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In Vitro Antimicrobial Activity of New 3-Substituted 5H-(1,2,4)Triazolo(3',4':2,3) (1,3,4)Thiadiazino(5,6-B)Quinoxaline Derivatives

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ABSTRACT

Background and objectives: Antimicrobial resistance is a serious threat to global public health. The overuse and misuse of antibiotics are the most important contributing factors to development of antibiotic resistance. Thus, there is an urgent need to identify and discover new compounds against drug-resistant microorganisms. We have previously synthesized new series of 3-substituted 5H-(1,2,4)triazolo(3',4':2,3) (1,3,4)thiadiazino(5,6-b)quinoxaline derivatives (4a-4f). Here, we evaluate the antimicrobial activity of these derivatives against methicillin-resistant *Staphylococcus aureus*, *S. aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*, *Candida tropicalis* and *Candida krusei*.

Methods: The agar well diffusion and agar dilution methods were used for determining inhibition zone diameter and minimum inhibitory concentration during preliminary evaluation of antimicrobial activity.

Results: All synthesized compounds exhibited antibacterial and antifungal activity against the tested microorganisms.

Conclusion: Our findings indicate the antimicrobial potential of the six novel synthetic triazolo thiadiazin quinoxaline compounds.

Keywords: Antimicrobial, Anti-bacterial agents, Antifungal agents, Triazolo, Thiadiazin, Quinoxaline.

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INTRODUCTION

Antibiotics are powerful drugs that are used to treat infectious diseases. Resistance of pathogenic bacteria to antibiotics and the subsequent treatment difficulty are important global health issues. Therefore, discovering new classes of antimicrobial compounds is urgently needed to overcome the growing threat of drug-resistant bacterial and fungal infections. For this purpose, a combination of different active components of a molecule can be employed through which each drug moiety is designed to bind independently to different biological targets and synchronously accumulate at both target sites (1). Among different classes of nitrogen-containing heterocyclic compounds, quinoxaline and triazolo are well known for their broad antibacterial (2,3), antifungal (4,5), anti-mycobacterial (6,7), antiviral (8,9), anti-inflammatory (10,11), anticancer (12,13) and antimalarial (14,15) properties. In our previous study, we synthesized 3-substituted 5H-(1,2,4)triazolo(3',4':2,3) (1,3,4)thiadiazino(5,6-b)quinoxaline derivatives under solvent-free conditions (16). Since the synthesis and evaluation of antimicrobial activity are important parts of our research program (15,16), herein, we evaluated antibacterial and antifungal activity of these derivatives against some Gram-positive (*Staphylococcus aureus*, *Enterococcus faecalis* and methicillin-resistant *S. aureus*) and Gram-negative (*Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis* and *Klebsiella pneumoniae*) bacteria as well as some fungi (*Candida albicans, Candida tropicalis, Candida krusei*).

MATERIALS AND METHODS

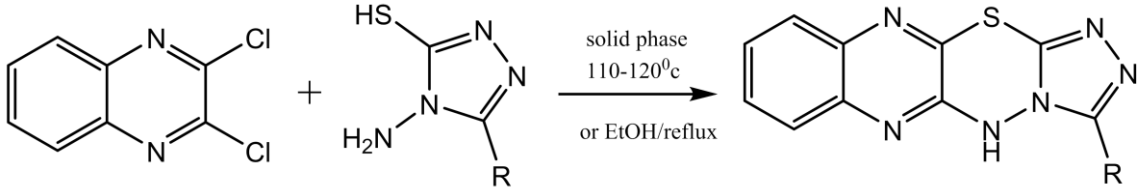
First, 3-alkyl-5H-(1,2,4)triazolo(3',4':2,3)(1,3,4)thiadiazino(5,6-b)quinoxaline derivatives were synthesized through the reaction of 4-amino-5-alkyl-4H-1,2,4-triazole-3-thiol with 2,3-dichloroquinoxaline under solvent-free conditions in moderate yields or refluxing in EtOH in the presence of K_2CO_3 in excellent yields (Scheme1) (16).

The antibacterial activity of the synthesized compounds (1 mg/ mL dissolved in dimethyl sulfoxide) against *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *E. faecalis* ATCC 29212, *K. pneumoniae* ATCC 7881, *S. aureus* ATCC 25923 and methicillin-resistant *S. aureus* ATCC 700698 was evaluated by the agar diffusion method (17, 18). The cultures of the mentioned bacteria were inoculated separately on the surface of Mueller Hinton agar (Merck, Germany) plates using a sterile cotton swab. Susceptibility to the synthesized compounds was tested using the agar well diffusion method (18). For this purpose, 6-mm diameter wells were created on the agar using a sterile glass borer. Then, about 70 µl of the compounds solution were poured into the wells. Ceftizoxime and ciprofloxacin (Sigma Aldrich, USA) were used as standard antibiotics, while dimethyl sulfoxide was used as the solvent control. The plates were incubated at 37 °C for 24 h, and the diameter of inhibition zone (mm) was measured. Results were reported as mean of duplicate data.

Determining minimum inhibitory concentration (MIC)

Minimum inhibitory concentration of the compounds was determined using the two-fold serial dilution agar method.

Scheme 1- Synthesis of 3-substituted 5H-(1,2,4)triazolo(3',4':2,3)(1,3,4) thiadiazino(5,6-b)quinoxalines.



4a:R=Furan, 4b: R=Butyl, 4c: R=Ethyl, 4d: R=H, 4e:R= Propyl, 4f: R=Methyl

Concentrations of 31.5, 62.6, 125, 250 and 500 µg/ml were prepared in sterile molten Muller Hinton agar from the stock solution. The microplates were inoculated with approximately 1.5×10^6 CFU/mL of bacterial suspension at 37 °C for 24 h. The MIC was defined as the lowest concentration of the compounds that prevented visible growth of microorganisms after overnight incubation. The results were compared with ceftizoxime and ciprofloxacin as standard antibacterial agents. The well diffusion test was performed using Sabouraud dextrose agar (Merck, Germany).

Yeasts suspension was made and adjusted to turbidity equivalent to a 0.5 McFarland standard (1.5×10^6 CFU/mL). The inoculated agar was poured into the assay plate and allowed to cool down on a leveled surface. Once the medium solidified, wells were created on the agar, and 70 µl of the antifungal agents were poured into each well. After adding the synthesized compounds, the plate was incubated at 35 ^{o}C for 48 h (19). Antifungal susceptibility test was performed by the agar dilution method. Stock solutions of triazolo derivatives were prepared at concentration of 5 mg/ml in dimethyl sulfoxide. Next, 1.6 ml of molten Sabouraud dextrose agar was poured into sterilemicroplates and allowed to cool to 50 °C. Then, 0.4 ml of dilutions prepared from the stock solutions of the drugs was added in descending order of concentration. In addition, 10 µl of the standardized fungal inoculum were added to all microplates except for the sterility control. The microplates were incubated at 35 °C for 7 days and visualized macroscopically for growth. The lowest concentration that inhibited growth of fungi was defined as the MIC. To determine the minimum fungicidal concentration (MFC) of the synthesized compounds, a loopful was taken from the MIC tubes and streaked on Sabouraud dextrose agar. Growth was observed after incubation at 37 ºC for 24 h. The lowest concentration at which no growth was observed was determined as the MFC (20).

All experiments were performed in duplicate and results were reported as mean ± standard deviation.

RESULTS

The antibacterial activity of the novel series of 3-alkyl-5H-(1,2,4) triazolo (3',4':2,3)(1,3,4)thiadiazino(5,6-b)quinoxaline derivatives is shown in table 1 and figure 1.

Figure 1- Inhibition zones of the synthesized compounds (1 mg/ml) against *E. coli* and methicillin-resistant *S. aureus*

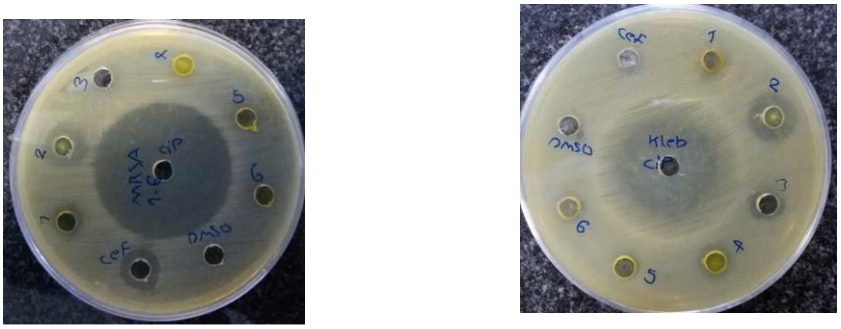


Table 1- Minimum inhibitory concentrations (µg/mL) and inhibition zone diameter (1mg/ml) of the synthesized compounds against the tested bacteria

	MIC(IZ)					
Compounds	*Staphylococcs uaureus*	*Escherichi coli*	*MRSA*	*Pseudomons aeruginosa*	*Enterococcs faecalis*	*Klebsiella pneumonae*
4a	1000 (12≥)	250 (13)	(12≥)	500 (12≥)	1000(12≥)	1000(12≥)
4b	500 (13)	250 (13)	500 (14)	500 (12≥)	1000 (12≥)	500 (15)
4c	1000 (12≥)	250 (13)	1000 (12≥)	500 (12≥)	1000 (12≥)	1000 (12≥)
4d	1000 (12≥)	1000 (12≥)	1000 (12≥)	500 (12≥)	1000 (12≥)	1000 (12≥)
4e	1000 (12≥)	1000 (12≥)	1000 (12≥)	500 (12≥)	1000 (12≥)	1000 (12≥)
4f	1000 (12≥)	1000 (12≥)	1000 (12≥)	500 (12≥)	1000 (12≥)	1000 (12≥)
Ceftizoxim	125 (25)	100 (16)	1000 (12≥)	500 (14)	100 (12≥)	100 (12≥)
Ciprofloxacin	0/07 (40)	15 (45)	125 0/07 (7)	1/5 (42)	35(35)	35 (36)

All tested compounds exhibited some antibacterial activity. Based on the inhibition zone diameter measured for the stock solution, compound 4b had the highest antimicrobial activity against Gram-negative and Gram-positive bacteria. Compounds 4a and 4c showed moderate antibacterial activity while compounds 4d, 4e and 4f showed less antibacterial activity. The antibacterial activity of all synthesized compounds was weaker than that of ciprofloxacin and ceftizoxim. The antifungal activity of the tested compounds was compared with that of fluconazole (Table 2 and Figure 2).

As shown in table 2, all synthesized compounds exhibited significant antifungal activity against *C. albicans*, *C. tropicalis* and *C. glabrata* with inhibition zone diameters ranging from 15 to 17 mm. However, the MICs of the synthesized compounds were lower than the MIC obtained for fluconazole. The MFC of the synthesized compounds was similar or two-fold higher than the corresponding MIC values.

Figure 2- Inhibition zone of the synthesized compounds (1 mg/ml) against *C. tropicali* and *C. glabrata*

Table 2- Minimum inhibitory concentrations (µg/mL) and inhibition zone diameter (1mg/ml) of the synthesized compounds against the tested fungi

	MIC (IZ)		
Compounds	*Candida tropicalis*	*Candida albicans*	*Candida glabrata*
4a	500 (17)	500 (15)	500 (17)
4b	1000 (15)	500 (14)	1000 (15)
4c	1000 (15)	1000 (12)	1000 (15)
4d	1000 (15)	1000 (12)	1000 (15)
4e	500 (15)	1000 (12)	1000 (15)
4f	1000 (15)	1000 (12)	1000 (15)
Flucanazole	200(15)	100 (17)	200 (15)

MIC= Minimum Inhibitory Concentration
IZ= Mean inhibition zone of derivatives in 1mg/ml concentration.

DISCUSSION

The objective of this study was to evaluate the in vitro antimicrobial activity of quinoxaline derivatives against some pathogenic bacteria and fungi. In recent years, a number of new 1,2,3-triazole quinoline analogues have been introduced as antimicrobial agents. The main modifications on these compounds were made on the 6-fluoro-carboxylic acid groups. A study reported that 1, 2, 3-triazole appended quinoline derivatives with electron withdrawing substituent can exhibit significant antimicrobial activity (21). In our investigation, 3-(butyl -2-yl)-5H-(1,2,4) triazolo (3',4':2,3) (1,3,4) thiadiazino (5,6-b) quinoxalines (4b), was the most active antifungal compound with inhibition zone diameters similar or comparable to that of the reference drugs. This may be due to the presence of butyl substituents (longer side chain of a compound) with a triazol moiety. Indeed, increasing the side chains length will improve antimicrobial properties of a compound. These findings are in line with findings of a study by Chil et al. on triazolo scaffold (22). In our study, the newly synthesized quinoxaline derivatives showed inhibitory properties against *P. aeruginosa*. However, the rest of the tested compounds did not show inhibitory properties against Gram-positive bacteria. Nevertheless, they showed moderate inhibitory effects on the tested

fungal strains. In our previous study (23), the presence of a naphthol ring combined triazol moiety caused a significant increase in the antibacterial and antifungal properties. In the present study, the presence of quinoxaline ring combined triazol moiety caused a considerable decrease in the antimicrobial potential of the compound.

Therefore, it can be inferred that in similar structures the presence of a naphthol ring enhances the antimicrobial activity.

CONCLUSION

Based on our findings, the synthesized compounds could be utilized in designing more potent antimicrobial agents for therapeutic applications. Further studies are required to assess cytotoxicity of such compounds.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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