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Short communication

ANTIFUNGAL EFFECTS OF SOME OXADIAZOLE DERIVATIVES ON PATHOGENIC MOLDS

Zafer Asim KAPLANCIKLI[1,*], Mehlika Dilek ALTINTOP[1], Rasime DEMİREL[2]

[1]Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 26470 Eskişehir, TURKEY

[2]Anadolu University, Faculty of Science, Department of Biology, 26470 Eskişehir, TURKEY

Abstract

*Some oxadiazole derivatives were tested in vitro against Aspergillus parasiticus, Aspergillus flavus, Aspergillus niger, Fusarium solani, Fusarium moniliforme and Stachybotrys chartarum. All compounds showed the highest antifungal activity against S. chartarum. Among these compounds (**3a-j**), compounds **3a**, **3c**, **3d**, **3e**, **3i**, **3j** exhibited the same level of antifungal activity against all tested mold species. Compounds **3a**, **3c**, **3d**, **3e** possess 4-methoxyphenoxymethyl moiety on oxadiazole ring, whereas compounds **3i** and **3j** carry 2-cyclohexylethyl moiety on oxadiazole ring.*

Key words: *Oxadiazole, Antifungal activity, Aspergillus, Fusarium.*

Bazı Oksadiazol Türevlerinin Patojenik Küfler üzerindeki Antifungal Etkileri

*Bazı oksadiazol türevleri Aspergillus parasiticus, Aspergillus flavus, Aspergillus niger, Fusarium solani, Fusarium moniliforme and Stachybotrys chartarum'a karşı in vitro olarak test edilmiştir. Bütün bileşikler en yüksek antifungal etkiyi S. chartarum'a karşı göstermiştir. Bu bileşikler (**3a-j**) arasında, **3a, 3c, 3d, 3e, 3i** ve **3j** bileşikleri test edilen küf türlerine karşı aynı seviyede antifungal etki göstermiştir. **3a**, **3c**, **3d**, **3e** bileşikleri oksadiazol halkası üzerinde 4-metoksifenoksimetil parçasına sahipken, **3i** ve **3j** bileşikleri oksadiazol halkası üzerinde 2-siklohekziletil parçası taşımaktadır.*

Anahtar kelimeler: *Oksadiazol, Antifungal etki, Aspergillus, Fusarium.*

***Correspondence:** E-mail: zakaplan@anadolu.edu.tr; Tel: +90-222-3350580/3776;

INTRODUCTION

Systemic fungal infections due to pathogenic yeasts and molds have emerged as important causes of morbidity and mortality in immunocompromised patients (1).

Aspergillus species, which cause a wide range of diseases including allergic syndromes, chronic pulmonary and nasal sinus aspergillosis and acute and subacute invasive disease, are the second most frequent cause of systemic fungal infections after *Candida* species. Among the human pathogenic species of *Aspergillus*, *A. fumigatus* is the primary causative fungus of human infections, followed by *A. flavus*, *A. terreus*, *A. niger*, and the model organism, *A. nidulans* (2-5).

Fusarium species also cause a broad spectrum of infections, including superficial, locally invasive, or disseminated infections in immunocompromised patients and allergic diseases (sinusitis) in immunocompetent individuals and mycotoxicosis in humans and animals following the ingestion of food contaminated by toxin-producing *Fusarium* species (6).

There are many drugs belonging to different chemical classes (polyenes, pyrimidines, azoles, and echinocandins) as therapeutic options for fungal infections, the treatment of these infections still remains a challenging problem due to narrow spectrum of activity, low tolerability, or high toxicity accompanying the use of these agents.

Azoles, especially triazoles, play a leading role in the treatment of systemic fungal infections owing to their broad spectrum and improved safety profile. But the widespread use of these drugs has led to the development of resistance in recent years. As a consequence of this situation, medicinal chemists focused on the development of more effective agents with fewer adverse effects (7-10).

Many researchers have focused on oxadiazoles due to the fact that new effective compounds can be obtained by the bioisosteric replacement of triazole ring with oxadiazole ring. Some studies have confirmed that oxadiazole derivatives possess antifungal activity (11-20).

Previously we reported the discovery of new oxadiazole derivatives (Table 1), which were tested *in vitro* against various bacteria species and *Candida albicans* (21). In continuation of our previous work, herein we evaluated antifungal effects of these compounds on pathogenic molds.

EXPERIMENTAL

Chemistry

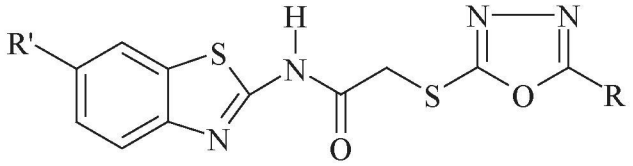
5-Substituted-1,3,4-oxadiazolin-2-thiones were synthesized via the ring closure reactions of appropriate acid hydrazides with carbon disulphide. *N*-(Benzothiazol-2-yl)-2-[[5-substituted-1,3,4-oxadiazol-2-yl]sulfanyl]acetamide derivatives (**3a-j**) were obtained by the nucleophilic substitution reactions of 5-substituted-1,3,4-oxadiazolin-2-thiones with *N*-(benzothiazol-2-yl)-2-chloroacetamides in the presence of potassium carbonate. The synthetic protocol and spectral data of the compounds (**3a-j**) were reported previously by our research group (21).

Microbiology

The antifungal activities of the compounds (**3a-j**) were tested using the microbroth dilution method with some modifications (22,23,26). Tested fungal strains were *Aspergillus parasiticus* (NRRL-465), *Aspergillus flavus* (NRRL-980), *Aspergillus niger* (ATCC-1094), *Fusarium solani* (NRRL-13414), *Fusarium moniliforme* (NRRL 1866) and *Stachybotrys chartarum* (wild culture) (24). Microbroth dilution-susceptibility assay was used for antifungal evaluation of the compounds. Stock solutions of the samples were prepared in dimethyl sulfoxide. Dilution series using sterile distilled water were prepared from 4 mg/mL to 0.0039 mg/mL in micro-test tubes that were transferred to 96-well microtiter plates. Fungal strains grown on Potato Dextrose Agar (PDA) at 25 °C for 5 suspensions in double-strength Potato Dextrose Broth (PDB) were standardized to 10^5 spores/mL with Thoma counting slide. Hundred microliter of each spore

suspension was then added into the wells. The last well-chain without a fungus was used as a negative control. Sterile distilled water and the medium served as a positive growth control. After incubation at 25 ºC for 48-72 h, antifungal activity was detected by investigation of mycelia growing under stereo microscope. Minimum inhibitory concentration (MIC) was defined as the lowest concentration of compounds that inhibited visible mycelia growth. Ketoconazole and nystatin were used as standard antifungal agents.

Table 1. The chemical structures of the test compounds (**3a-j**)



3a-j

Compound	R	R'	Molecular formula	Molecular weight
3a	4-methoxyphenoxymethyl	H	$C_{19}H_{16}N_4O_4S_2$	428
3b	4-methoxyphenoxymethyl	Cl	$C_{19}H_{15}ClN_4O_4S_2$	462.5
3c	4-methoxyphenoxymethyl	CH_3	$C_{20}H_{18}N_4O_4S_2$	442
3d	4-methoxyphenoxymethyl	OCH_3	$C_{20}H_{18}N_4O_5S_2$	458
3e	4-methoxyphenoxymethyl	OC_2H_5	$C_{21}H_{20}N_4O_5S_2$	472
3f	2-Cyclohexylethyl	H	$C_{19}H_{22}N_4O_2S_2$	402
3g	2-Cyclohexylethyl	Cl	$C_{19}H_{21}ClN_4O_2S_2$	436.5
3h	2-Cyclohexylethyl	CH_3	$C_{20}H_{24}N_4O_2S_2$	416
3i	2-Cyclohexylethyl	OCH_3	$C_{20}H_{24}N_4O_3S_2$	432
3j	2-Cyclohexylethyl	OC_2H_5	$C_{21}H_{26}N_4O_3S_2$	446

RESULTS AND DISCUSSION

In this study, all compounds were tested *in vitro* against *A. parasiticus*, *A. flavus*, *A. niger*, *F. solani*, *F. moniliforme* and *S. chartarum* and compared with ketoconazole and nystatin. The MIC values of the compounds and control drugs are given in Table 2.

All compounds (**3a-j**) showed less antifungal activity than ketoconazole and nystatin. The results indicated that all of the compounds (**3a-j**) exhibited the highest antifungal effect on *S. chartarum*, which is a significant mycotoxigenic airborne mold (25). The compounds (**3a-j**) exhibited the inhibitory activity against *S. chartarum* with a MIC value of 125 μg/mL, whereas ketoconazole exhibited the inhibitory activity with a MIC value of 31.25 μg/mL.

Table 2. Antifungal activities of the compounds (**3a-j**) (μg/mL)

Compound	A	B	C	D	E	F
3a	250	125	250	250	250	125
3b	250	250	250	250	250	125
3c	250	125	250	250	250	125
3d	250	125	250	250	250	125
3e	250	125	250	250	250	125
3f	500	125	250	250	500	125
3g	250	500	250	250	250	125
3h	250	250	250	250	250	125
3i	250	125	250	250	250	125
3j	250	125	250	250	250	125
Reference 1	7.81	15.62	31.25	62.5	31.25	31.25
Reference 2	1.95	3.90	7.81	3.90	3.90	1.95

Reference 1: Ketoconazole; **Reference 2:** Nystatin
A: *A. parasiticus* (NRRL 465), **B**: *A. flavus* (NRRL 980), **C**: *A. niger* (ATCC 1094), **D**: *F. solani* (NRRL 13414), **E**: *F. moniliforme* (NRLL 1866), **F**: *S. chartarum* (wild culture)

Among these compounds (**3a-j**), the compounds bearing 4-methoxyphenoxymethyl moiety on oxadiazole ring (**3a-e**) except **3b** are equally active against *A. flavus*. Although compounds **3f**, **3i** and **3j** do not carry 4-methoxyphenoxymethyl moiety, they showed the same level of antifungal activity against *A. flavus*. They showed the inhibitory activity against *A. flavus* with a MIC value of 125 μg/mL, whereas ketoconazole exhibited the inhibitory activity with a MIC value of 15.62 μg/mL.

All compounds except compound **3f** are equally active against *A. parasiticus*, which is an important food-borne aflatoxigenic mold and *F. moniliforme*, which is a plant pathogenic fungus (25). They exhibited the inhibitory activity with a MIC value of 250 μg/mL, whilst ketoconazole exhibited the inhibitory activity against *A. parasiticus* and *F. moniliforme* with MIC values of 7.81 μg/mL and 31.25 μg/mL, respectively.

All of the compounds exhibited the same level of inhibition against *A. niger* and *F. solani* with a MIC value of 250 μg/mL.

CONCLUSION

In conclusion, we evaluated the *in vitro* antifungal activities of some oxadiazole derivatives against *A. parasiticus*, *A. flavus*, *A. niger*, *F. solani*, *F. moniliforme* and *S. chartarum*.

The biological results indicated that *S. chartarum* was the most susceptible fungus to the compounds. All compounds were equally active against *S. chartarum*.

Among these derivatives (**3a-j**), all compounds except **3b, 3f, 3g, 3h** showed the same level of antifungal activity against all tested mold species. Compounds **3a**, **3c**, **3d**, **3e** carry 4-methoxyphenoxymethyl moiety on oxadiazole ring, whereas compounds **3i** and **3j** possess 2-cyclohexylethyl moiety on oxadiazole ring.

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