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Investigation of Antimicrobial and Antitubercular Activities of Some Hydrazone Derived Compounds

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Hydrazone,
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Abstract: A series of hydrazone derivatives were synthesized to investigate antifungal, antimicrobial and antitubercular activities. These activities were investigated against *Escherichia coli*, *Escherichia coli* isolate, *Pseudomonas aeruginosa*, *Pseudomonas aeruginosa* isolate (resistant to gentamicin), *Staphylococcus aureus*, *Staphylococcus aureus* isolate (MRSA), *Enterococcus faecalis*, *Enterococcus faecalis* isolate (VRE), *Candida albicans*, *Candida krusei* and *Mycobacterium tuberculosis*. Among the synthesized compounds **B23** had the best activity against *Candida albicans* with 16 µg/mL MIC value and **B24** had the best activity against *Staphylococcus aureus* isolate (MRSA) with 16 µg/mL MIC value. The most effective compound against *Mycobacterium tuberculosis* is found to be **E9** with 32 µg/mL MIC value, a chalcone derivative. However, all compounds were determined as ineffective against *Escherichia coli* and *Escherichia coli* isolate.

Bazı Hidrazon Türevi Bileşiklerin Antimikrobiyal ve Antitüberküler Etkilerinin Araştırılması

Anahtar Kelimeler
Hidrazon,
Antitüberküler etki,
Antimikrobiyal etki

Özet: Bir seri hidrazon türevi, antifungal, antimikrobiyal ve antitüberküler etkilerini araştırmak için sentezlendi. Bileşiklerin bu etkileri, *Escherichia coli*, *Escherichia coli* izolat (ESBL), *Pseudomonas aeruginosa*, *Pseudomonas aeruginosa* izolat (gentamisine dirençli), *Staphylococcus aureus*, *Staphylococcus aureus* izolat (MRSA), *Enterococcus faecalis*, *Enterococcus faecalis* izolat (VRE), *Candida albicans*, *Candida krusei ve Mycobacterium tuberculosis* karşısında araştırıldı. Sentez edilen bileşiklerden **B23** *Candida albicans* karşısında 16 µg/mL MİK değeri ile, **B24** ise *Staphylococcus aureus* izolatı (MRSA) karşısında 16 µg/mL MİK değeri ile en iyi etkiyi gösterdi. *Mycobacterium tuberculosis* karşısında en etkin bileşiğin ise 32 µg/mL MİK değeri ile bir şalkon türevi olan **E9** olduğu bulundu. Bununla birlikte, tüm bileşiklerin *Escherichia coli ve Escherichia coli* isolat karşısında etkisiz olduğu saptandı.

1. Introduction

Tuberculosis causes an estimated one million six hundred and seventy thousand deaths annually [1]. The estimated number of infected people is 1.7 billion, but only some of them are developing the disease [2]. Since the duration of treatment protocols is very long, resistance develops to the drugs used for treatment. While old drugs are usually used in the treatment of tuberculosis, there are several new drugs such as bedaquiline, delamanide, pretomanide and rifapentine that have reached phase 3 clinical trials [1,3,4]. The microorganism which is resistant to rifampin and isoniazid is called Multidrug-resistant tuberculosis (MDR-TB), and this resistance is increasing gradually [5,6]. It is very important to shorten the long treatment period, reduce the side effects of combined drug therapy, and discover new, effective drugs with less side-effects, instead of drugs that have lost their effectiveness due to resistance development.

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S. aureus is a gram-positive human pathogen leading to community and hospital-acquired infections, which is commensally present in human skin and mucous membranes [7-9]. Since infections of *Staphylococcus aureus* may cause deaths, these bacterial infections are very dangerous [7,10,11]. The main routes of transmission of this microorganism are; patients who are colonized or infected with this microorganism, contaminated medical instruments, contaminated media surfaces, contaminated clothing of healthcare workers. In addition, airborne transmission occurs in the presence of patients who cannot control their secretions [12]. These bacteria can cause many infections in humans, such as bacteremia, skin and tissue infections, septic arthritis, infective endocarditis, lung infections, osteomyelitis, gastroenteritis, meningitis and urinary tract infection [7]. Although these bacteria do not cause infection on healthy skin, they can cause very serious infections mentioned above, if they enter the bloodstream and internal tissues [13,14].

S. aureus infections were successfully treated with penicillin derivatives in the 1940s. However, resistance to penicillin derivatives has developed with the emergence of the strains that synthesize beta-lactamase enzyme. Thereafter, treatment was started with methicillin, a semisynthetic penicillin derivative resistant to beta-lactamase enzyme. Over time, strains resistant to methicillin have also been evolved [9].

Candida are fungi that may be found in the microbiota. However, they are opportunistic pathogens and may cause diseases in case of immun suppression. They may also be resistant to antifungal agents by different mechanisms, especially by forming biofilm [15].

Since the development of resistance to antibiotics and antifungal drugs increases rapidly, many of these drugs lose their effectiveness in a short time. Therefore, the discovery of new effective antibiotics has gained great importance. Hydrazone group compounds are remarkable for their antimicrobial, antifungal and antitubercular properties [16-18]. In this study, we investigated the antimicrobial, antifungal and antitubercular effects of a group of hydrazone compounds.

2. Material and Method

2.1. Synthesis

Chalcone derivatives were obtained by reacting acetophenone and benzaldehyde derivatives in KOH/methanol. The hydrazone compounds were obtained by reacting the chalcone derivatives with the hydrazide compounds. Reaction and product details, have been previously published [19].

2.2. Determination of antibacterial and antifungal activity

Antibacterial and antifungal susceptibility testing were performed through the guidelines of CLSI-M100-S16 [20] and M27-A3 [21] standards, respectively. *E. coli* ATCC 25922, *E. coli* ATCC 35218, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *C. albicans* ATCC 10231 and *C. krusei* ATCC 6258 and their clinical isolates were used in the study. *E. coli* isolate has extended spectrum beta lactamase enzyme and used as the ESBL strain. *C. krusei* ATCC 6258 strain was used because it is resistant to fluconazole naturally. A methicillin resistant *S. aureus* isolate which is resistant to all beta lactam antibiotics (MRSA), an *E. faecalis* isolate which is resistant to vancomycin (VRE) and a *P. aeruginosa* isolate which is resistant to gentamicin were also included in the study. The lowest concentration of the compounds that completely inhibits macroscopic growth was determined as minimum inhibitory concentrations (MICs). Details of the method is given in the literature [22].

2.3. Determination of antimycobacterial activity

Mycobacterium tuberculosis H37RV (ATCC 27294) was used as the quality control strain. MABA method was used to determine the antituberculosis activity as described by Franzblau et al.. 1:1 mixture of 10X Alamar Blue reagent were added to wells. A blue color in the well showed no growth and a pink color showed growth. The MIC was defined as the lowest drug concentration which prevented a color change from blue to pink [23]. Details of the method is given in our previous publication [22].

3. Results

Initially, compounds **E9** and **E13** were synthesized by reaction of acetophenone and aldehyde derivatives. Then, the compounds **E9** and **E13** were reacted with 2-furoic hydrazide, 2-thienyl hydrazide and 4-methyl-1,2,3-thiadiazol 5-carbo hydrazide to form compounds **B22**, **B23**, **B24**, **B25** (Figure 1).

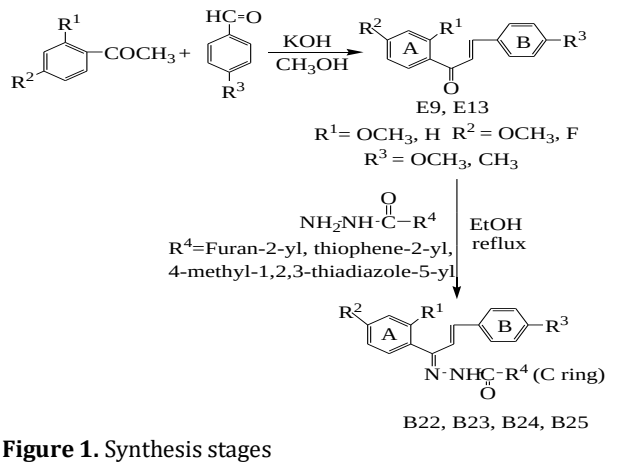

Figure 1. Synthesis stages

Table 1 shows the formulas of the compounds.

Table 1. Formulas of compounds

Compound	R^1	R^2	R^3	R^4
E9	-H	-F	$-OCH_3$	-
E13	$-OCH_3$	$-OCH_3$	$-CH_3$	-
B22	$-OCH_3$	$-OCH_3$	$-CH_3$	Thiophene-2-yl
B23	$-OCH_3$	$-OCH_3$	$-CH_3$	Furan-2-yl
B24	-H	-F	$-OCH_3$	Thiophene-2-yl
B25	-H	-F	$-OCH_3$	4-Methyl-1,2,3-thiadiazole-5-yl

Antitubercular and antimicrobial activities of synthesized compounds were determined. In determining these effects, ampicillin, gentamicin, ofloxacin, meropenem, vancomycin, ampicillin / sulbactam (1/1), Amoxicillin / clavulonic acid (2/1), fluconazole, amphotericin B, ethambuthol and isoniazid standards were compared, obtained results are displayed in Table 2.

4. Discussion and Conclusion

Compounds **B22** and **B23** contain methoxyl at positions 2 and 4 in ring A, and methyl at para position in ring B. Of the two compounds, **B22** carries the thiophene ring as the C ring, while **B23** carries the furan ring. When the compounds **B22** and **B23** were compared, an increase was observed in the activity against MRSA and *C. albicans* with the substitution of the furan ring instead of thiophene as the C ring. However, the presence of furan ring in the structure was found to increase the effect on *E. coli*, *P. aeruginosa* isolate (gentamicin resistant), *S. aureus, E. faecalis isolate* (VRE) and *C. krusei*. Compounds **B22** and **B23** were obtained by reacting compound **E13** with different hydrazides. Compound **E13**, which is a chalcone, showed better activity only against *S. aureus,* when compared with the compounds **B22** and **B23** which are formed by its transformation. Apart from this, it has the same or worse effect against all other bacterial and fungal strains, suggesting that the reaction of the chalcone with hydrazides generally increases the effect. Compounds **B24** and **B25** both carry a fluorine atom at para position of ring A and a methoxyl group at p-position of ring B. Compound **B24** contains thiophene as ring C, while ring C of compound **B25** contains a thiadiazole ring. Compound **B24** was found to be 2-fold more potent against *S. aureus* and *C. krusei* than compound **B25**. In addition, compound **B24** was found to be 4 times more effective against MRSA than compound **B25**. The presence of thiophene in the C ring is preferred because it increases the effect on many strains. The presence of thiadiazole in ring C increased the effect on *E. faecalis*. Compound **E9**, the starting material of compounds **B24** and **B25**, was found to be more effective on *C. albicans* and *M. tuberculosis* than the hydrazone derivative. For better antibacterial effect, the presence of the hydrazone structure and the use of thiophene as the C ring were required. Also, when the effect on tuberculosis is

Table 2. Antimicrobial and antifungal activities of the compounds

MIC (µg/mL)												
Compounds	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
B22	64	128	128	64	64	128	64	128	64	64	32	64
B23	128	128	128	64	128	256	32	128	128	16	64	64
B24	64	128	128	64	64	64	16	128	128	64	32	64
B25	64	128	128	64	64	128	64	64	128	64	64	64
E9	64	128	128	64	128	128	32	128	128	32	64	32
E13	128	128	128	128	128	64	64	128	128	128	64	64
Ampicilin	2	-	>1024	-	-	0.5	-	0.5	0.5	-	-	-
Gentamicin	0.25	-	256	1	64	0.5	128	8	8	-	-	-
Ofloxacin	0.015	-	16	1	1	0.125	0.5	1	4	-	-	-
Meropenem	0.008	-	0.015	0.25	0.015	0.03	-	4	8	-	-	-
Vancomycin	-	-	-	-	-	0.5	1	1	8	-	-	-
Ampicilin/ sulbactam (1/1)	-	16	-	-	-	-	-	-	-	-	-	-
Amoxicilin/ clavulonic acid (2/1)	-	16	-	-	-	-	-	-	-	-	-	-
Fluconazole	-	-	-	-	-	-	-	-	-	0.0625	32	-
Amphotericin B	-	-	-	-	-	-	-	-	-	<0.03	0.5	-
Ethambuthol												4
Isoniazid												0.125

1: *E.coli* ATCC 25922, 2: *E.coli* ATCC 35218, 3: *E.coli* isolate (ESBL), 4: *Pseudomonas aeruginosa* ATCC 27853, 5: *P.aeruginosa* isolate (resistant to gentamicin), 6: *Staphylococcus aureus* ATCC 29213, 7: *S.aureus* isolate (MRSA), 8: *Enterococcus faecalis* ATCC 29212, 9: *E.faecalis* isolate (VRE), 10: *Candida albicans* ATCC 10231, 11: *C.krusei* ATCC 6258, 12: *Mycobacterium tuberculosis* H37RV ATCC 27294

considered, the importance of preserving the chalcone structure emerges. In this group of compounds, it is seen that the chalcone structure is more effective against *C. albicans* which is a common fungal species.

When the compounds **B22** and **B24** carrying thiophene as ring C are compared, the compound **B24** having fluorine at the p-position of ring A and the methoxyl structure at the p-position of the ring B were found to be more effective against *S. aureus* and MRSA, with respect to compound **B22**, with methoxyl at the 2nd and 4th positions of ring A and methyl at the 4th position of the ring B. This study is important for the development of new drug active substances that can be used to treat *S. aureus*, a bacterium that causes many serious infections, including nosocomial infections. **B22** was found to be more effective only against VRE than **B24**.

When **E9** and **E13** compounds were compared, it was found that **E9** was more effective than **E13** in many bacteria *(E. coli, P. aeruginosa,* MRSA, *C. albicans*, *M. tuberculosis*). Compound **E9** was found to be 4 times more potent against *C. albicans* than **E13**. Compound **E13** was found to be twice as effective against *S. aureus* as compared to compound **E9**. It is known that the fluorine atom in the structure of compound **E9** increases the effect of the chalcone compounds [11]. Therefore, it is thought that compound **E9** is more effective.

In this study, antimicrobial and antifungal effects of some hydrazone derivatives and chalcones as starting materials of these compounds, were investigated. All the compounds showed a better effect against MRSA than gentamicin. Compound **B24** showed the best effect against MRSA. The presence of the fluorine atom in ring A and the thiophene group in ring C is important for activity against MRSA. Compounds **B22** and **B24** showed the same effect against *C. krusei* as fluconazole. Compounds **E9** and **B23** are the most effective compounds against *C. albicans*. The presence of fluorine atom in the 4th position in ring A and the preference of the furan ring as ring C increased the effect against *C. albicans*. The most effective compound against *M. tuberculosis* is the compound **E9**, which is a chalcone containing a fluorine atom. All other compounds showed a similar effect against *M. tuberculosis*.

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