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Potansiyel Antifungal Ajanlar Olarak Yeni Benzotiyazol-Ditiyokarbamat Türevlerinin Sentezi ve Karakterizasyonu

Synthesis and Characterization of New Series Benzothiazole-Dithiocarbamate Derivatives As Potential Antifungal Agents

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Öz

İnvaziv mantar enfeksiyonları (IFI'lar) insan için büyük bir tehdit olmuştur ve olmaya devam edecektir. Günümüzde birçok tıbbi araştırmanın amacı yeni daha güvenli ve daha etkili antifungal ajanlar bulmaktır. Benzotiyazol bileşikleri, çok çeşitli biyolojik aktiviteleri nedeniyle farmasötik kimyada önemli bir farmakofor yapıdır. Ayrıca, ditiyokarbamat bileşikleri kimyasal yapıları nedeniyle birçok aktivite için önemli hale gelmiştir. Bu çalışmada, yeni sentezlenen benzotiyazol-ditiyokarbamat bileşiklerinin antifungal etkinliklerinin belirlenmesi amaçlanmıştır. Elde edilen bileşiklerin yapıları, ^{1}H-NMR, ^{13}C-NMR ve kütle spektroskopisi kullanılarak aydınlatılmıştır.

Anahtar Kelimeler: *Benzotiyazol, Ditiyokarbamat, NMR, Antifungal aktivite*

Abstract

Invasive fungal infections (IFIs) have been and will continue to be a great threat to human. Nowadays the aim of many medical research is to discover new safer and more effective antifungal agents. Benzothiazole compounds are important fragments in medicinal chemistry because of their wide range of biological activities. Furthermore, dithiocarbamate compounds have become important for many activities due to their chemical structure. In this study, it was aimed to determine being antifungal agents capacity of newly synthesized benzothiazole compounds including dithiocarbamate moiety. The structures of the obtained compounds were elucidated using by ^{1}H-NMR, ^{13}C-NMR and mass spectroscopy.

Keywords: *Benzothiazole, Dithiocarbamate, NMR, Antifungal activity*

1. Giriş

Invasive fungal infections (IFIs) have been and will continue to be a great threat to human. The growing practice of organ and hematopoietic cell transplantation and the increasing use of immunosuppressive, antiviral, and antineoplastic therapies opens the door to adventitious systemic fungal infections. The incidence fungal infections has increased markedly in humans, which are considered as human "hidden killers" and frequently happen in immunocompromised humans such as patients undergoing anticancer chemotherapy or organ transplants and patients with AIDS. It is estimated that more than 300 million people suffer from IFIs. There is also a dramatic reality in the world that results in the death of more than 1 million infected people each year. The high mortality rates for mycoses such as candidiasis and aspergillosis underscore the need for new antifungal therapies. Furthermore, drug-resistant strains of fungal infections, the most common fungal pathogens of the genus Candida, in particular *Candida albicans* and *Candida glabrata*, are increasing. Most of the antifungal drugs in the clinic have serious side effects, and the use of irregular and unnecessary antibiotics has brought resistance to pathogenic fungi. The World Health Organization has launched a global action plan that calls on all countries to take measures against drug-resistant microbes, and the discovery of effective and safer antimicrobials with new or multiple mechanisms of action has been an urgent need to combat resistant strains. Therefore, developing an antifungal drug against new, safe and resistant strains has been an important field of study for researchers [1-11].

Benzothiazole scaffold possess a broad range of biological activities such as anticonvulsant, anti-inflammatory, antitumor, antipsychotic, schictosomicidal, diuretic, antidiabetic, carbonic anhydrase inhibitor and antimicrobial activities [12-17]. Moreover, dithiocarbamates have gained much attention because oftheir interesting chemistry and extensive utility and they have also been used in medicine because they have been found in a variety of biologically active compounds [18].

From this point of view, in the present study new benzothiazole derivatives including dithiocarbamate moiety were synthesized by aiming at the identification of new chemical entities and evaluated antifungal activity against *Candida spp*..

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 synthesized compounds were measured by MP90 digital melting point apparatus (Mettler Toledo, Ohio, USA) and were presented as uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded by a Bruker 300 MHz and 75 MHz digital FT-NMR spectrometer (Bruker Bioscience, Billerica, MA, USA) in DMSO-d_6, respectively. In the NMR spectra splitting patterns were designated as follows: s: singlet; d: doublet; t: triplet; m: multiplet. Coupling constants (*J*) were reported as Hertz. LC-MS-MS studies were performed on a Schimadzu, 8040 LCMSMS spectrophotometer (Shimadzu, Tokyo, Japan). The purities of compounds were checked by TLC on silica gel 60 F254 (Merck KGaA, Darmstadt, Germany).

2.1.1. Synthesis of 2-chloro-N-(6-substitutedbenzothiazol-2-yl)acetamide (**1a-1c**)

Chloroacetyl chloride was added as dropwise at a mixture of 6-substituebenzothiazol-2-amine (0.6 gr, 0.004 mol) and TEA (0.692 ml) in THF (10 ml) at iced-bath. After completion of reaction, tetrahydrofuran was removed under reduced pressure, precipitated product was washed with water in order to removed obtained salts.

2.1.2. Synthesis of sodium 4-(4-substitutedbenzyl)piperazine-1-carbodithioate (**2a, 2b**)

A mixture of carbon disulphide in ethanol was added to the mixture of appropriate piperazine (0.005 mol) and NaOH in EtOH (10 mL) and stirred at ice-bath for 2h. After the reaction was complete, the precipitated product was filtered, washed with diethylether and dried.

2.1.3. Synthesis of target compounds (**3a-3f**)

2-chloro-*N*-(6-substitutedbenzothiazol-2-yl)acetamide (**1a, 1b**) (0.0013 mol), sodium 4-(4-substitutebenzyl)piperazine-1-carbodithioate **(2a, 2b)** (0.0013 mol) were stirred for 4h in acetone. After completion of the reaction, acetone was evaporated under reduced pressure. The precipitated product was washed with water in order to removed uncovered salt, dried and recrystallized from EtOH.

2-(Benzothiazol-2-ylamino)-2-oxoethyl 4-(4-methylbenzyl)piperazine-1-carbodithioate (**3a**)

Yield: 81 %, ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.28 (3H, s, -CH_3), 2.44-2.47 (4H, m, piperazine), 3.48 (2H, s, -CH_2-), 3.93-4.19 (4H, m, piperazine), 4.43 (2H, s, -CH_2-), 7.13 (2H, d, *J*=7.8 Hz, 1,4-disubstitutebenzene), 7.20 (2H, d, *J*=7.9 Hz, 1,4-disubstitutebenzene), 7.27-7.33 (1H, m, Benzothiazole-H), 7.41-7.46 (1H, m, Benzothiazole-H), 7.75 (1H, d, *J*=7.9 Hz, Benzothiazole-H), 7.97 (1H, d, *J*=7.6 Hz, Benzothiazole-H), 12.57 (1H, s, -NH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 21.19, 40.40, 50.41, 51.77, 52.32, 61.48, 121.02, 122.17, 124.02, 126.59, 129.29, 129.41, 131.89, 134.85, 136.66, 149.01, 158.39, 167.41, 194.37. ESI-MS $[M+H]^+$: 457

2-(Benzothiazol-2-ylamino)-2-oxoethyl 4-(4-methoxybenzyl)piperazine-1-carbodithioate (**3b**)

Yield: 81 %, ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.44-2.46 (4H, m, piperazine), 3.46 (2H, s, -CH_2-), 3.73 (3H, s, -OCH_3), 3.94-4.19 (4H, m, piperazine), 4.42 (2H, s, -CH_2-), 6.89 (2H, d, *J*=8.6 Hz, 1,4-disubstitutebenzene), 7.22 (2H, d, *J*=8.6 Hz, 1,4-disubstitutebenzene), 7.30-7.33 (1H, m, Benzothiazole-H), 7.41-7.46 (1H, m, Benzothiazole-H), 7.75 (1H, d, *J*=7.9 Hz, Benzothiazole-H), 7.97 (1H, d, *J*=7.6 Hz, Benzothiazole-H), 12.65 (1H, s, -NH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 40.36, 50.40, 51.74, 52.23, 55.48, 61.12, 114.08, 121.03, 122.17, 124.04, 126.61, 129.70, 130.70, 131.87, 148.99, 158.35, 158.88, 167.40, 194.35. ESI-MS $[M+H]^+$: 473

2-((6-methoxybenzothiazol-2-yl)amino)-2-oxoethyl 4-(4-methylbenzyl)piperazine-1-carbodithioate (**3c**)

Yield: 84 %, ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.28 (3H, s, -CH_3), 2.43-2.46 (4H, m, piperazine), 3.47 (2H, s, -CH_2-), 3.79 (3H, s, -OCH_3), 3.94-4.20 (4H, m, piperazine), 4.40 (2H, s, -CH_2-), 7.02 (1H, dd, J_1=2.5 Hz, J_2=8.8 Hz, Benzothiazole-H), 7.13 (2H, d, *J*=7.9 Hz, 1,4-disubstitutebenzene), 7.19 (2H, d, *J*=7.9 Hz, 1,4-disubstitutebenzene), 7.55 (1H, d, *J*=2.5 Hz, Benzothiazole-H), 7.63 (1H, d, *J*=8.8 Hz, Benzothiazole-H). ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 21.16, 50.38, 51.66, 52.31, 56.04, 61.48, 105.16, 115.33, 121.58, 129.28, 129.29, 129.41, 133.23, 134.85, 136.65, 143.13, 156.55, 167.17, 194.46. ESI-MS $[M+H]^+$: 487.

2-((6-methoxybenzothiazol-2-yl)amino)-2-oxoethyl 4-(4-methoxybenzyl)piperazine-1-carbodithioate (**3d**)

Yield: 79 %, ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.45-2.46 (4H, m, piperazine), 3.45 (2H, s, -CH_2-), 3.73 (3H, s, -OCH_3), 3.80 (3H, s, -OCH_3), 3.95-4.19 (4H, m, piperazine), 4.41 (2H, s, -CH_2-), 6.88 (2H, d, *J*=8.6 Hz, 1,4-disubstitutebenzene), 7.02 (1H, dd, J_1=2.6 Hz, J_2=8.8 Hz, Benzothiazole-H), 7.22 (2H, d, *J*=8.6 Hz, 1,4-disubstitutebenzene), 7.55 (1H, d, *J*=2.6 Hz, Benzothiazole-H), 7.63 (1H, d, *J*=8.8 Hz, Benzothiazole-H), 12.52 (1H, s, -NH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 50.31, 51.66, 52.20, 55.47, 56.04, 56.08, 61.10, 105.16, 114.07, 115.39, 121.64, 129.61, 130.71, 133.21, 143.08, 156.30, 156.61, 158.89, 167.06, 194.41. ESI-MS $[M+H]^+$: 503.

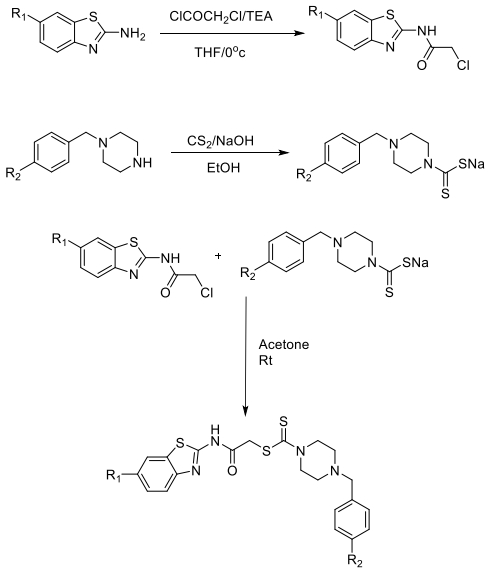
2.2. Antifungal Activity

The *in vitro* antifungal activities of all resynthesized derivatives **3a**–**3d** 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 [19,20].

3. Results and Discussion

3.1. Chemistry

In this study, we have synthesized new compounds, which possessed Benzothiazole-dithiocarbamate moiety. The synthesis procedure was carried out via three steps. And synthetic pathway was outlined in Scheme 1. Firstly, 2-chloro-N-(6-substitutebenzothiazol-2-yl)acetamide derivatives (**1a, 1b**) were obtained by means of acetylation reaction. Secondly, dithiocarbamate derivatives (**2a, 2b**) were obtained result of reaction between carbonsulfide and secondary amines. Finally, target compounds (**3a**-**3f**) were obtained with the reaction between compounds **1a-1b** and compounds **2a, 2b**. The final compounds were purified, and their structures were characterized by spectroscopic methods (^{1}H-NMR, ^{13}C-NMR, and LCMSMS). The ^{1}H-NMR spectra of compounds showed signals at 2.43-4.20 ppm for piperazine protons. N-H had peaks between 12.52 ppm and 12.65 ppm. In the ^{13}C NMR spectrum, aliphatic peaks belonging to substituents were observed between 21.16 ppm and 61.48 ppm. Aromatic carbons were gained between 105.16 ppm and 194.46 ppm. All masses were accorded with the estimated M+H values.



Comp.	R_1	R_2
3a	-H	4-Methylbenzyl
3b	-H	4-Methoxybenzyl
3c	-OCH_3	4-Methylbenzyl
3d	-OCH_3	4-Methoxybenzyl

Scheme 1: Synthesis way of the compounds **3a-3d**.

3.2. Antifungal Activity

According to the protocol of the EUCAST reported previously by our research group [20,21], all obtained compounds **3a**-**3d** 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. The minimal inhibitory concentration (MIC) values of the compounds range between 100-800 µg/ml. The tested compounds possessed moderate or weak antifungal activities.

Table 1. MIC_{50} (µg/mL) values of compounds **3a**-**3d**.

Comp.	*C. albicans*	*C. glabrata*	*C. krusei*	*C. parapsilosis*
3a	800	800	100	100
3b	800	800	100	800
3c	800	800	100	800
3d	800	400	100	200
Ket.	0.98	1.95	1.95	1.95
Flu.	0.98	1.95	1.95	0.98

4. Conclusion

In summary, a series of benzothiazole-dithiocarbamate derivatives were synthesized and evaluated for their antifungal activity. The antifungal activity potential of the synthesized compounds (**3a-3d**) against four fungus strains at various concentrations (800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.75, 1.875 µg/ml) was evaluated. All compounds tested showed antifungal activity against *C. krusei* with a MIC of µg/mL

Teşekkür

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