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RESEARCH ARTICLE

Evaluation of Antifungal Activity of Some Benzothiazole Derivatives

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ABSTRACT

The antifungal activity of the previously synthesized compounds was evaluated in order to provide solutions to the candida-induced diseases in animals. In the present study, 10 benzothiazole derivatives (4a-4j) were re-synthesized to evaluate their antifungal activity. IR, 1HNMR, 13C-NMR and HRMS (Infrared Spectroscopy, ^{1}H Nuclear Magnetic Resonance Spectroscopy, ^{13}C Nuclear Magnetic Resonance Spectroscopy, High Resolution Mass Spectrometry) spectroscopic methods, determined the structure of the synthesized compounds. MIC_{50}(Minimum Inhibitory Concentration) values of the re-synthesized compounds against *Candida* species were evaluated by *in vitro* experiments. As a result of activity studies, it was found that compounds 4c and 4d showed significant activity. Compound 4d was found to be the most potent derivative against *Candida krusei* with a MIC_{50} value of 1.95 μg / mL.

Keywords: Benzothiazole, Antifungal Activity, *Candida krusei*

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Bazı Benzotiyazol Türevlerinin Antifungal Aktivitesinin Değerlendirilmesi

ÖZ

Hayvanlarda oluşan candida kaynaklı hastalıklara çözüm üretmek amacıyla daha önceden sentezi yapılmış bileşikler benzer metot kullanarak tekrar sentezlenmiş ve antifungal etkinlikleri değerlendirilmiştir. Mevcut çalışmada, 10 tane benzotiyazol türevi bileşik (4a-4j), antifungal aktivitelerini değerlendirmek üzere yeniden sentezlenmiştir. Sentezlenen bileşiklerin yapı tanımlamaları IR, $^{1}HNMR$, ^{13}C-NMR ve HRMS (Kızılötesi Spektroskopi, ^{1}H Nükleer Manyetik Rezonans Spektroskopisi, ^{13}C Nükleer Manyetik Rezonans Spektroskopisi, Yüksek Çözünürlüklü Kütle Spektrometresi) spektroskopik yöntemleri kullanılarak gerçekleştirilmiştir. Yeniden sentezlenmiş bileşiklerin *Candida* türlerine karşı MIC_{50} (Minimum İnhibitör Konsantrasyon) değerleri in vitro deneyler yapılarak değerlendirilmiştir. Yapılan aktivite çalışmaları sonucunda 4c ve 4d bileşikleri önemli aktivite göstermiştir. 4d bileşiğinin *Candida krusei*'ye karşı 1.95 μg / mL MIC_{50} değeri ile güçlü bir türev olduğu bulunmuştur.

Anahtar Kelimeler: Benzotiyazol, Antifungal Aktivite, *Candida krusei*

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INTRODUCTION

There are many species of fungi including cutaneous (*Microsporum spp*., *Trichophyton spp*., *Epidermaphyton floccosum*, *Dermatophilus congolensis*), subcutaneous (*Rhinosporidium seeberi*, *Sporotrichum schenckii*) and systemic (*Aspergillus spp*., *Blastomyces dermatitidis*, *Histoplasma spp*.) that can cause infections in humans and animals. (Arda et al. 1999). Fungal infections can spread very quickly by direct contact, causing general condition disturbances in animals and deaths by generalized infections in young animals. Moreover, parasitic, viral, bacterial other infective agents cause secondary infections, which make the differential diagnosis of the disease more gruelling, and worsen the course of the disease. Candida species are taxonomically found in Fungi realm, Ascomycete branch, Saccharomycetes class, Saccharomycetales order, Saccharomycetaceae family and Candida genus. (Wilson 2019). Humans and animals such as cats, dogs, cattle, horses, sheep, goats, poultry, rodents and pigs are susceptible to infections (Edelmann et al. 2005). *C. glabrata, C. tropicalis, C. parapsilosis, C. krusei* (Pappas et al. 2018)*, C. aloffii, C. bovina, C. pseudotropicalis*'in (Arda et al. 1999) especially *C. albicans* have been reported to cause diseases in animals and humans. (Pappas et al. 2018). In addition to these species, C. *guilliermondii*, *C. lusitaniae*, *C. kefyr*, *C. famata*, *C. inconspicua*, *C. rugosa*, *C. dubliniensis* and *C. norvegensis* have also been reported to cause infections. (Sanguinetti et al. 2015). It has also been reported in many countries (Japan, India, Pakistan, England, Spain, Colombia, Venezuela, Panama, the United States) that *Candida auris* is transmitted by the nasocomial route and causes very serious infections in humans in recent years. (Pappas et al. 2018). Factors found in the skin and intestinal mucosa of healthy people (Pappas et al. 2018) cause invasive infections, especially in patients; where the immune system is suppressed and post-surgical operations. (Fidel et al. 1999, Perlin 2015). The diseases caused by fungi of the genus *Candida* are called Candidiasis. Although the term candidiasis means cutaneous, disease agents may affect the mucosa and organs. (Pappas et al. 2018). Even in healthy people, after gastrointestinal system operation or when the immune system is suppressed, the causative agents in the intestine can cross from the intestinal barrier to the blood, and cause serious infections in different organs such as brain, eye, bone marrow, lung, heart, liver, spleen, pancreas, kidney, peritoneum (Pappas et al. 2018). In animals, it has also been reported to cause infections in different organs such as mouth, oesophagus, stomach, intestines, skin, subcutaneous tissue, uterus, breast, testis (Arda et al. 1999). It is reported that the species and strains causing Candida infections in animals and humans do not differ genetically from each other. Factors that can be found in normal conditions in the oral cavity and intestines of healthy living organisms are thought to cause infections especially in immunocompromised individuals and those animals can be a source for infections in such immunosuppressive individuals. (Edelmann et al. 2005).

The virulence and antifungal susceptibility of each species differ. Most of the clinical infections are revealed due to *C. albicans*. Candida spp., which are dispersed in the body-entangled blood, can cause many clinical symptoms. (Pappas et al. 2018).

Candida albicans has been reported to cause serious infections of the feet, footpad, nail and skin in dogs with *Malassezia spp.* (McEwan 2001). *Candida albicans* and some other *Candida* species have been identified in urinary tract infections in cats and dogs (Pressler et al. 2003, Jin and Lin 2005). It was reported that, *Candida glabrata* (together with *Fusarium oxysporum*) were isolated in a patient who started with foot soles and skin lesions and continued myocardial, liver, renal interstitium in dog (Rothenburg et al. 2017). In addition, *Candida albicans* was isolated from samples of internal organs of sepsis foals with new born necrotizing enterocolitis, renal insufficiency and incompatibility syndrome. Systemic infection has been identified to originate from this species (Reilly and Palmer 1994).

Echinocandin (micafungin, anidulafungin, caspofungin) and azole group drugs are most preferred drugs against fungal infections. However, these drugs may be ineffective in infections caused by Candida species, especially *C. glabrata*. (Sanguinetti et al. 2015; Pappas et al. 2018). Resistance to azoles has been reported in a dog diagnosed with urinary tract infection from *Candida tropicalis* (Álvarez-Pérez et al. 2016). It is also observed that *C. auris*, which is a newly identified global threat, is resistant to many drugs. (Pappas et al. 2018). Antifungal resistance between Candida species is terrifying (Perlin 2015). Therefore, it is necessary to discover new alternative drugs to be used in cases of candidiasis. The aim of this study was to introduce new antifungal drugs for potential clinical use.

MATERIALS and METHODS

Chemistry

The compounds previously synthesized and tested for anticancer activity were re-synthesized to evaluate their antifungal activity. All chemicals used in the syntheses were purchased either from Sigma-Aldrich Chemicals (Sigma-Aldrich Corp., St. Louis, MO, USA) or Merck Chemicals (Merck KGaA, Darmstadt, Germany) (Osmaniye et al. 2018).

General procedure for the synthesis of target compounds (4a-4j)

Compounds 2-((5-substituted)benzothiazol-2-ylthio) acetohydrazide (3a, 3b) (0.002 mol) re-synthesized

according to the indicated method (Osmaniye et al. 2018) and reacted with suitable aldehydes (0.002 mol) in butanol (20 mL). After the completion of the reaction was judged by TLC, the reaction contents were cooled and the precipitated product was filtered off. The yield was increased by 3-5% in comparison with the method involving ethanol as solvent (Osmaniye et al. 2018).

Analysis Studies

Melting degrees, IR, ^{1}HNMR, ^{13}CNMR and HRMS spectra of the synthesized compounds were obtained and were found to be in agreement with the previous study. (Osmaniye et al. 2018). In addition, HMBC (Heteronuclear multiple-bond correlation spectroscopy), HSQC (Heteronuclear single-quantum correlation spectroscopy) spectra for compound 4d were obtained and the structure determination of the compound was evaluated in detail.

Antifungal Activity

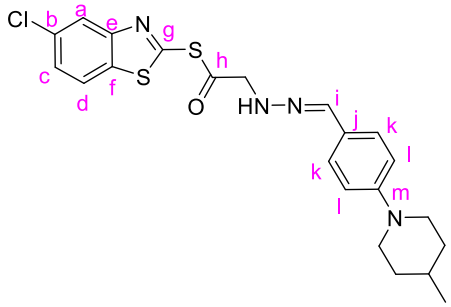
In vitro antifungal activity of all derivatives (4a-4j) was evaluated against *C. albicans* (ATCC 90030), *C. glabrata* (ATCC 90030), *C. krusei* (ATCC 6258) ve *C. parapsilopsis* (ATCC 22019) at concentrations between 1 mg/mL–1.95 µg/mL. Activity studies were conducted following EUCAST protocol in accordance with previous studies reported by our research group (Karaburun et al. 2018, Karaburun et al. 2019).

RESULT and DISCUSSION

Chemistry

Compounds were re-synthesized as shown in Scheme-1. 4-fluorobenzaldehyde and secondary amine derivatives were reacted to obtain 4-substituted benzaldehyde derivatives (1a-1e). The 2-mercapto-5-substitutedbenzothiazole derivatives were refluxed with ethyl chloroacetate to obtain ethyl 2-(5-substitutedbenzothiazol-2-yl-thio)acetate derivatives. The obtained esters (2a, 2b) were reacted with excess of hydrazine hydrate to give hydrazide derivatives (3a, 3b). The hydrazide derivatives (3a, 3b) were reacted with 4-substituted benzaldehydes (1a-1e) in butanol and the reaction was performed with higher yield than the previously reported results.

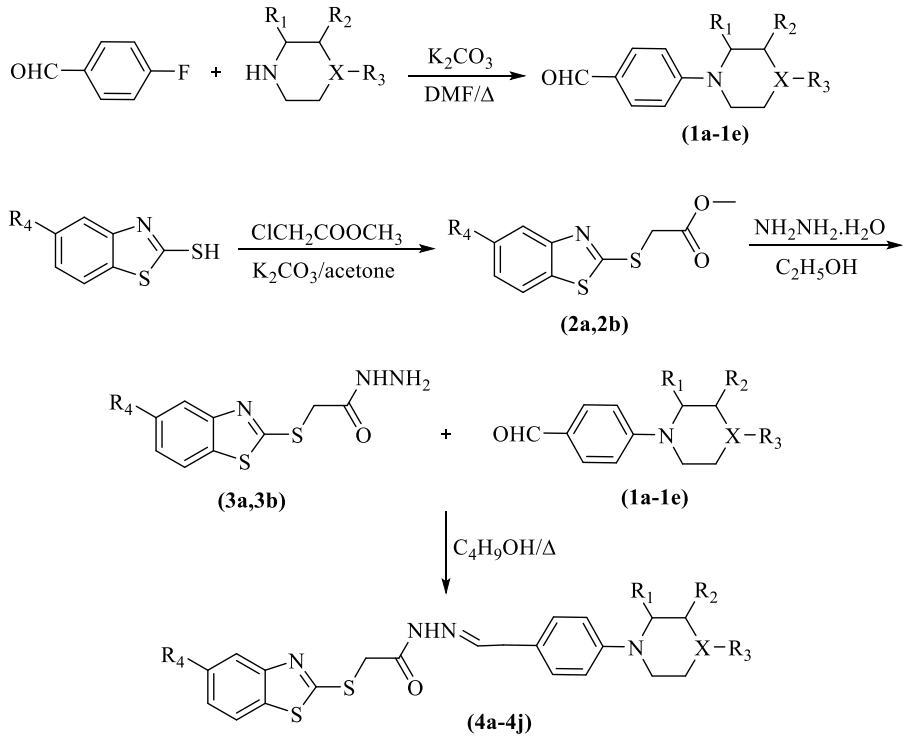
Analysis Studies



The melting points, IR, ^{1}HNMR, ^{13}CNMR and HRMS spectra of the synthesized compounds were in agreement with the reported study. 2D-NMR technique was used to clarify the structure of the compounds. The two-dimensional NMR method is one of the most commonly used NMR techniques when one-dimensional NMR techniques cannot be used for precise structure determination. HSQC (Heteronuclear single-quantum correlation spectroscopy) and HMBC (Heteronuclear multiple-bond correlation spectroscopy) are heteronuclear spectroscopy methods obtained from the correlation of protons in one axis and carbon spectrum in the other axes. HSQC provides information about the interactions between the carbon to which the proton is directly connected, while HMBC shows the interactions between hydrogen and carbon at a distance of 2 to 4 bonds. 2D NMR studies (HSQC, HMBC) were performed for the compound 4d i.e. the compound having the highest activity among the synthesized compounds (Figure 1 and Figure 2). In the light of the data obtained from HSQC, it was find that methyl group gave proton peaks at 0.90 ppm gave carbon peak at 22.19 ppm. The carbon of the piperidine group of which its hydrogens were resonated at 1.09-1.22 ppm and 1.63-1.67 ppm yielded peaks at 33.64 ppm. The carbon peak of the piperidine group attached to the methyl group, of which its protons resonated at 1.48-1.55 ppm was observed at 22.19 ppm. It was observed that the carbon peaks of the piperidine group, of which its protons at 2.68-2.75 ppm and 3.74-3.79 ppm, were observed at 48.20 ppm. The peak of methylene group carbon was observed at 35.79 ppm. Using HSQC data, it was found that the carbons coded a, c, k, 1 were resonated at 121.06 ppm, 124.86 ppm, 128.63 ppm and 152.47 ppm, respectively. In the light of the information obtained from HMBC, the carbons coded e, f, g, h, i, j and m were found to be 153.98 ppm, 131.65 ppm, 168.08 ppm, 148.18 ppm, 123.66 ppm and 152.47 ppm, respectively.

Antifungal Activity

In vitro antifungal activities of all obtained compounds (4a-4j) were evaluated against four pathogenic fungi (*C. albicans* (ATCC 90030), *C. glabrata* (ATCC 2001), *C. krusei* (ATCC 6258), *C. parapsilosis* (ATCC 22019) according to the EUCAST protocol. Ketoconazole and fluconazole were used as reference drugs. The activity results obtained are presented in Table-1. The MIC_{50} value of the compound 4c was observed as 7.81 µg / ml against *C. krusei*. Compound 4d were determined to have MIC_{50} value of 1.95 µg/ml against *C. krusei*. This MIC_{50} value is the same as the MIC_{50} value of reference drugs. As a result of the activity studies, it was found that compounds carrying chlorine substituents (4c, 4d) on the benzothiazole ring were more effective than the compounds bearing methoxy substituent (4h, 4i) on the benzothiazole ring.



Scheme 1. Synthesis pathway of target compounds

Bileşik	R_1	R_2	R_3	R_4	X	%Verim
4a	-H	-H	-H	-Cl	-CH	86
4b	$-CH_3$	-H	-H	-Cl	-CH	83
4c	-H	$-CH_3$	-H	-Cl	-CH	85
4d	-H	-H	$-CH_3$	-Cl	-CH	88
4e	-H	-H	4-methoxyphenyl	-Cl	-N	82
4f	-H	-H	-H	$-OCH_3$	-CH	85
4g	$-CH_3$	-H	-H	$-OCH_3$	-CH	82
4h	-H	$-CH_3$	-H	$-OCH_3$	-CH	85
4i	-H	-H	$-CH_3$	$-OCH_3$	-CH	82
4j	-H	-H	4-methoxyphenyl	$-OCH_3$	-N	85

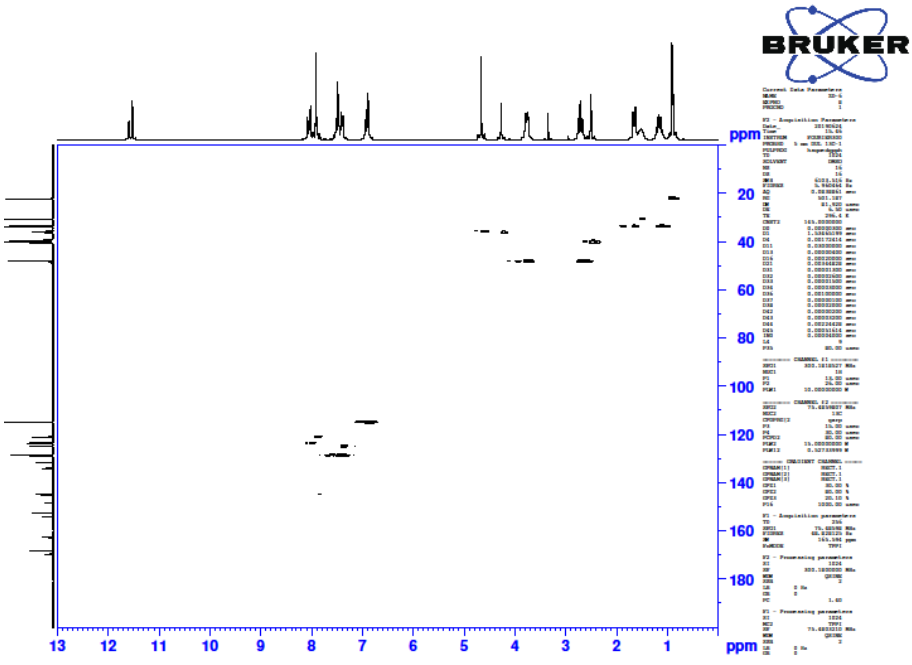


Figure 1. HSQC spectra of compound 4d.

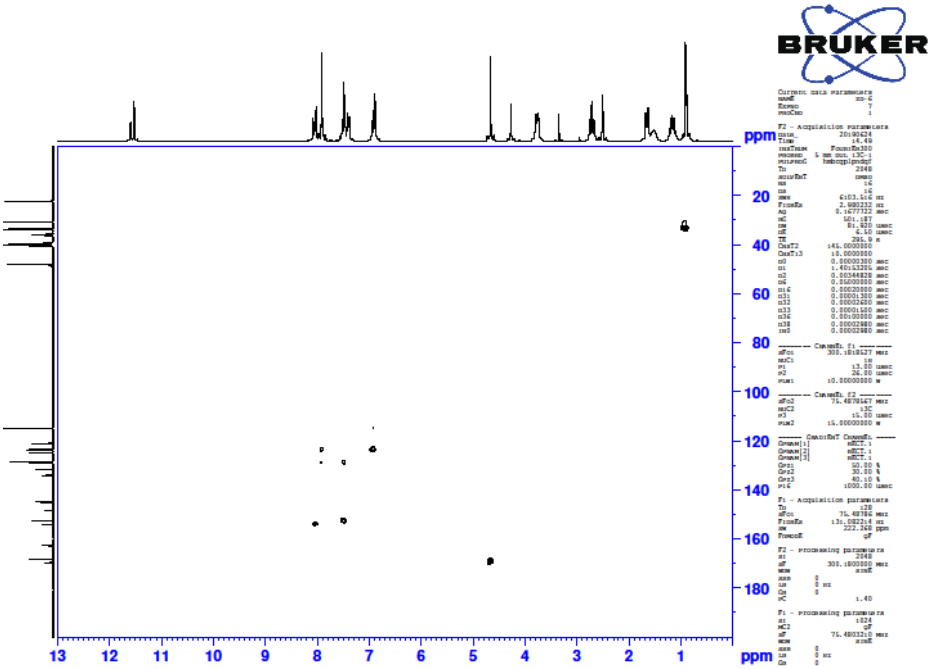


Figure 2. HMBC spectra of compound 4d

Table 1. MIC_{50} (µg/mL) values of compounds **4a-4j** and reference drugs.

Comp.	*C. albicans*	*C. glabrata*	*C. krusei*	*C. parapsilosis*
4a	>1000	>1000	>1000	>1000
4b	>1000	>1000	>1000	>1000
4c	>1000	125	7.81	>1000
4d	>1000	125	**1.95**	>1000
4e	>1000	>1000	>1000	>1000
4f	>1000	>1000	>1000	>1000
4g	>1000	>1000	>1000	>1000
4h	>1000	>1000	>1000	>1000
4i	>1000	>1000	>1000	>1000
4j	>1000	>1000	>1000	>1000
Ketoconazole	0.98	1.95	1.95	1.95
Fluconazole	0.98	1.95	1.95	0.98

CONCLUSION

In summary, 10 benzothiazole-hydrazone derivatives that were synthesized previously were re-synthesized for their potential utilisation in candidal infections in animals and their in vitro antifungal activities were evaluated. Structure elucidation of the synthesized compounds was performed using spectroscopic methods (IR, ^{1}HNMR, ^{13}CNMR, HRMS) and it was found to be in agreement with the previous study. In addition, structure determination was completed using 2D NMR technique (HMBC, HSQC). As a result of the activity studies, it is found that compound 4d was the most active derivative in the series with MIC_{50} value of 1.95 µg/ml. This information suggests that derivatives carrying chlorine substituents on benzothiazole are more active than derivatives bearing methoxy substituents, and the methyl group at position 4 of the piperidine ring contributes to activity.

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