

ANTIMICROBIAL AND ANTI-PATHOGENIC ACTIVITY EVALUATION OF SOME 2-(TRIMETHOXYPHENYL)-4-AR₁-5-R₂-THIAZOLES

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Abstract

A series of 9 compounds with a 2-(3,4,5-trimethoxyphenyl) thiazolyl structure were tested in order to determine their antimicrobial potential, using a qualitative screening method followed by a quantitative assay in order to determine the antimicrobial activity. The anti-pathogenic activity was investigated by the study of anti-biofilm activity of the obtained molecules. The tested substances showed a low to moderate microbicidal activity, but a good anti-biofilm activity.

Rezumat

O serie de 9 compuși cu structură 2-(3,4,5-trimetoxifenil) tiazolică au fost testați pentru stabilirea potențialului antimicrobian, prin teste de *screening* calitative, urmate de o evaluare cantitativă a activității antimicrobiene. Activitatea anti-patogenică a fost evaluată prin studiul influenței moleculelor testate asupra dezvoltării biofilmului microbian. Rezultatele au arătat pentru compușii nou sintetizați o acțiune antimicrobiană modestă, dar o bună acțiune anti-biofilm.

Keywords: antimicrobial; anti-biofilm; aryl-thiazoles

Introduction

Antibiotic resistance is an increasingly concerning issue for the medical and scientific community, the need for new powerful antibiotic agents, especially those active against multidrug resistant bacteria, being strongly acknowledged [5, 12, 15]. Considering that the presence of the thiazole nucleus in the structures of a series of known antibacterial agents (e.g., ceftriaxone, ceftazidime, cefixime, aztreonam) is required for their antimicrobial activity [4], we have focused on obtaining new antimicrobial agents bearing this ring. Following our research group's past interest for synthesizing innovative molecules containing the thiazole nucleus [1, 2, 13] we decided to investigate the influence of the 3,4,5-trimethoxyphenyl moiety on the spectrum and intensity of the antibacterial activity, directed especially towards microbial biofilm development followed by the inert substratum colonization. Also, we aimed to compare how the removing of a thiazole nucleus from the structures of the previously assessed series of 4,2 and 5,2 bisthiazoles [2] would influence the antimicrobial activity.

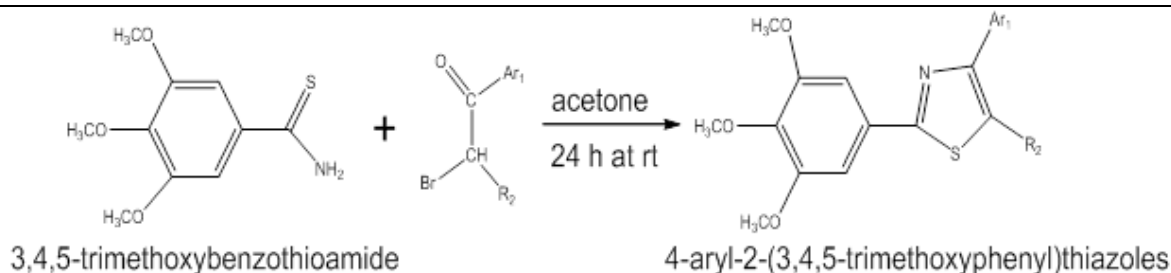
The potential antimicrobial activity was evaluated by using both qualitative and quantitative assays. Also, a special focus was given to the evaluation of bacterial adherence inhibition as a possible mean to reduce the burden of biofilm associated infections [7-9, 14, 16].

Materials and Methods

Chemistry

All 9 compounds have a 2-(3,4,5-trimethoxyphenyl)thiazolyl structure and were synthesized by our team [1] through the Hantzsch condensation of the 3,4,5-trimethoxybenzothioamide, with various α -bromo-ketones, as presented in Figure 1. As shown in our previous work, all new compounds were characterized using IR, ¹H-RMN, mass spectra and elemental analysis, in order to confirm the assumed structures (see Table I) [1].

Compounds were referred to as **MTZ 1-9** as shown in Table I. All compounds have the 4-aryl-2-(3,4,5-trimethoxyphenyl)thiazole scaffold with various substituents on the 4-aryl nucleus. The compound **MTZ1** is the only one bearing in the 5 position of the thiazole ring (R₂) a methyl substituent.

**Figure 1.**

The general reaction for the synthesis of the 2-(3,4,5-trimethoxyphenyl) thiazoles as described in our previous paper [1].

Table I

The chemical structures of the tested compounds as described in reference [1].

Compound	Ar ₁	R ₂
MTZ1		-CH ₃
MTZ2		H
MTZ3		H
MTZ4		H
MTZ5		H
MTZ6		H
MTZ7		H
MTZ8		H
MTZ9		H

Antimicrobial activity evaluation

The antimicrobial activity was tested on the following microbial strains: *Enterococcus faecium* E5, *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6683, *Pseudomonas aeruginosa* ATCC 27857, *Klebsiella pneumoniae* IC 13420, *Escherichia coli* ATCC 25922, *Candida albicans* 393. These strains are either reference strains (those bearing the ATCC indicative) or recently isolated strains from clinical specimens. The VITEK 1 automatic system was used to identify the microbial strains. All determinations were performed in accordance with the manufacturer's recommendations. The

results were interpreted using the AMS RO9.1 software [3].

The qualitative screening of the antimicrobial activity

The qualitative antimicrobial activity screening was performed by an adapted disk diffusion technique [6, 17]. Petri dishes containing the solid Muller-Hinton medium were seeded with a saline suspension of 0.5 McFarland microbial density, obtained from 15-18 h cultures on a solid medium. An amount of 5 μ L of the chemical compounds, previously solubilised in dimethyl sulfoxide (DMSO) to a concentration of 10 mg/mL, was spotted directly on the solid medium seeded with different microbial *inocula*. The resulting plates were left at room temperature for 20-30 minutes and then were incubated for 24 h at 37°C. The occurrence of a growth inhibition area, surrounding the spot, was interpreted as an antibacterial effect. The diameters of the inhibition zones were measured and expressed in mm.

Quantitative assay of the antimicrobial activity

The quantitative assay was performed by using a serial binary micro-dilution method [10, 11, 18]. Multi-well plates were filled with liquid Mueller-Hinton medium. Serial binary dilutions of the tested compounds, ranging from 1 to 0.015 mg/mL, were performed in a total volume of 200 μ L liquid medium. All measurements were performed using both a positive control (wells containing the culture medium and the microbial inocula) and a set of blanks (wells containing only the culture medium). A volume of 20 μ L of 0.5 McFarland microbial inocula suspension, prepared in sterile saline solution using 24 h bacterial cultures developed on a solid medium was added to each well, excepting the negative control.

The plates were incubated for 24 h at 37°C, and then subjected to macroscopic evaluation. The concentration of the tested chemical compounds from the last well that did not show the signs of culture growth (the culture medium remained clear) was considered the Minimum Inhibitory Concentration (MIC) for that molecule.

The study of the influence of the tested molecules against microbial biofilm development and inert substratum colonization

The anti-biofilm properties were measured by the biofilm microtiter method [6, 10, 11, 18]. This measurement was applied only for some of the initial compounds, which have shown a good antimicrobial activity in the preliminary qualitative assay. The microbial cells were incubated for 24 h at 37°C for bacterial strains and 48 h at 28°C for fungal strains, in the presence of two fold dilutions of the tested compounds performed in nutrient broth. The 96 wells plates were subsequently emptied and washed 3 times with phosphate buffered saline and then fixed with cold methanol 80%.

In order to stain the cells a violet crystal solution was added in every well and allowed to act for 30 minutes. Afterwards, the staining solution was removed by several water rinses.

The biofilm formed on the plastic plates was resuspended in a 33% acetic acid solution. The intensity of the stained suspension was assessed by measuring its absorbance at 490 nm. The smallest concentration of the tested compound that inhibited biofilm development was considered the minimum biofilm eradicating concentration (MBEC), expressed in mg/mL.

Results and Discussion

The qualitative screening of the antimicrobial activity

The qualitative screening can be considered a preliminary investigation, as these results usually offer a good insight into the antimicrobial spectrum of a compound, but limited information about the potency is provided.

A typical result obtained through the adapted disk diffusion technique is presented in Figure 2.

The results for the initial qualitative screening of the antimicrobial activity are shown in Table II. The new compounds exhibited different antimicrobial

activities. For most of them, the activity spectrum included gram negative (*P. aeruginosa* ATCC 27857, *E. coli* ATCC 25922), gram positive (*B. subtilis* ATCC 6683) bacterial and the fungal *C. albicans* 393 strains.

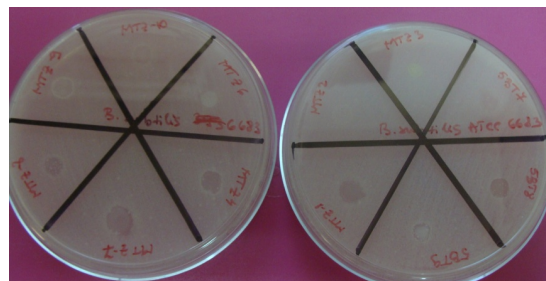


Figure 2.

Qualitative screening of the antimicrobial against *B. subtilis* ATCC 6683 – adapted disk diffusion technique

The compounds **MTZ3**, **MTZ5**, **MTZ6**, **MTZ7** expressed the widest antimicrobial spectrum, including gram negative and gram positive bacterial, as well as fungal strains.

All tested compounds have been active against *Bacillus subtilis* 6683 and *Candida albicans* 393 strains.

The tested compounds were less effective on the gram positive cocci, the **MTZ5** being the only active compound against *Enterococcus faecium* E5, and in case of *Staphylococcus aureus* ATCC 6538, only the **MTZ1**, **MTZ3** and **MTZ5** compounds proved to be active.

The **MTZ5** compound proved to have the largest antimicrobial spectrum, being active against all tested strains.

Table II

The diameters (expressed in mm) of the growth inhibition zones induced by different compounds on the bacterial and fungal strains

Microbial strains Chemical Compound	<i>Enterococcus faecium</i> E5	<i>Staphylococcus aureus</i> ATCC 6538	<i>Bacillus subtilis</i> ATCC 6683	<i>Pseudomonas aeruginosa</i> ATCC 27857	<i>Klebsiella pneumoniae</i> IC 13420	<i>Escherichia coli</i> ATCC 25922	<i>Candida albicans</i> 393
MTZ1	0	6	7	0	0	0	7
MTZ2	0	0	6	0	0	0	7
MTZ3	0	4	4	5	4	5	4
MTZ4	0	0	6	6	4	0	6
MTZ5	6	6	4	6	6	6	4
MTZ6	0	0	8	7	6	6	7
MTZ7	0	0	7	6	6	8	6
MTZ8	0	0	7	6	4	8	6
MTZ9	0	0	6	6	0	0	6
DMSO	0	0	0	0	0	0	0

Quantitative assay of the antimicrobial activity

The results of minimum inhibitory concentrations (MIC) assay are presented in Table III. They show a low antibacterial activity for the tested compounds, with MIC equal or higher than 1 mg/mL. As

exceptions, the only significant antimicrobial action was PROVEN for compounds **MTZ1** against the fungal strain *C. albicans* 393, **MTZ5** against *K. pneumoniae* IC 13420, and **MTZ7** against *E. coli* ATCC 25922, with MIC 500 µg/mL.

Table III

The minimum inhibitory concentrations (mg/mL) obtained from the quantitative antimicrobial assay

Microbial strains Chemical Compound	<i>Enterococcus faecium</i> E5	<i>Staphylococcus aureus</i> ATCC 6538	<i>Pseudomonas aeruginosa</i> ATCC 27857	<i>Klebsiella pneumoniae</i> IC 13420	<i>Escherichia coli</i> ATCC 25922	<i>Candida albicans</i> 393	<i>Bacillus subtilis</i> ATCC 6683
MTZ1	-	>1	-	-	-	0.5	>1
MTZ2	-	-	-	-	-	1	>1
MTZ3	-	-	>1	-	>1	-	-
MTZ4	-	-	>1	-	-	>1	>1
MTZ5	>1	>1	>1	0.5	>1	-	-
MTZ6	-	-	>1	>1	>1	1	>1
MTZ7	-	-	>1	>1	0.5	>1	>1
MTZ8	-	-	>1	-	>1	>1	>1
MTZ9	-	-	>1	-	-	>1	>1

The influence of the tested molecules against microbial biofilm development and inert substrate colonization

Microbial biofilm formation is due to the ability of the different microorganisms to adhere to different substrata, represented either by eukaryotic cells or by inanimate surfaces. This dynamic process is considered a virulence factor. All substances that are able to inhibit the microbial adherence, and thus the biofilm formation, will also render harmless the microbial strains. By blocking biofilm formation, the substratum colonization is also blocked and thus the evolution of the infectious process is stopped in its initial phase.

Our assays aimed at evaluating the ability of the tested compounds to inhibit biofilm formation. The

minimum biofilm eradicating concentrations (MBEC) are presented in Table IV. The obtained results showed that the inhibitory effect on microbial adhesins synthesis was expressed against a diverse spectrum of microbial strains, at concentrations as low as 0.062 mg/mL.

The most active antibiofilm compound proved to be **MTZ5** with a MBEC=0.062 mg/mL against *Staphylococcus aureus* ATCC 6538. However, this significant antibiofilm activity does not correlate with a good MIC in the antimicrobial quantitative test (MIC>1mg/mL). This suggests that **MTZ5** could be further developed only as an adjuvant molecule for supporting the antibiotic therapy.

Table IV

The MBEC (mg/mL) obtained from the quantitative assay of the antibiofilm activity

Microbial strains Chemical Compound	<i>Enterococcus faecium</i> E5	<i>Staphylococcus aureus</i> ATCC 6538	<i>Bacillus subtilis</i> ATCC 6683	<i>Pseudomonas aeruginosa</i> ATCC 27857	<i>Klebsiella pneumoniae</i> IC 13420	<i>Escherichia coli</i> ATCC 25922	<i>Candida albicans</i> 393
MTZ1	-	>1	0.5	-	-	-	0.5
MTZ2	-	-	>1	-	-	-	1
MTZ3	-	-	-	>1	-	>1	-
MTZ4	-	-	0.25	>1	-	-	>1
MTZ5	>1	0.062	-	>1	0.5	>1	-
MTZ6	-	-	0.25	>1	>1	>1	1
MTZ7	-	-	>1	1	>1	0.125	>1
MTZ8	-	-	>1	1	-	>1	>1
MTZ9	-	-	>1	>1	-	-	>1

The tested molecules showed a good antibiofilm activity MBEC especially against *Bacillus subtilis* ATCC 6683 (**MTZ1**, **MTZ4**, **MTZ6**) with MBEC values of 0.25-0.5 mg/mL. However, this activity did not correlate with a similar profile in the previous quantitative assay.

MTZ7 showed a good inhibition of *Escherichia coli* ATCC 25922 adhesins synthesis (MBEC=0.125 mg/mL) that also correlated with a moderate antimicrobial activity (MIC=0.5 mg/mL). The only compound active against the biofilm formed by the fungal tested strain remains **MTZ1** with identical MIC and MBEC values. Similarly, **MTZ5** was the only compound active against *Klebsiella pneumoniae* IC 13420 with a MIC and MBEC of 0.5 mg/mL.

When compared with structurally related compounds [3, 4], the presence of the 3,4,5-trimethoxyphenyl moiety does not seem to have a specific influence on the antimicrobial activity of the respective compounds. However, it is apparent that this moiety could be responsible for the increase in the antibiofilm activity of these molecules, when compared with the previously assessed bisthiazoles molecules [4]. When comparing the related 4,2 and 5,2 bisthiazole molecules with the current series, a mild reduction in antibacterial activity against *Pseudomonas aeruginosa* ATCC 27857 and *Escherichia coli* ATCC 25922 has occurred. This could be explained by the elimination of one of the 2 thiazole rings from the structures of the synthesized compounds.

Conclusions

A total of 9 molecules bearing the 4-aryl-2-(3,4,5-trimethoxyphenyl) thiazole moiety were investigated for their antimicrobial and anti-pathogenic activity. The tested molecules showed a low to moderate antimicrobial activity, but a good anti-biofilm effect, in a wide range of concentrations. These results are encouraging for the further investigation of some of the tested compounds for the development of new anti-biofilm molecules and surfaces.

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