

# Biological activity screening of some hydrazone and chalcone derivatives

## Bazı hidrazon ve kalkon türevlerinin biyolojik aktivite taraması

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### ABSTRACT

**Objective:** Today, the development of resistance to antibiotics is increasing rapidly. Loss of antibiotics effectiveness due to the development of resistance causes serious hazards. Especially methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecalis* create life-threatening conditions in patients with a weak immune system. For this reason, it is important to develop new drug active substances against these microorganisms. In this article, hydrazones and chalcones were synthesized and some biological activities of these compounds such as antimicrobial and antituberculosis were investigated. This study was conducted to investigate new antimicrobial and antituberculous drug-active substances instead of antibiotics that lost their effectiveness due to the rapidly increasing resistance to existing antibiotics.

**Methods:** The compounds were obtained by synthesis of chalcones via Claisen Schmidt condensation and then by reacting these chalcones with hydrazides to form hydrazone. Antimicrobial and antituberculosis activities of the compounds were evaluated by microdilution method and microplate alamar blue assays, respectively.

**Results:** All compounds displayed the same activity against *Mycobacterium tuberculosis* (MIC: 64 µg/mL). E4,

### ÖZET

**Amaç:** Günümüzde antibiyotiklere karşı direnç gelişimi hızla artmaktadır. Direnç gelişmesi nedeniyle antibiyotik etkinliğinin kaybı ciddi tehlikelere neden olmaktadır. Özellikle metisiline dirençli *Staphylococcus aureus* ve vankomisine dirençli *Enterococcus faecalis* izolatu, bağışıklık sistemi zayıf olan hastalarda yaşamı tehdit eden durumlar oluşturmaktadır. Bu nedenle, bu mikroorganizmalara karşı yeni ilaç etken maddeleri geliştirmek önemlidir. Bu makalede, hidrazonlar ve kalkonlar sentezlenmiş ve bu bileşiklerin antimikrobiyal ve antitüberküloz gibi bazı biyolojik aktivitelerinin araştırılması yapılmıştır. Bu çalışma, mevcut antibiyotiklere karşı hızla artan direnç nedeniyle etkinliğini kaybeden antibiyotiklerin yerine antimikrobiyal ve antitüberküloz etkili yeni ilaç etken maddelerinin araştırılması için yapılmıştır.

**Yöntem:** Bileşikler, Claisen Schmidt kondensasyonu yoluyla kalkonların sentezi ve daha sonra bu kalkonların hidrazon oluşturmak üzere hidrazidlerle reaksiyona girmesiyle elde edilmiştir. Bileşiklerin antimikrobiyal ve antitüberküloz aktiviteleri, sırasıyla mikrodilüsyon yöntemi ve mikropak alamar mavisi deneyleri ile değerlendirilmiştir.

**Bulgular:** Tüm bileşikler *Mycobacterium tuberculosis*'e karşı aynı etkiyi göstermiştir (MİK: 64 ug/

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4'-Bromo-4-methyl chalcone, displayed the best activity against Meticilline resistant-*Staphylococcus aureus* (MIC: 16 µg/mL). The most effective compounds against *Enterococcus faecalis* isolate and *Enterococcus faecalis* were found B26 (N-(1-(4-fluorophenyl)-3-p-tolylallylidene) thiophene-2-carbohydrazide) and E8 (4'-fluoro-3-methyl chalcone), respectively (MIC: 32 µg/mL).

**Conclusion:** Hydrazones have been found to be more effective than chalcones against *S. aureus* ATCC 29213. However, chalcones exhibited a remarkable effect against meticilline resistant-*Staphylococcus aureus* and *Enterococcus faecalis*. In terms of tuberculosis bacillus, it did not differ whether the compounds had a chalcone or hydrazone structure. Both chalcones and hydrazones exhibited the same activity against candida species. All compounds were observed to be more effective than gentamicin against methicillin-resistant *Staphylococcus aureus*.

**Key Words:** Hydrazone, chalcone, antitubercular, antimicrobial, microbial resistance

mL). E4, 4'-Bromo-4-metil kalkon, metisiline dirençli-*Staphylococcus aureus*'a karşı en iyi etkinliği göstermiştir (MİK: 16 ug/mL). *Enterococcus faecalis* izolatu ve *Enterococcus faecalis*'e karşı en etkili bileşikler sırasıyla B26 (N-(1-(4-florofenil)-3-p-tolilaliden)tiyofen-2-karbonhidrazit) ve E8 (4'-floro-3-metil kalkon) olarak bulunmuştur.

**Sonuç:** Hidrazonların *S. aureus* ATCC 29213 suşu üzerine kalkonlardan daha etkili olduğu bulunmuştur. Bununla birlikte kalkonlar, metisiline dirençli-*Staphylococcus aureus* ve *Enterococcus faecalis* üzerine dikkate değer bir etki göstermiştir. Tüberküloz basili açısından, bileşiklerin kalkon veya hidrazon yapısında olması fark göstermemiştir. Hem kalkonlar hem de hidrazonlar kandida türlerine karşı aynı aktiviteyi sergilemiştir. Tüm bileşiklerin metisiline dirençli *Staphylococcus aureus* üzerine gentamisinden daha etkili olduğu gözlenmiştir.

**Anahtar Kelimeler:** Hidrazon, kalkon, antitüberküloz, antimikrobiyal, mikrobiyal direnç

## INTRODUCTION

*Staphylococcus aureus* is a microorganism that is important due to its pathogenicity from the beginning of the microbiological era to the present day. This bacterium, which can be found in the skin and mucous flora, can cause fatal diseases such as bacteremia and pneumonia when immunity is weakened. *S. aureus*, which was previously treatable with penicillins, began to develop resistance to methicillin in the 1960s, resulting in Meticilline resistant-*Staphylococcus aureus* (MRSA) strains. MRSA has *mecA* gene which encodes the penicillin-binding protein of MRSA. Thus, resistance to  $\beta$ -lactam antibiotics develops (1-3). Also, MRSA, which started to develop resistance against antibiotics such as linezolid and daptomycin, is a very important health threat (2, 3). Resistance was not developed only by *S. aureus* but also by other bacteria that neutralize antibiotics which makes the

discovery of new antibiotics is essential.

Balsacone C (figure 1), a dihydrochalcone derivative, draws attention with its activity against MRSA (4). Balsacone C displayed very good activity against 34 MRSA isolates, which are resistant to many antibiotics [Minimum Inhibitory Concentration (MIC): 3-11.6 µg/mL] (3). Licochalcone A (Figure 1), which is a natural chalcone as balsacone C, also shows antibacterial properties (5). Beside its many activities such as antidepressant (6,7) anticonvulsant (8, 9), analgesic, antiinflammatory, antiplatelet (10-12) and anticancer (13-16), hydrazones attract researchers with their antibacterial activities. In this study, we synthesized a group of hydrazones and their starting materials, chalcones, and investigated the antimicrobial and antituberculosis activities of these compounds.

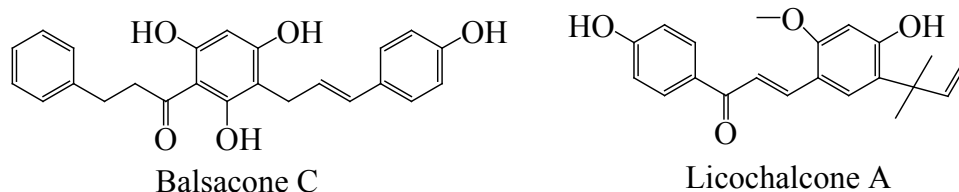


Figure 1. Some natural antibacterial chalcones

## MATERIAL and METHOD

Details of used chemicals for synthesis (17) and for biological activities (18) and used apparatus to prove the structures of the compounds (17) are given in our previous publications.

### Synthesis

Chalcone derivatives were synthesized by reacting of equimolar amounts of 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 (18).

### Biological activities of compounds

#### Antibacterial and antifungal activity

Antibacterial and antifungal susceptibility testing were applied through CLSI-M100-S16 guidelines (19) and M27-A3 (20) standards, respectively. *E. coli* ATCC 25922 [1], *E. coli* ATCC 35218 [2], *E. coli* isolate (ESBL) [3], *P. aeruginosa* ATCC 27853 [4], *P. aeruginosa* isolate (resistant to gentamicin) [5], *S. aureus* ATCC 29213 [6], *S. aureus* isolate (MRSA) [7], *E. faecalis* ATCC 29212 [8], *E. faecalis* isolate (VRE) [9], *C. albicans* ATCC 10231 [10] and *C. krusei* ATCC 6258 [11] and their clinical isolates were used in the study. *E. coli* isolate produces 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), and *E. faecalis* isolate

which is resistant to vancomycin (VRE) were also used in the study. The lowest concentrations inhibiting macroscopic growth was determined as MICs. Details of the method is given in the literature (18).

#### Antitubercular activity

*Mycobacterium tuberculosis* H37RV (ATCC 27294) [12] was used as the quality control strain. MABA method was used in order to assess the antituberculosis activity as described by Franzblau et al, 1:1 mixture of 10X Alamar Blue reagent were added to wells. A blue color or a pink color formation indicated no proliferation and proliferation, respectively. The MIC was determined as the lowest drug concentration preventing formation of pink color (21). Details of the method is given in our previous publication (18).

## RESULTS

### Chemistry

Chalcones (E4, E5, E7, E8) (Table 1) were synthesized via Claisen-Schmidt condensation. Then, chalcones were reacted with appropriate hydrazide to give corresponding hydrazones (Table1) as shown in the diagram (Figure 2).

### Biological Activity

Antimicrobial and antitubercular activities of the compounds were examined by using different standards. Antimicrobial activities were examined by microdilution method while antitubercular activities were assessed by microplate alamar blue assay (MABA). Conclusions were given in Table 2.

Table 1. Formula of compounds

Compound	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>
B26	-F	-H	-CH <sub>3</sub>	Thiophene-2-yl (T)
B27	-F	-CH <sub>3</sub>	-H	T
B28	-Br	-H	-CH <sub>3</sub>	T
B29	-Br	-H	-CH <sub>3</sub>	4-Methyl-1,2,3-thiadiazole-5-yl (MT)
B31	-Br	-CH <sub>3</sub>	-H	MT
E4	-Br	-H	-CH <sub>3</sub>	-
E5	-Br	-CH <sub>3</sub>	-H	-
E7	-F	-H	-CH <sub>3</sub>	-
E8	-F	-CH <sub>3</sub>	-H	-

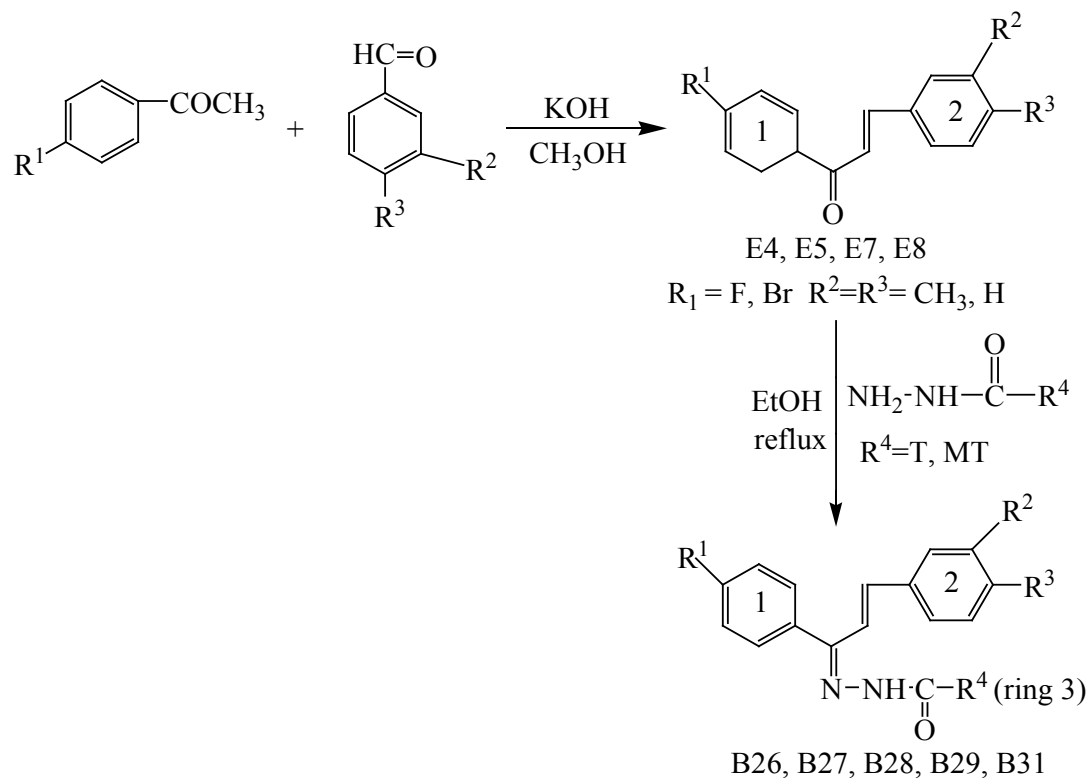


Figure 2. Synthesis of compounds

**Table 2.** Antimycobacterial, antifungal and antibacterial activities of compounds

Compounds	1	2	3	4	5	6	7	8	9	10	11	12
B26	64	128	128	64	128	128	64	64	32	128	64	64
B27	128	128	128	128	128	128	64	128	64	128	64	64
B28	64	128	128	128	128	128	64	64	128	128	64	64
B29	64	128	128	128	128	128	64	128	128	128	64	64
B31	128	128	128	128	128	128	64	64	128	128	64	64
E4	128	128	128	64	128	256	16	128	128	128	64	64
E5	128	128	128	64	128	256	64	128	128	128	64	64
E7	128	128	128	64	128	256	64	128	128	128	64	64
E8	128	128	128	64	128	256	32	32	128	128	64	64
Gentamicin	0.25	-	256	1	64	0.5	128	8	8	-	-	-
Ampicilin	2	-	>1024	-	-	0.5	-	0.5	0.5	-	-	-
Meropenem	0.008	-	0.015	0.25	0.015	0.03	-	4	8	-	-	-
Ofloxacin	0.015	-	16	1	1	0.125	0.5	1	4	-	-	-
Fluconazol	-	-	-	-	-	-	-	-	-	0.0625	32	-
Vancomycin	-	-	-	-	-	0.5	1	1	8	-	-	-
Amphotericin B	-	-	-	-	-	-	-	-	-	<0.03	0.5	-
Ampicilin/ sulbactam (1/1)	-	16	-	-	-	-	-	-	-	-	-	-
Amoxicilin/ clavulonic acid (2/1)	-	16	-	-	-	-	-	-	-	-	-	-
Ethambuthol												4
Isoniazid												0.125

Names of the bacteria and explanation of numbers in the table and article is given in Materials and Methods.

MIC: µg/mL

## DISCUSSION

Compounds **B26**, **B28** and **B29** were displayed the best activity against *E. coli* [1] (MIC= 64 µg/mL). Against this strain, hydrazone derivatives have been shown to be more effective than chalcones. **B27**, a derivative of hydrazone, had the same activity as chalcones. For this activity, it is important that ring 2 has methyl in the para position. Placing the methyl group in the meta position instead of para reduced the activity. Chalcones generally outperformed than hydrazones against *Pseudomonas aeruginosa* ATCC 27853. One of the hydrazones, compound **B26**, showed the same activity as chalcones. **E4**, a compound in the structure of chalcone containing bromine at 4th position of ring 1 and methyl at 4th position of ring 2, showed the best activity against MRSA (MIC=16 µg/mL). The **E8** compound, which is a chalcone carrying fluorine atom in the 4th position of ring 1 and carrying methyl in the meta position of ring 2, was the second compound showing the best activity against MRSA. According to Xu et al., electron-withdrawing (-Cl, - F and - Br) groups at the 2nd and 4th positions of phenyl ring is more preferable for antibacterial activity than electron-donating (-Me and -OMe) substituents (22).

The **E8** compound was found to be the most active compound against *E. faecalis* [8] (MIC=32 µg/mL). It is observed that the activity decreases in **B27**, which is the hydrazone formed by the reaction of **E8** with thiophene hydrazide. Against this microorganism, compounds **B26**, **B28** and **B31** showed the same activity (MIC=64 µg/mL). **B26** is the most effective compound with 32 µg/mL MIC against VRE, which

contains fluorine atom in position 4 of the first ring, methyl in position 4 of the 2nd ring, and thiophene as the 3rd ring. Unlike **B26**, compound **B27** maintains the methyl in the meta position of ring 2 instead of para. **B27** was found to be the second most effective compound (MIC=64 µg/mL) against VRE. Therefore, including p-methyl on the 2nd ring is important for the activity against VRE. All compounds except **B26** and **B27** displayed the same activity against VRE (MIC=128 µg/mL). Unlike **B26**, **B28** compound has p-bromine instead of p-fluorine of the 1st ring. The replacement of the fluorine atom with a bromine atom caused a serious decrease in the activity. For the activity against VRE, presence of p-fluorine at the 1st ring and the presence of the methyl group in the 4th position of the 2nd ring is necessary. When the methyl group attached to the second ring is taken into the meta position instead of para, the activity decreases. Both chalcone and hydrazone derivatives displayed the same activity against *E. coli* [2], ESBL, *P. aeruginosa* [5], *C. albicans*, *C. krusei* and *M. tuberculosis* strains. Hydrazones were found to be more effective than chalcones against *S. aureus* [6].

In summary, some chalcones and their hydrazones were designed, synthesized and their antitubercular and antimicrobial activities were examined. Compound **E4** with its activity against MRSA, compound **E8** against *E. faecalis* and compound **B26** against VRE came into prominence. It is hoped that our study can contribute to the development of new antibiotics in an environment where bacterial resistance development is so frequent. Further studies are necessary to find the antibiotics.

## ETHICS COMMITTEE APPROVAL

\* This study does not require Ethics Committee Approval.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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