

INVESTIGATION OF ANTIBACTERIAL ACTIVITY OF FIVE HETEROCYCLIC COMPOUNDS AGAINST SOME ORAL STREPTOCOCCAL STRAINS

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Abstract

The oral streptococci are commensal bacteria, but sometimes may be involved in infections which need antimicrobial treatment. In the general context of an increasing incidence of antibiotic resistant isolates, the investigation of the potential antimicrobial activity of different classes of heterocyclic compounds is considered of great interest. The aim of the study was to investigate the antimicrobial activity against 64 clinical isolates of oral streptococci belonging to different species of 5 compounds (*Ca*, *Cb*, *Cc*, *Cd* and *Ce*) belonging to the class of: 1,2,4-triazole, 1,3,4-thiadiazole or 1,3,4-oxadiazole, which have been recently reported as newly-synthesized compounds and characterized by spectral and elemental analysis. In the present study, the broth microdilution method was performed to determine the minimum inhibitory concentrations (MIC) of these compounds against the oral streptococci isolates, and afterwards, to determine their minimum bactericidal concentrations (MBC). The values of the MIC ranged between: 32-256 µg/mL for *Ca* and *Cc*, 8-256 µg/mL for *Cb*, 128-256 µg/mL for *Cd* and 64-256 µg/mL for *Ce*. The MBC/MIC ratios were less or equal to 4 in all cases. In conclusion, compared to the other 4 compounds, *Cb* exhibited the highest degree of growth inhibition against the tested strains and might be subjected to further chemical reactions in order to improve its antimicrobial activity.

Rezumat

Streptococii orali sunt bacterii comensale, însă uneori pot fi implicați în infecții ce ar necesita tratament antimicrobian. În contextul general al creșterii incidenței izolatelor rezistente la antibiotice, investigarea activității antimicrobiene a compușilor heterociclici

aparținând unor clase diferite se dovedește a prezenta un interes deosebit. Scopul prezentului studiu a fost investigarea activității antimicrobiene a 64 de izolate clinice aparținând unor specii diferite de streptococi orali în cazul a 5 compuși (notați *Ca*, *Cb*, *Cc*, *Cd* și *Ce*) din clasa: 1,2,4-triazolilor, 1,3,4-tiadiazolilor și 1,3,4-oxadiazolilor. În vederea determinării concentrațiilor minime inhibitorii (MIC) și ulterior, a concentrațiilor minime bactericide (MBC), a fost utilizată metoda microdiluției în bulion Müller-Hinton. Valorile MIC au fost cuprinse între: 32-256 μg/mL pentru *Ca* și *Cc*, 8-256 μg/mL pentru *Cb*, 128-256 μg/mL pentru *Cd* și 64-256 μg/mL pentru *Ce*. Rezultatele au indicat faptul că *Cb* a prezentat gradul cel mai înalt de inhibiție a creșterii microbiene în cazul tulpinilor testate și ar putea fi recomandat pentru a fi supus unor reacții chimice ulterioare, în scopul îmbunătățirii acțiunii antibacteriene.

Keywords: oral streptococci, heterocyclic compound, antibacterial activity

Introduction

The oral streptococci are the species within the genus *Streptococcus* that predominantly inhabit the oral cavity and the upper respiratory tract as commensals [9, 10]. Unfortunately, these streptococci may be involved alone or in association with other microorganisms in the production of different oral and non-oral diseases [5, 7, 9, 10, 11], such as: oral and maxillofacial pyogenic infections, deep-seated abscesses, infective endocarditis, ear-nose-throat infections, pulmonary infections, abdominal infections, urogenital infections, central nervous system infections, bone and joint infections etc., especially in immuno-compromised patients.

Previously, these bacteria have been considered uniformly sensitive to penicillin. At present, there are many studies which have shown that resistance to penicillin and other commonly used antimicrobial agents is increasing [5, 11]. The emergence of antibiotic resistance and multiresistance of the clinically significant isolates is a stringent health problem which stresses the necessity to continue the efforts in searching for new antimicrobial agents. Based on the data reported in the literature, the heterocyclic compounds still represent an important organic chemistry field to be explored for this purpose [1, 3, 6, 8, 12, 13, 14].

Therefore, the aim of the present study was to investigate the antibacterial activity against oral streptococcal clinical isolates in case of 5 newly synthesized heterocyclic compounds of: 1,2,4-triazole, 1,3,4-thiadiazole and 1,3,4-oxadiazole class, which have been recently reported by Bărbuceanu et al. [2] and their structures have been previously confirmed by spectral methods and elemental analysis. Thus, the first compound, the acylthiosemicabazide, was obtained by the reaction of 4-(phenylsulfonyl)-benzoic acid hydrazide with 4-fluorophenyl isothiocyanate. Afterwards, the 1,2,4-triazole and 1,3,4-thiadiazole were obtained from intramolecular

cyclization of the acylthiosemicarbazide in basic and acidic medium, respectively, while the 1,3,4-oxadiazole was synthesized by cyclodesulfurization of the same acylthiosemicarbazide in the presence of yellow mercury oxide. The treatment of 1,2,4-triazole with ethyl bromoacetate in basic medium led to the synthesis of 1,2,4-triazole S-alkylated.

Materials and Methods

The 5 heterocyclic compounds investigated for their antibacterial activity against some oral streptococcal clinical isolates were the following: 4-(4-fluorophenyl)-1-(4-(phenylsulfonyl) benzoyl)-thiosemicarbazide (*Ca*), 4-(4-fluorophenyl)-5-(4-(phenylsulfonyl) phenyl)-2*H*-1,2,4-triazole-3(4*H*)-thione (*Cb*), ethyl 2-(4-(4-fluorophenyl)-5-(4-(phenylsulfonyl) phenyl)-4*H*-1,2,4-triazol-3-ylthio)acetate(*Cc*), N-(4-fluorophenyl)-5-(4-(phenylsulfonyl) phenyl) -1,3,4- thiadiazol -2- amine (*Cd*), and N- (4-fluorophenyl) -5- (4-(phenylsulfonyl) phenyl)-1,3,4-oxadiazol-2-amine (*Ce*). The structures of these compounds have been previously characterized by elemental analysis and different spectral studies (IR, UV-VIS, ¹H-NMR, ¹³C-NMR, MS), and they have been tested for their antimicrobial activity against some type/reference strains of oral streptococci and several clinical isolates of pneumococci [2].

In the present study these compounds were investigated for their antimicrobial action against 64 clinical isolates of oral streptococci from the collection of isolates of the Microbiology Department, Faculty of Dental Medicine, University of Medicine and Pharmacy “Carol Davila”, Bucharest. All 64 strains had been isolated in 2009 from paediatric patients diagnosed with different respiratory infections (mostly in the otorhinolaryngeal sphere) at 3 hospitals in Bucharest, Romania: “Gr. Alexandrescu” Emergency Clinical Hospital for Children, “Dr. Victor Babeş” Clinical Hospital of Infectious and Tropical Diseases and “Sf. Maria” Clinical Hospital. These isolates were stored on cryo-bead tubes (AES Laboratoire, France) at -70°C, during the first stage of an exploratory research project, contract no.1136/2009, granted by the National University Research Council (CNCSIS) and the Executive Agency for Higher Education and Research Funding (UEFISCSU) from Romania. According to the results of the identification by the Rapid ID 32 STREP system, reported in an article in press [4], the isolates belonged to different species as follows: *S. oralis* - 33 strains, *S. mitis* - 17 strains, *S. sanguinis* - 9 strains, *S. parasanguinis* - 2 strains, *S. constellatus* - 2 strains and *S. anginosus* - 1 strain.

In the present study, the broth microdilution method was chosen to determine the minimum inhibitory concentration (MIC) and afterwards, the minimum bactericidal concentration (MBC) of the tested heterocyclic compounds against the isolates belonging to the 6 species of oral streptococci. Stock solutions at the concentration of 2048 µg/mL of each compound were performed in dimethyl sulfoxide (DMSO), and no antimicrobial activity could be observed when testing DMSO against the clinical isolates. The broth microdilution test was carried out in 96 wells microplates (Nunc, Denmark), starting with a series of two-fold dilutions of the compounds, from 1:2 to 1:1024, in cation-adjusted Müller-Hinton broth supplemented with 3% lysed horse blood, in a 50 µL volume per well.

The inoculum of each strain was obtained by transferring several colonies from a 24 h culture on Columbia blood agar (BA) into a tube with Müller-Hinton broth (MHB) (Sanimed International Impex, Romania) and adjusting the suspension at 0.5 McFarland standard turbidity in order to achieve a bacterial density of about 1.5×10^8 colony forming units (CFU)/mL. Afterwards, the inoculum was diluted 1:100 by transferring 100µL into a tube with 9900µL MHB in order to obtain a density of 1×10^6 CFU/mL. Aliquots of 50µL from the last dilution were added into all wells containing the tested compounds and into the well representing the positive growth control, which already contained 50µL of MHB without any compound. The final microbial density became in each well 5×10^5 CFU/mL. The negative growth control (the sterility control) well was filled in only with 100µL MHB. The final liquid volume in every well was also 100µL.

An inoculum control was done by removing 10 µL from each growth control well (just after adding the bacterial inoculum within the well), diluting them into 10 mL MHB and spreading of 100µL of this dilution onto BA plates. Both the inoculum control plates and the microplates sealed with sterile adhesive sheets and covered with proper lids were incubated at 37°C for 24 h. The microbial growth in the wells containing the tested compounds was examined macroscopically and compared regarding the aspect with the positive and negative growth control.

The MIC was considered the lowest concentration of the compound which inhibited the visible microbial growth (showing neither turbidity nor growth button). For the determination of MBC, 10 µL were taken away from the wells with no visible microbial growth and also from the negative and positive growth control, and were applied in spots onto BA plates through an electronic pipette. After an incubation period of 48h in a 5% CO₂ atmosphere at 37°C, the MBC was considered the lowest concentration of

the respective compound able to kill at least 99.9% of the bacterial amount measured previously in the inoculum control plate.

Streptococcus pneumoniae ATCC 49619 was used as quality control and the MIC of amoxicillin was tested against this reference strain using the same broth microdilution method.

Results and Discussion

The minimum and maximum values of MIC (MIC_{min} and MIC_{max} , respectively) of the 5 heterocyclic compounds tested against the oral streptococcal isolates belonging to 6 different species are presented in Table I. As it can be seen, the MIC values ranged between: 32-256 $\mu\text{g/mL}$ for *Ca* and *Cc*, 8-256 $\mu\text{g/mL}$ for *Cb*, 128-256 $\mu\text{g/mL}$ for *Cd* and 64-256 $\mu\text{g/mL}$ for *Ce*.

Table I

The range of the minimum inhibitory concentration (MIC) values of the 5 heterocyclic compounds against 64 oral streptococcal clinical isolates

Compound	$MIC_{min}^* - MIC_{max}^{**} (\mu\text{g/mL})$					
	<i>S. oralis</i> (33 isolates)	<i>S. mitis</i> (17 isolates)	<i>S. sanguinis</i> (9 isolates)	<i>S. parasanguinis</i> (2 isolates)	<i>S. constellatus</i> (2 isolates)	<i>S. anginosus</i> (1 isolate)
<i>Ca</i>	64 - 256	64 - 256	32 - 256	64 - 256	32 - 64	256
<i>Cb</i>	8 - 256	8 - 256	8 - 128	32 - 256	16 - 64	256
<i>Cc</i>	64 - 256	64 - 256	64 - 256	64 - 256	32 - 128	256
<i>Cd</i>	128 - 256	128 - 256	128 - 256	128 - 256	128 - 256	256
<i>Ce</i>	128 - 256	128 - 256	64 - 256	128 - 256	128 - 256	256

* MIC_{min} = the minimum value of MIC; ** MIC_{max} = the maximum value of MIC

The minimum and maximum values of the MBC (MBC_{min} and MBC_{max} , respectively) of the same heterocyclic compounds are presented in Table II. The MBC values ranged between: 32-512 $\mu\text{g/mL}$ for *Ca* and *Cb*, 64-512 $\mu\text{g/mL}$ for *Cc* and 128-512 $\mu\text{g/mL}$ for *Cd* and *Ce*.

Table II

The range of the minimum bactericidal concentration (MBC) values of the 5 heterocyclic compounds against 64 oral streptococcal clinical isolates

Compound	$MBC_{min}^* - MBC_{max}^{**} (\mu\text{g/mL})$					
	<i>S. oralis</i> (33 isolates)	<i>S. mitis</i> (17 isolates)	<i>S. sanguinis</i> (9 isolates)	<i>S. parasanguinis</i> (2 isolates)	<i>S. constellatus</i> (2 isolates)	<i>S. anginosus</i> (1 isolate)
<i>Ca</i>	64 - 512	128 - 512	32 - 512	128 - 512	64 - 128	512
<i>Cb</i>	32 - 256	32 - 256	32 - 128	32 - 256	32 - 128	512
<i>Cc</i>	128 - 512	64 - 512	64 - 512	128 - 512	64 - 128	512
<i>Cd</i>	128 - 512	128 - 512	128 - 512	256 - 512	256 - 512	512
<i>Ce</i>	128 - 512	128 - 512	128 - 512	256 - 512	256 - 256	512

* MBC_{min} = the minimum value of MBC; ** MBC_{max} = the maximum value of MBC

Of the tested compounds, *Cb* presented the best growth inhibition activity against all isolates, except for the single strain of *S. anginosus*, when the same MIC and MBC values were obtained for all 5 compounds: 256 µg/mL and 512 µg/mL, respectively.

For all species represented by more than 2 isolates (*S. oralis*, *S. mitis* and *S. sanguinis*), the concentrations of *Ca*, *Cb* and *Cc* which inhibited 50% of the isolates (MIC₅₀, equivalent to the medium value of MIC) was 32 µg/mL, except for *Cb* against *S. sanguinis* (MIC₅₀ of 16 µg/mL), while the MIC₅₀ of *Cd* and *Ce* was 128 µg/mL. The concentration which killed 50% of the isolates (MBC₅₀, equivalent to the medium value of MBC) was of: 64 µg/mL for *Cb*, 128 µg/mL for *Ca* and *Cc* and 256 µg/mL *Ce*. Regarding the *Ca*, *Cb* and *Cc* concentrations which inhibited or killed 90% of the isolates (MIC₉₀ and MBC₉₀, that corresponded to the 90th percentile of MIC and MBC distributions, respectively) the values found against the 3 above mentioned species were of 64 µg/mL and 128 µg/mL, respectively. The value for both MIC₉₀ and MBC₉₀ found for *Cd* and *Ce* against *S. oralis*, *S. mitis* and *S. sanguinis* was 256 µg/mL.

As it can be seen, the alkylation of *Cb* failed to improve the antimicrobial effect of *Cc*. However, the latter compound behaved similarly to compound *Ca* against the isolates of *S. oralis*, *S. mitis*, *S. parasanguinis* and *S. anginosus*, concerning the degree of bacterial growth inhibition. In contrast, *Cd* and *Ce* showed by far the lowest antibacterial action against the tested isolates.

The MBC/MIC ratio was less or equal to 4 for every compound tested against all isolates.

Conclusions

The results of this study indicated that acylthiosemicarbazide (compound *Ca*) and S-alkylated 1,2,4-triazole derivative (*Cc*) showed a better growth inhibition action compared to the thiadiazole (*Cd*) and oxadiazole (*Ce*) derivatives. However, the highest degree of antibacterial activity against all oral streptococcal isolates, except for *S. anginosus* strains, was found in the case of 1,2,4-triazole-3(4*H*)-thione (*Cb*).

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