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Antifungal Activity of Some Benzimidazole-Hydrazones

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Abstract. In the present work, 15 4-(1-(prop-2-in-1-yl)-1*H*-benzoimidazole-2-yl)-*N*'-(substitutedmethylene)benzohydrazide derivatives (**4a**-**4o**) were re-synthesized to evaluate their antifungal activity. Structure identification of synthesized compounds was elucidated by ^{1}H-NMR, ^{13}C-NMR, and HRMS spectroscopic methods. Inhibitory potential of the re-synthesized compounds was investigated against *Candida supp*. The compounds **4e** and **4l** showed significant anti fungal activity. Consistent with the activity studies, **4e** was found to be potent derivative with its MIC_{50} value of (1.95 µg/mL) against *Candida glabrata*. And **4l** was found to be potent derivative with its MIC_{50} value of (1.95 µg/mL) against *Candida krusei*.

***Keywords*:** Benzimidazole, Hydrazone, Antifungal activity, NMR, HRMS

Bazı Benzimidazol-Hidrazonların Antifungal Etkinliği

Özet. Mevcut çalışmada, 15 4-(1-(prop-2-in-1-il)-1*H*-benzimidazol-2-il)-*N*'-(sübstitüemetilen) benzohidrazit türevleri **(4a**-**4o),** antifungal aktivitelerini değerlendirmek üzere yeniden sentezlendi. Sentezlenen bileşiklerin yapı tanımlamaları ^{1}H-NMR, ^{13}C-NMR ve HRMS spektroskopik yöntemlerle açıklandı. Yeniden sentezlenen bileşiklerin antifungal etkinlikleri Candida türlerine karşı değerlendirildi. Bileşik **4e** ve **4l** önemli aktivite gösterdi. Aktivite çalışmaları ile uyumlu olarak, **4e** bileşiği *Candida glabrata*'ya karşı 1.95 µg / mL MIC_{50} değeri ile güçlü bir türev olarak bulundu. Aynı zamanda **4l** bileşiği *Candida krusei*'ye karşı 1.95 µg / mL MIC_{50} değeri ile güçlü bir türev olarak bulundu.

***Anahtar Kelimeler*:** Benzimidazol, Hidrazon, Antifungal aktivite, NMR, HRMS

1. INTRODUCTION

Life loss depending on fungal infections are more than 1.35 million per year worldwide [1]. *Aspergillus spp*., *Cryptococcus neoformans* and *Candida spp*. are still the three main pathogens of fungal infections. Over the last few decades, the occurrence of systemic fungal infections has increased considerably in immunocompromised hosts, particularly patients who receive cancer chemotherapy or patients with AIDS and undergone organ transplantation [1-3]. Some research teams stated that *Candida albicans* (*C. albicans*) which caused severe mucosal infections with potentially fatal invasive infections was the most common fungus in these patients [4, 5]. On the other hand, only a restricted number of antifungal agents are formerly accessible to treat these life-threatening fungal infections [6]. Presently, clinical antifungal agents can be separated into four classes: Echinocandins (such as Micafungin and Caspofungin), antimetabolites (such as 5-fluorocytosine) polyenes (such as Nystatin and Amphotericin B) and azoles (such as Itraconazole and Fluconazole) [2,3].

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Azoles inhibits lanosterol 14-α-demetilase enzyme; which is responsable for converting lanosterol into ergosterol. Ergosterol is the major component of fungal cytoplasmic membranes and responsable for bioregulation of membrane assymmetry [7]. Azoles are first-line drug for fungal infections due to their wide antifungal spectrum, high potency and low toxicity [8]. However, some problems remain, such as severe drug resistance, cytotoxicity. However, emergence of drug resistance, environmental hazards and other problems objectively indicate the urgent need for novel antifungal agents [2,9].

Nitrogen heterocycles are common structural motifs in compounds with antifungal activity [4]. Benzimidazole that was an important heterocyclic nucleus that has been used extensively in medicinal chemistry is a component of vitamin B12 and is related to the DNA base purine and the stimulant caffeine. Substituted benzimidazole displays a broad spectrum of potential pharmacological activities such as antiinflammatory, anti-tubercular, anticancer, antibacterial, antiviral and antifungal activity. [5-7, 9, 10-17]. In addition to benzimidazole derivatives, hydrazone based compounds, which bear an azomethine -NHN=CH- functional group, have showed antifungal activity [18,19].

Based on the above information, antifungal activities of the compounds containing benzimidazole and hydrazone residues were the subject of curiosity. For this purpose, we resynthesized some benzimidazole-hydrazone based upon the studies that reported the antifungal potentials [20].

2. MATERIALS AND METHODS

2.1. Chemistry

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). Melting points of the obtained compounds were determined by MP90 digital melting point apparatus (Mettler Toledo, OH, USA) and were uncorrected. The IR spectra were obtained on a Shimadzu, IR Prestige-21 (Shimadzu, Tokyo, Japan). ^{1}H NMR and ^{13}C NMR spectra of the synthesized compounds were registered by a Bruker 300 MHz and 75 MHz digital FT-NMR spectrometer (Bruker Bioscience, Billerica, MA, USA) in DMSO-d_6, respectively. Splitting patterns were designated as follows: s: singlet; d: doublet; t: triplet; m: multiplet in the NMR spectra. Coupling constants (J) were reported as Hertz. M+1 peaks were determined by Shimadzu LC/MS ITTOF system (Shimadzu, Tokyo, Japan). All reactions were monitored by thin-layer chromatography (TLC) using Silica Gel 60 F254 TLC plates (Merck KGaA, Darmstadt, Germany).

2.1.1. General procedure for the synthesis of target compounds **(4a-4o)**

In order to determine the antifungal activity, synthesis studies were carried out for compound **4a**-**4o**. Synthesis studies was performed by means of previous study reported by our research group [20].

2.2. Antifungal Activity

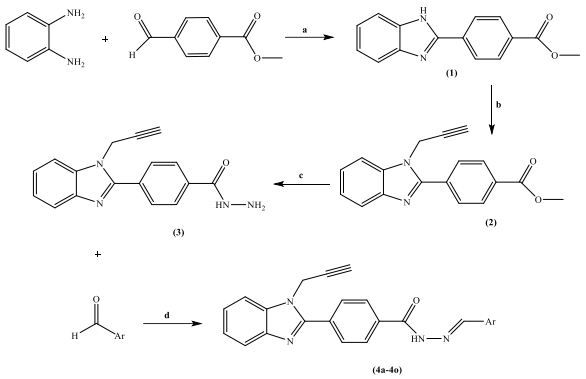
The *in vitro* antifungal activities of all resynthesized derivatives **4a**–**4o** were screened at between 1 mg/mL–1.95 µg/mL concentrations using various Candida strains including *C. albicans (ATCC 90030), C. glabrata (ATCC 90030) C. krusei (ATCC 6258)* and *C. parapsilopsis (ATCC 22019)* and following the protocol of the EUCAST in keeping with the previous studies reported by our research group [21, 22].

3. RESULTS AND DISCUSSIONS

3.1. Chemistry

The compounds **4a**-**4o** were re-synthesized as summarized in **Scheme 1**. Compound **1** was synthesized under microwave irradiation using 1,2-phenylenediamine and methyl 4-formylbenzoate. Reaction of Methyl 4-(1*H*-benzo[d]imidazol-2-yl)benzoate and propargyl bromide gave the compound **2**. Reaction of the methyl 4-(1-(prop-2-in-1-yl)-1*H*-benzo[d]imidazole-2-yl)benzoate (**2)** and hydrazine hydrate gave 4-(1-(prop-2-in-1-yl)-1*H*-benzo[d]imidazole-2-yl)benzohydrazide (**3**). Reaction of compound 3 and aldehydes afforded to the target compounds (**4a**-**4o**).

Structural elucidation of the synthesized compounds (**4a-4o**) was carried out by spectral analyses including IR, ^{1}H-NMR, ^{13}C-NMR and HRMS. All the results of the analysis are in accordance with our previous report [20].



Comp.	Ar	Comp.	Ar
4a	Thiophen-2-yl	**4i**	4-Methoxyphenyl
4b	5-Nitro-thiophen-2-yl	**4j**	2-Methoxyphenyl
4c	Pyrrol-2-yl	**4k**	3-Methoxyphenyl
4d	1-Methyl-pyrrol-2-yl	**4l**	3-Hydoxyphenyl
4e	Pyridin-3-yl	**4m**	3,5-Dihydoxyphenyl
4f	4-Fluorophenyl	**4n**	3,5-Dimethoxyphenyl
4g	4-Chlorophenyl	**4o**	2,3-Dimethoxyphenyl
4h	4-Hydroxyphenyl		

Scheme 1: Synthesis way of the compounds **4a-4o**. **Reagents and Conditions: a:** $Na_2S_2O_5$ /DMF, Microwave irradiation, 240 ^{o}C, 10 bar, 5 + 5 min. **b:** NaH/DMF, propargyl bromide, r.t, 20 h. **c:** $NH_2NH_2.H_2O$ (%100), Microwave irradiation, 150^{o}C, 10 bar, 10 min. **d:** EtOH/CH_3COOH, reflux, 2 h.

Table 1. MIC_{50} (µg/mL) values of compounds **4a**-**4o**.

Comp.	*C. albicans*	*C. glabrata*	*C. krusei*	*C. parapsilosis*
4a	>1000	>1000	62.50	>1000
4b	>1000	>1000	>1000	>1000
4c	>1000	>1000	62.50	>1000
4d	**1.95**	>1000	>1000	>1000
4e	125	**1.95**	>1000	>1000
4f	>1000	>1000	>1000	>1000
4g	>1000	>1000	>1000	>1000
4h	**1.95**	>1000	>1000	15.63
4i	>1000	>1000	>1000	>1000
4j	**1.95**	>1000	>1000	>1000
4k	>1000	>1000	>1000	>1000
4l	15.63	>1000	**1.95**	>1000
4m	>1000	125	7.81	>1000
4n	>1000	>1000	>1000	>1000
4o	>1000	>1000	31.25	>1000
Ketoconazole	0.98	1.95	1.95	1.95
Fluconazole	0.98	1.95	1.95	0.98

3.2. Antifungal Activity

According to the protocol of the EUCAST reported previously by our research group [21, 22], all obtained compounds **4a-4o** were screened for their *in vitro* antifungal activity against four pathogenic fungi; *C. albicans (ATCC 90030), C.glabrata (ATCC 2001), C. krusei (ATCC 6258), C. parapsilosis (ATCC 22019*). Ketoconazole and fluconazole were used as reference drugs. The results of antifungal activities are listed in **Table-1**. Compounds **4d**, **4h** and **4j** were determined to have MIC_{50} values of 1.95 µg/ml against *C. albicans*. On the other hand, Compounds **4e** and

4l were determined to have MIC_{50} values of 1.95 µg/ml against *C. glabrata* and *C. krusei*, respectively. This MIC_{50} values is the same as the MIC_{50} values of reference drugs against *C. glabrata* and *C. krusei*. Compound **4e** bearing pyridin-3-yl possessed good activity against *C. glabrata* with an MIC value of 1.95 µg/mL. Compound **4l** including 3-hydroxyphenyl possessed good activity against *C. krusei* with an MIC value of 1.95 µg/mL. However compound **4m** bearing 3,5-dihydoxyphenyl had weak antifungal activity. This information suggests that the hydroxy group brought to the fifth position of phenyl ring reduces the antifungal activity. The lack of activity of the methoxy-containing derivative in the third position of phenyl (**4k**) indicates that the replacement of the hydrogen and methyl group of the hydroxyl group decreases the This information suggests that hydroxyl hydrogen in the third position of phenyl ring enhances activity propably by making hydrogen bonds.

4. CONCLUSION

In summary, a series of benzimidazole-hydrazone derivatives were re-synthesized and evaluated for their antifungal activity. The primary results indicated that obtained compounds **4a**–**4o** displayed antifungal activities within different ranges. Compounds **4e** and **4l** displayed significant activity against *C. glabrata* and *C. krusei*, respectively. Compound **4e** bearing pyridine-3-yl and compound **4l** bearing 3-hydroxyphenyl came to the fore in the series. We assume that the aromatic ring bound to the hydrazone group contributes to the antifungal activity by means of long-pair electron in third position. However, if the hydroxyl group at position third of phenyl ring is replaced by a methoxy group, the activity decreases activity. This information suggests that the steric hindrance of the methyl group abolishes the effect of the long-pair electron, or that the hydroxyl hydrogen's ability to form hydrogen bonds contributes to activity.

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