromine atom in their molecule.

Table III

The MIC values of the compounds against the 30 isolates of *C. albicans*

Compound	Values of MIC (μg/mL)			
	MICmin[a]	**MICmax**[b]	**MIC_{50}**[c]	**MIC_{90}**[d]
C1	32	256	64	128
C2	128	>512	256	512
C3	32	256	64	256
C4	128	>512	256	512
C5	256	>512	512	>512
C6	256	>512	512	>512

[a]*The minimum value of MIC among the tested isolates;*
[b]*the maximum value of MIC among the tested isolates;*
[c]*the MIC which inhibited the growth of 50% of the tested isolates;*
[d]*the MIC which inhibited the growth of 90% of the tested isolates.*

Conclusions

Comparing the antimicrobial activity of these 6 previously synthetised compounds against the 10 microbial reference strains and 30 clinical isolates of *C. albicans*, it was clearly noticed that the thiosemicarbazide C1 and the corresponding triazole C3, which contained

not a bromine atom in their molecule, showed the best antimicrobial activity. However, it is recommended to test the antimicrobial action of both compounds on a larger sample of strains. The derivatives C2 and C4 (belonging to the thiosemicarbazide and triazole classes, respectively) containing a bromine atom in their molecule showed a lower antimicrobial activity than the derivatives without halogen in their molecule, belonging to the same classes. Thiadiazole derivatives had the worst antimicrobial activity comparatively to the triazole and thiosemicarbazide compounds.

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