

Investigation of the Antimicrobial Activity of Some Ethyl Paraben Hydrazide-Hydrazone Derivatives

Short Title in English: Hydrazide-hydrazone Activity

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ABSTRACT

Introduction: The development of antimicrobial molecules that have been discussed with big achievement over the past decades provided many classes of semisynthetic or synthetic compounds. The resistance to many antimicrobial agents requires the discovery of novel molecules.

Methods: In this study, ten previously reported ethyl paraben hydrazide-hydrazone derivatives 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 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 (μ g/mL) to *Staphylococcus aureus* strain (ATCC 29213).

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

Keywords: Antimicrobial activity, ethyl paraben, hydrazide-hydrazone, Broth micro-dilution method, *in vitro*.

INTRODUCTION

Since the beginning of the last century, various antimicrobial molecules have been systematically introduced to use, both experimentally and also by trial and error. Because of the exceptional

genetic plasticity of the microorganisms, misuse, and world population, resistance to bacterial strains have appeared and have radiated throughout the world^[1]. Today, antibiotic resistance has become a big clinical and public health problem and resistance rates are climbing dangerously all over the world. 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 are required to 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-hydrazone 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 the present study, 10 previously reported ethyl paraben hydrazide-hydrazone derivatives were tested for their antibacterial and antifungal activity with using various microorganism strains.

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 (CAMHB) and RPMI-1640 mediums were used for the determination of potential antibacterial and antifungal activity, respectively.

The compounds were dissolved in 1 mg/0.976 ml DMSO with a final concentration of 1024 µg/ml and the serial dilutions of each compound at the range of 2-512 µg/mL were prepared in 96-well microplates, after placing broth medium in each well. Suspension of each microorganism was prepared using McFarland:0.5 standard and as a result 10⁵ cfu/ml densities were reached. Microplates containing bacteria and fungus were incubated for 16-20 hours at 37°C and 48 hours 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 mediums were tested. The 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 minimum inhibition concentrations. The detection was made by visual evaluation using dye MTT^[15]. Each test was repeated 3 times.

Chemistry

All compounds have been reported our previous study^[16]. Chemical route for synthesis of compounds is shown in Figure 1.

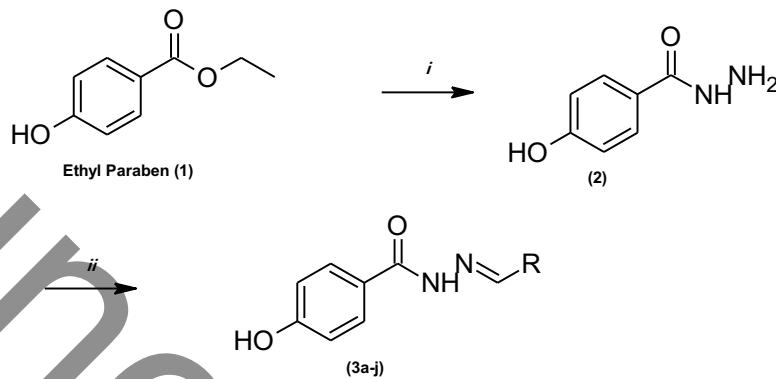


Figure 1. Synthesis route of hydrazide-hydrazone derivatives (**3a-j**) (i): $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}/\text{C}_2\text{H}_5\text{OH}$, (ii) $\text{C}_2\text{H}_5\text{OH}/\text{glacial CH}_3\text{COOH}/\text{R-CHO}$.

Table 1. Substituents of compounds 3a-j

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

RESULTS AND DISCUSSIONS

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

The MIC values determined for each substance and reference antimicrobial agents as a result of the experiment are shown in Table 2. The MIC values of the compounds were compared to reference antimicrobials (Ampicillin, gentamicin, and vancomycin for antibacterial; fluconazole

for antifungal activity). The antimicrobial activity of 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 the *S. aureus* (ATCC 29213) strain was 2 µg/mL, which is equivalent to the MIC of the ampicillin reference antibiotic. Compound **3g** was shown to have the best antibacterial activity among the compounds in ours tudy. All compounds except compound **3g** had antimicrobial activity at the range of 64-256 µg/mL. Therefore, other compounds in the study were also found to have moderate antimicrobial activity. We have detected that Compound **3b** had shown 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. It was determined that 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* when compared to other synthesized compounds. It was observed that other substituents did not effect on the activity. In the literature, there are many studies investigating the antimicrobial activities of synthesized chemical compounds. In one of these, Noshiranzadeh et al. evaluated antibacterial activity of some new hydrazide-hydrazone of lactic acid. In this study, which used the broth micro-dilution method, it was stated that the MIC value of the compounds was 64-128 µg/mL against some bacterial strains^[17]. In another study, Abdelrahman et al. tested in vitro antibacterial activity of novel hydrazide-hydrazone derivates. In this study it was found that some compounds were reported to exhibit better antibacterial activity compared to ampicillin and ciprofloxacin, respectively. For example, a compound in the study (MIC = 0.49 µg/mL) exceeded the MIC of ampicillin(0.98 µg/mL) which is the reference agent against *S. pneumoniae*^[18].

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

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
Ampicilin	2	32	16	16	-	-
Gentamycin	1	8	1	16	-	-
Vancomycin	1	4	-	-	-	-
Fluconazole	-	-	-	-	1	1

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

CONCLUSION

In this study, several ethyl paraben hydrazide-hydrazone derivatives were screened for their antibacterial and antifungal activities. Among the synthesized molecules, compound **3g** showed the best antimicrobial activity at 2 ($\mu\text{g}/\text{mL}$) MIC value to *S. aureus* strain (ATCC 29213). The MIC value of Compound **3b** on *C. albicans* (ATCC 10231) and its clinical isolate was determined to be 64 and 64 $\mu\text{g}/\text{mL}$, respectively. Ethyl paraben is currently used as an antibacterial agent and preservative in preparations. Also 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 take place in the therapeutic use.

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AUTHOR CONTRIBUTIONS

Uİ and MİH performed all the experiments, interpreted data and prepared the manuscript; Uİ and MİH helped in procuring sample and edited the manuscript. Uİ and MİH conceptualized the research, designed the experiments, wrote and edited the manuscript.

CONFLICT OF INTEREST STATEMENT

Authors declare there is no conflict of interest.

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