



Araştırma Makalesi/ Research Article

Synthesis and Antifungal Activity of New Heterocyclic Compounds

Yeni Heterosiklik Bileşiklerin Sentezleri ve Antifungal Etkileri

Zafer Asım Kaplıncıklı^{1,2}, Mehlika Dilek Altıntop², Gülhan Turan-Zitouni², Ahmet Özdemir², Rasime Demirel³, Usama Abu Mohsen⁴, Weiam Hussein¹

¹Anadolu University, Graduate School of Health Sciences, ²Pharmacy, ESKİŞEHİR

³Anadolu University, Faculty of Science, Eskişehir, Turkey

⁴Al-Azhar University, Faculty of Pharmacy, Gaza, Palestine

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ABSTRACT

Purpose: The objective of this study was to describe the synthesis of 2-arylthio-*N*-(2-methoxydibenzo[b,d]furan-3-yl)acetamide derivatives as heterocyclic compounds and focused on their potential antifungal effects against fungi species.

Method: The reaction of 2-chloro-*N*-(2-methoxydibenzo[b,d]furan-3-yl)acetamide with various aromatic thiols gave 2-arylthio-*N*-(2-methoxydibenzo[b,d]furan-3-yl)acetamide derivatives. Antifungal activities of the synthesized compounds have been evaluated using microbroth dilution method.

Results: Four new heterocyclic compounds were synthesized. All compounds were as effective as ketoconazole against *T. harzianum*, *A. ochraceus*, *C. albicans*, whilst the compounds were more effective than ketoconazole against *F. solani*, *F. moniliforme*, *F. culmorum*.

Conclusion: All synthesized compounds had significant antifungal activity when compared with ketoconazole.

Key Words: Antifungal activity, Microbroth dilution method, Dibenzofuran, Heterocyclic compound.

ÖZET

Amaç: Bu çalışmanın amacı, heterosiklik bileşik olarak, 2-arilthio-*N*-(2-metoksidibenzo[b,d]furan-3-il)asetamid türevlerinin sentezleri ve mantar türlerine karşı etkilerini araştırmaktır.

Yöntem: 2-Kloro-*N*-(2-metoksidibenzo[b,d]furan-3-il)asetamid ile uygun aromatik tiyollerin reaksiyonu 2-arilthio-*N*-(2-metoksidibenzo[b,d]furan-3-il)asetamid türevlerini verdi. Sentezlenen bu bileşiklerin antifungal aktiviteleri microbroth dilution method kullanılarak araştırıldı.

Bulgular: Dört yeni heterosiklik bileşik sentezlendi. Bileşiklerin tamamı *F. solani*, *F. moniliforme*, *F. culmorum*'a karşı Ketokonazol'den daha aktif çıkarken, *C. albicans*, *T. harzianum*, *A. ochraceus*'e karşı Ketokonazol ile aynı etkiyi göstermiştir.

Sonuç: Ketokonazol ile kıyaslandığında, sentezlenen tüm bileşikler önemli derecede antifungal etki göstermişlerdir.

Anahtar Kelimeler: Antifungal aktivite, Microbroth dilution method, Dibenzofuran, Heterosiklik bileşik.

INTRODUCTION

Invasive fungal infections are becoming increasingly implicated as a cause of crucial and fatal diseases. This is especially the case in immunocompromised patients, who have a tendency to infections caused by opportunistic fungal pathogens that are normally kept in check by a functioning immune system. Antifungal therapies for curative, pre-emptive or prophylactic intentions have been developed to overcome the threat of fungal infection, but have also led to the development of resistance to the currently available antifungal agents^{1,2,3}.

As known, not only biochemical similarity of the human cell and fungi forms is a handicap for selective activity, but also the easily gained resistance is the main problem encountered in developing safe and effective antifungals. The incidence of systemic fungal infections has been increasing. The choice of suitable antifungal agents remains relatively limited, although the advent of the new echinocandin class is a welcome development as the expansion of members of the azole antifungals as heterocyclic compounds^{4,5,6}.

There are two basic approaches to develop a new antifungal drug: (i) synthesis of analogues, modifications or derivatives of existing compounds for shortening and improving fungal infection treatment and, (ii) searching novel structures, that the fungus organisms have never been presented with before, for the treatment of fungal infection⁷.

To pursue this goal, our research efforts are directed to finding new chemical classes of antifungal agents. The methods of investigation using structure–activity relationships (SAR) enabled us to find some new pharmacophores of the above-mentioned activity. Many studies have been carried out on heterocyclic systems bearing an arylthio group as a pharmacophore^{8,9,10}.

In view of this data, we aimed at the synthesis and antifungal evaluation of new arylthio-dibenzofuran derivatives.

MATERIAL and METHOD

General procedure for the synthesis of the compounds

2-Chloro-N-(2-methoxydibenzo[b,d]furan-3-yl)acetamide (1)

Chloroacetyl chloride (0,1 mol) was added dropwise with stirring to a mixture of 3-amino-2-methoxydibenzofuran (0,1 mol) and triethylamine in toluene at 0-5°C. The solvent was evaporated under reduced pressure. The residue was washed with water and crystallized from ethanol¹¹.

2-Arylthio-N-(2-methoxydibenzo[b,d]furan-3-yl)acetamide derivatives (2a-d)

A mixture of 2-chloro-N-(2-methoxydibenzo[b,d]furan-3-yl)acetamide (2 mmol) and aromatic thiol (2 mmol) in acetone (10 mL) was stirred at room temperature for 8 hours in the presence of potassium carbonate (2 mmol) and filtered. The residue was washed with water and crystallized from ethanol.

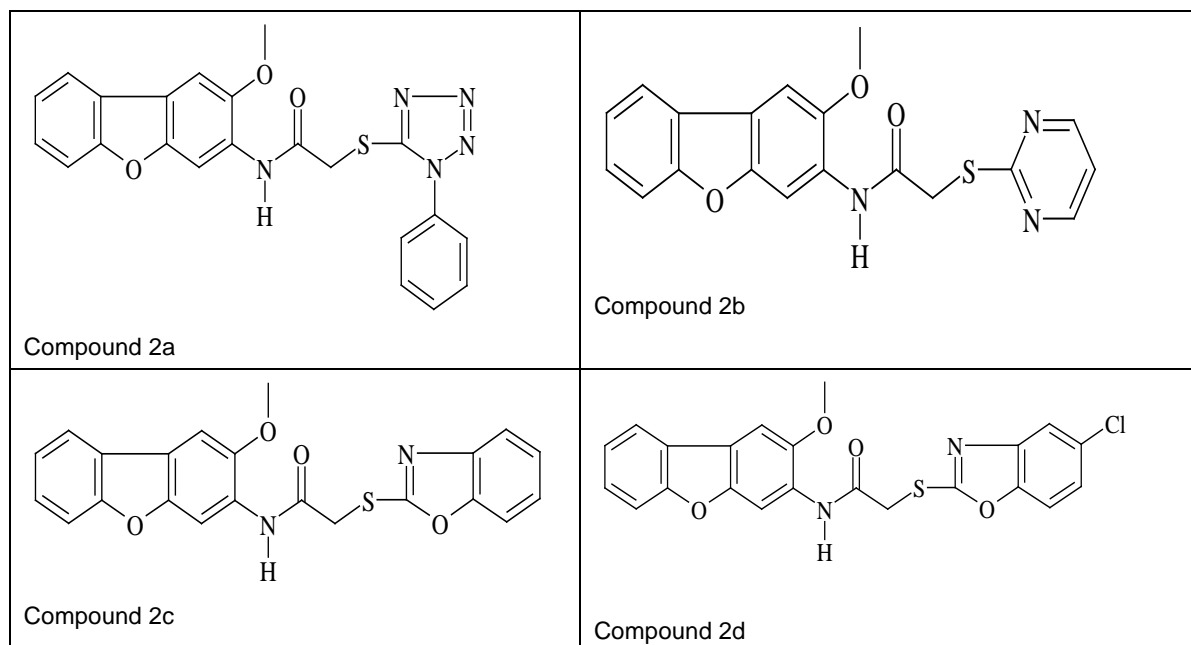


Figure 1. The general structure of the compounds

Microbiology

Anticandidal activity

The antimicrobial activities of the compounds were tested using the microbroth dilution method¹². Tested microorganism strain was *Candida albicans* (ATCC-22019). Microbroth dilution-susceptibility assay was used for antimicrobial 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. Overnight-grown *C. albicans* suspension in double-strength Mueller–Hinton broth was standardized to 108 CFU/mL using McFarland No: 0.5 standard solutions. Hundred microliter of *C. albicans* suspension was then added into the wells. The last well-chain without a microorganism was used as a negative control. Sterile distilled water and the medium served as a positive growth control. After incubation at 37°C for 18-24 h, antimicrobial activity was detected by spraying of 0.5% TTC (2,3,5-triphenyl tetrazolium chloride, Merck)

aqueous solution. MIC was defined as the lowest concentration of compounds that inhibited visible growth, as indicated by the TTC staining. Ketoconazole was used as standard antifungal agent.

Antifungal activity against molds

The antifungal activities of the compounds against molds were tested using the microbroth dilution method with some modifications^{12,13}. Tested mold strains were *Aspergillus parasiticus* (NRRL 465), *Penicillium chrysogenum* (NRRL 1951), *Trichoderma harzianum* (NRRL 20565), *Aspergillus ochraceus* (NRRL 3174), *Fusarium solani* (NRRL-13414), *Fusarium moniliforme* (NRLL 1866), *Fusarium culmorum* (wild culture). Fungal strains grown on PDA at 25°C for 5 suspensions in double-strength Potato Dextrose Broth (PDB) were standardized to 105 spores/mL. 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°C for

48-72 h, antifungal activity was detected by investigation of mycelia growing under stereo microscope.

Minimum fungicidal concentration (MFC) was defined as the lowest concentration of compounds that inhibited visible mycelia growth. Ketoconazole was used as an antifungal agent.

Table 1. Antifungal activity (MFC) of the compounds ($\mu\text{g/mL}$)

Compound	A	B	C	D	E	F	G	H
2a	250	250	250	250	250	250	250	250
2b	250	125	250	250	250	250	250	250
2c	250	250	250	250	250	250	250	250
2d	250	250	250	250	250	250	250	250
Ketoconazole	125	7.8	250	250	500	500	500	250

A: *A. parasiticus* (NRRL 465), **B:** *P. chrysogenum* (NRRL 1951), **C:** *T. harzianum* (NRRL 20565), **D:** *A. ochraceus* (NRRL 3174), **E:** *F. solani* (NRRL-13414), **F:** *F. moniliforme* (NRLL 1866), **G:** *F. culmorum* (wild culture), **H:** *C. albicans* (ATCC-22019)

RESULTS

2-Arylthio-*N*-(2-methoxydibenzo[b,d]furan-3-yl)acetamide derivatives were synthesized via the reaction of 2-chloro-*N*-(2-methoxydibenzo[b,d]furan-3-yl)acetamide with aromatic thiols. The structures of compounds 2a-2d were confirmed by $^1\text{H-NMR}$, mass spectral data and elemental analysis. The general structure of the compounds is given in Figure 1.

All compounds were tested *in vitro* against *A. parasiticus*, *P. chrysogenum*, *T. harzianum*, *A. ochraceus*, *F. solani*, *F. moniliforme*, *F. Culmorum*, *C. albicans*. Observed data on the antifungal activity of the compounds and control drug are given in Tables 1.

DISCUSSION

All compounds showed significant antifungal activity against *C. albicans*. The compounds and ketoconazole showed the inhibitory activity against *C. albicans* with a MFC value of 250 $\mu\text{g/mL}$.

All compounds were as effective as ketoconazole against *T. harzianum*, *A. ochraceus*, whilst the compounds were more effective than ketoconazole against *F. solani*, *F. moniliforme*, *F.*

culmorum. All compounds showed the inhibitory activity against *Fusarium* species with a MFC value of 250 $\mu\text{g/mL}$.

CONCLUSION

All compounds had significant antifungal activity. It is worthwhile to extend the bioactivity evaluation from *in vitro* whole cells to a mechanistic enzymatic level as well as to further *in vivo* studies. In future research, the cytotoxic effects of the compounds can also be investigated.

REFERENCES

1. Pfaller MA. The epidemiology of invasive mycoses-narrowing the gap. Clin Infect Dis. 1998; 27:1148-50.
2. Marr KA, Carter RA, Crippa F, Wald A, Corey L. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. Clin Infect Dis. 2002; 34:909-17.
3. Hood S, Denning DW. Treatment of fungal infection in AIDS. J Antimicrob Chemoth. 1996; 37:71-85.
4. Rees JR, Pinner RW, Hajjeh RA. The epidemiological features of invasive mycotic infections in the San Francisco, 1992-1993: Results of population-based laboratory active surveillance. Clin Infect Dis. 1993; 27:1138-40.

5. Polak A. The past, present and future of antimycotic combination therapy. *Mycoses*. 1999; 42:355-70.
6. Musiol R, Jampilek J, Buchta V, Silva L, Niedbala H, Podeszwa B, et al. Antifungal properties of new series of quinolone derivatives. *Bioorg Med Chem*. 2006; 14:3592-8.
7. Rao VS, Srinivas K. Modern drug discovery process. *J Bioinform Seq Anal*. 2011; 2: 89-94.
8. Ryu CK, Kim YH, Im HA, Kim JY, Yoon JH, Kim A. Synthesis and antifungal activity of 6,7-bis(arylthio)-quinazoline-5,8-diones and furo[2,3-f]quinazolin-5-ols. *Bioorg Med Chem Lett*. 2012; 22:500-3.
9. Ryu CK, Shim JY, Chae MJ, Choi IH, Han JY, Jung OJ, et al. Synthesis and antifungal activity of 2/3-arylthio- and 2,3-bis(arylthio)-5-hydroxy-/5-methoxy-1,4-naphthoquinones. *Eur J Med Chem*. 2005; 40:438-44.
10. Ryu CK, Sun YJ, Shim JY, You HJ, Choi KU, Lee H. Synthesis and antifungal activity of 6,7-bis-[s-(aryl)thio]-5,8-quinolinediones. *Arc Pharmacol Res*. 2002; 25:795-800.
11. Venkatachalam TK, Barreto J, Kreher U, Reutens DC, Spiccia L. Synthesis, characterization and coordination chemistry of dibenzofuran derivatives of 1,4,7,10-tetraazacyclododecane. *Inorg Chim Acta*. 2010; 363:2896-904
12. Winn WC, Allen SD, Janda WM, Koneman EW, Procop GW, Schreckenberger PC, et al. *Koneman's Color Atlas and Textbook of Diagnostic Microbiology*, 6th Ed. Baltimore and Philadelphia, Lippincott Williams & Wilkins, 2006.
13. Espinel-Ingroff A. In Vitro Fungicidal Activities of Voriconazole, Itraconazole, and Amphotericin B against Opportunistic Moniliaceous and Dematiaceous Fungi. *J Clin Microbiol*. 2001; 39:954-8.

Yazışma Adresi / Address for Correspondence:

Dr. Zafer Asım Kaplancıklı
Anadolu University
Graduate Scholl of Health Sciences
ESKİŞEHİR
e-mail: zakaplan@anadolu.edu.tr.

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