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ORIGINAL ARTICLE

Investigation of Antimicrobial Activity of Some Ethylparaben Hydrazide-Hydrazone Derivatives

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ABSTRACT

Objectives: The development of antimicrobial molecules discussed with considerable achievement over the past decades provided many classes of semisynthetic or synthetic compounds. Resistance to many antimicrobial agents requires the discovery of novel molecules.

Materials and Methods: In this study, ten ethylparaben hydrazide-hydrazone derivatives, the previously reported, were evaluated for their *in vitro* antibacterial and antifungal activities. The microbroth dilution method was used for the determination of the minimum inhibitory concentration (MIC) values of the novel molecules.

Results: The antimicrobial activities of the molecules were found in a wide range with MIC values of 2-256 µg/mL. The synthesized compounds showed good to moderate antimicrobial activity compared with the standards. Among the synthesized molecules, compound 3g showed the best antimicrobial activity at 2 µg/mL against *Staphylococcus aureus* strain (ATCC 29213).

Conclusion: Ethylparaben hydrazide-hydrazone compounds in our study were found to have antimicrobial activities. Ethylparaben is currently used as an antibacterial agent and preservative for preparations. These studies are necessary since they detect the relationship between the substitutions and activity.

Key words: Antimicrobial activity, ethylparaben, hydrazide-hydrazone, microbroth dilution method, *in vitro*

INTRODUCTION

Since the beginning of the last century, various antimicrobial molecules have been systematically introduced for use, both experimentally and by trial and error. Because of the exceptional genetic plasticity of the microorganisms, misuse, and world population, resistance to bacterial strains has appeared and has radiated throughout the world.[1] Today, antibiotic resistance has become a big clinical and public health problem and resistance rates are climbing dangerously worldwide. Meanwhile, minimizing toxicity and development of drug resistance, an optimal antimicrobial dose ensures enough drugs to access a clinical response. Better methods to pursue and rapidly adjust antimicrobial dosing must understand, although current approaches to antimicrobial dose optimization address fixed variability.[2] Resulting in high morbidity and mortality reports, the antibiotic treatment diversity is restricted for existing hard-to-treat multidrug-resistant bacterial infections.[3] For human and veterinary pathogens, antibiotic-resistant genes constituting the environmental "resistome" get transferred.[4] On developing new antimicrobial drugs, all scientists, governments, health sectors, and societies must take the necessary precautions and support investigations.

Hydrazide-hydrazone compounds are molecules that result in the formation of a Schiff base on the structure by the reaction of hydrazides with various aldehydes and ketones. It is known that hydrazide-hydrazones have various pharmacological activities.[5] According to the literature above, it was shown that hydrazide-hydrazone compounds have antimicrobial activity.[6-12]

Meanwhile, we tested our compounds for their antimicrobial activity. In this study, ten ethylparaben hydrazide-hydrazone derivatives, previously reported, were tested for their antibacterial and antifungal activity using various microorganism strains.

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MATERIALS AND METHODS

Antimicrobial activity tests

The synthesized ten compounds were investigated for their potential antimicrobial activities against *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922) (Gram-positive and Gram-negative bacteria, respectively), *Candida albicans* (ATCC 10231) (fungus), and the clinical isolates of these microorganisms. The study was conducted according to the Clinical Laboratory Standards Institute (CLSI) M100-S28 protocol for bacteria[13] and CLSI M27-A3 protocol for fungi.[14] In the study, cation-adjusted Mueller Hinton Broth and RPMI-1640 media were used for the determination of potential antibacterial and antifungal activities, respectively.

The compunds (1 mg) were dissolved in 0.976 mL of 10% dimethyl sulfoxide (DMSO) with a final concentration of 1024 µg/mL and the serial dilutions of each compound in the range of 2-512 µg/mL were prepared in 96 well microplates, after placing broth medium in each well. The suspension of each microorganism was prepared using McFarland: 0.5 standard and as result, 10^5 cfu/mL densities were reached. Microplates containing bacteria and fungus were incubated for 16-20 hours at 37°C and 48 h at 35°C, respectively. The reference antimicrobials were tested against these microorganisms at the same time. Besides, growth control of microorganisms and sterilization control of the media were tested. Antimicrobial activity of DMSO, which was used as a solvent in the study, was also tested. The wells with the lowest concentration with no microbial growth were determined as the minimum inhibition concentrations (MIC). The detection was made by visual evaluation using dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT).[15] Each test was repeated 3 times.

Chemistry

Synthesis of all compounds was reported in our previous study.[16] The chemical route for the synthesis of compounds is shown in Figure 1.

RESULTS AND DISCUSSIONS

Several ethylparaben hydrazide-hydrazone derivatives (3a-j) were synthesized and characterized (Figure 1 and Table 1). These compounds were previously studied and evaluated for their anticancer activity. In this study, all compounds were evaluated for antimicrobial activity.

The minimum inhibitory concentration (MIC) values determined for each substance and reference antimicrobial agents because of the experiment are shown in Table 2. MIC values of the compounds were compared to reference antimicrobials (*i.e.* ampicillin, gentamicin, and vancomycin for antibacterial; fluconazole for antifungal activity). The antimicrobial activity of 10% DMSO used as a solvent could not be determined. According to the result of the study, among the compounds with the best MIC value was compound 3g. The MIC of compound 3g on *S. aureus* (ATCC 29213) strain was 2 µg/mL, which is equivalent to MIC of the ampicillin, the reference antibiotic. Compound 3g was shown to have the best antibacterial activity among the compounds in our study. All compounds except compound 3g had antimicrobial activity in the range of 64-256 µg/mL. Therefore, other compounds in the study were also found to have moderate antimicrobial activity. We determined that compound 3b showed the highest antifungal activity. The MIC value of compound 3b on *C. albicans* (ATCC 10231) and its clinical isolate was determined to be 64 and 64 µg/mL, respectively. Other compounds other than 3b had antifungal activity in the range of 128-256 µg/mL.

Among our compounds, a bis-3,5-trifluoromethyl substituent (compound 3g) in this position increased the antibacterial activity against *S. aureus* compared to those of other synthesized compounds. It was observed that other substituents did not have effect on the activity. In the literature, there are many studies investigating the antimicrobial activities of synthesized chemical compounds. In one of these studies, Noshiranzadeh et al.[17] evaluated the antibacterial activity of some new

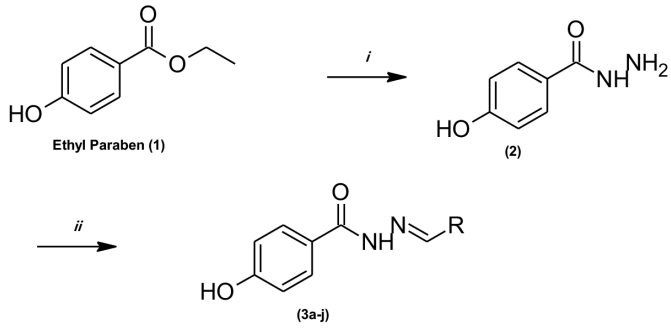


Figure 1. Synthesis route of hydrazide-hydrazone derivatives (3a-j) (*i*): $NH_2NH_2 \cdot H_2O/C_2H5OH$, (*ii*) C_2H_5OH/glacial CH_3COOH/R-CHO

Table 1. Substituents of compounds 3a-j

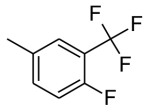
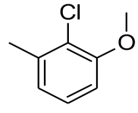
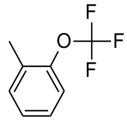
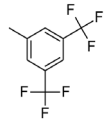
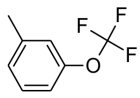
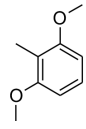
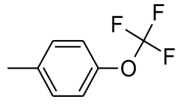
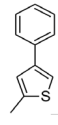
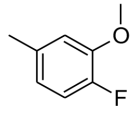
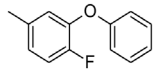
Compounds	-R	Compounds	-R
3a		3f	
3b		3g	
3c		3h	
3d		3i	
3e		3j	

Table 2. ***In vitro*** **MICs (µg/mL) observed of the compounds and reference antimicrobial drugs**

Compounds	Microorganisms					
	S.a.	S.a.*	E.c.	E.c.*	C.a.	C.a.*
3a	256	256	128	128	256	128
3b	256	256	128	128	64	64
3c	256	256	256	128	128	128
3d	256	256	256	128	128	128
3e	256	256	256	128	128	64
3f	256	256	128	64	256	256
3g	2	256	128	128	256	256
3h	256	256	256	256	256	256
3i	256	256	256	128	256	128
3j	256	256	256	128	256	256
Ampicillin	2	32	16	16	-	-
Gentamicin	1	8	1	16	-	-
Vancomycin	1	4	-	-	-	-
Fluconazole	-	-	-	-	1	1

S.a.: *Staphylococcus aureus* ATCC 29213, S.a.*: *Staphylococcus aureus* isolate (MRSA), E.c.: *Escherichia coli* ATCC 25922; E.c.*: *Escherichia coli* isolate (contains broad spectrum β-lactamase enzyme -GSB-), C.a: *Candida albicans* ATCC 10231, C.a.*: *Candida albicans* isolate

hydrazide-hydrazones of lactic acid. In that study, which used the microbroth dilution method, it was stated that MIC values of the compounds were 64-128 µg/mL against some bacterial strains. In another study, Abdelrahman et al.[18] tested the *in vitro* antibacterial activity of novel hydrazide-hydrazon derivates and found that some compounds exhibited better antibacterial activity compared to ampicillin and ciprofloxacin, respectively. For example; a compound in the study (MIC: 0.49 µg/mL) exceeded MIC of ampicillin (0.98 µg/mL), that was the reference agent against *S. pneumoniae*.[18]

In summary, the compounds in our study showed varying levels of antimicrobial activity. However, MIC value of the compound 3g, which captures the reference antibiotic, makes this compound stand out among others. Although studies in the literature show that hydrazide-hydrazone derivatives have variable antimicrobial MIC values, our study with these studies supports the idea that these derivatives are promising antimicrobial molecule candidates for the future.

CONCLUSION

In this study, ten ethylparaben hydrazide-hydrazone derivatives were screened for their antibacterial and antifungal activities. Among the synthesized molecules, compound 3g showed the best antimicrobial activity at 2 µg/mL MIC value to *S. aureus* strain (ATCC 29213). MIC value of compound 3b on *C. albicans* (ATCC 10231) and its clinical isolate was determined to be 64 and 64 µg/mL, respectively. Ethylparaben is currently used as an antibacterial agent and preservative for preparations. As these studies are necessary to understand the relationship between the substitutions and activity, which can lead to the design and synthesis of more potent antimicrobial compounds, which can occur in the therapeutic use.

Ethics

Ethics Committee Approval: Not applicable.

Informed Consent: Not required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: U.İ., M.İ.H., Design: U.İ., M.İ.H., Data Collection or Processing: U.İ., M.İ.H., Analysis or Interpretation: U.İ., M.İ.H., Literature Search: U.İ., M.İ.H., Writing: U.İ., M.İ.H.

Conflict of Interest: No conflict of interest was declared by the authors.

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