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Research paper

The synthesis, antifungal and apoptotic effects of triazole-oxadiazoles against *Candida species*

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ABSTRACT

In search of potent and safe antifungal agents, herein, we report the synthesis, characterization and biological activities of triazole-oxadiazole compounds. The structural verification of the molecules was carried out by ^{1}H NMR, ^{13}C NMR and mass spectral data. The *in vitro* antifungal and apoptotic activity were investigated against *C. albicans*, *C. parapsilosis*, *C. krusei* and *C. glabrata*. The compounds namely *N*-(4-nitrophenyl)-2-[(5-(2-((4-methyl-4*H*-1,2,4-triazol-3-yl)thio)ethyl)-1,3,4-oxadiazol-2-yl)thio]acetamide (**4e**) and *N*-(6-fluorobenzothiazol-2-yl)-2-[(5-(2-((4-methyl-4*H*-1,2,4-triazol-3-yl)thio)ethyl)-1,3,4-oxadiazol-2-yl)thio]acetamide (**4i**) were detected as the most potent compounds against *C. albicans* and *C. glabrata* (MIC_{90} = 62.5 μg/mL). According to studies on their mechanism of action, it was confirmed that compound **4i** has apoptotic effect on four *Candida* via Annexin V-PI with flow cytometry. The MTT assay revealed that all compounds were determined to be non-toxic against healthy cells in the tested concentrations.

1. Introduction

Candida infections represent a serious global health threat and are mainly attributed to the following species: *C. albicans*, *C. parapsilosis*, *C. krusei* and *C. glabrata*. More recently, the frequency of candidiasis has raised in accord with the number of immunosuppression cases including AIDS patients, organ transplantations, cancer therapy and invasive surgery [1–3]. Azoles (fluconazole, ketoconazole), polyene antibiotics (amphotericin B, nystatin), glucan synthesis inhibitors (caspofungin and micafungin), chitin synthesis inhibitors (nikkomycins), allylamines (terbinafine) and flucytosine are amongst currently used drugs against fungal infections [4]. Drug resistance, narrow spectrum and reduced bioavailability at the target tissues and toxicity impact the efficacy of antifungal agents and limit therapeutic options [5]. Taken together, these findings have important implications for developing novel, effective, broad-spectrum and low toxic antifungal agents.

Antifungal agents can act by inhibiting ergosterol synthesis pathway, blocking β-1,3-glucan, chitin production or other mechanisms of action [4,6]. Since most of *Candida* spp. are developing resistance to antifungal drugs in current clinical use, it is necessary to develop new antifungal strategies. Induction of apoptosis in fungal cells may well provide new development in the search for novel antifungal agents. Apoptosis, also termed as programmed cell death (PCD), is a regulated cellular suicide program that characterized by specific morphological and biochemical features. Yeast cells undergoing apoptosis exhibit characteristic markers related to apoptosis such as accumulation of ROS, cleavage of DNA, exposure of phosphatidylserine, chromatin condensation and nuclear fragmentation, which are in common with mammalian cells [7–10]. There are some studies that investigated the relationship between antifungal drugs and apoptosis [11–13].

Due to their high oral bioavailability and broad spectrum of activity against invasive fungal species, azole compounds are one of the most widely used groups of antifungal agents [14]. Among them, 1,3,4-triazole and 1,2,4-oxadiazole compounds were intensively investigated by researchers and incorporated into potent drugs including fluconazole, efinaconazole, zibotentan and nesapidil shown in Fig. 1. Benzothiazole ring also represent a key motif in medicinal chemistry. The antifungal activity data of 1,3,4-triazoles [15–18], 1,3,4-oxadiazoles [19–21] and benzothiazoles [22–24] encouraged us to envisage the combination of mentioned pharmacophores to obtain a new series of antifungal agents. Therefore, in this article, the design, synthesis and anticandidal

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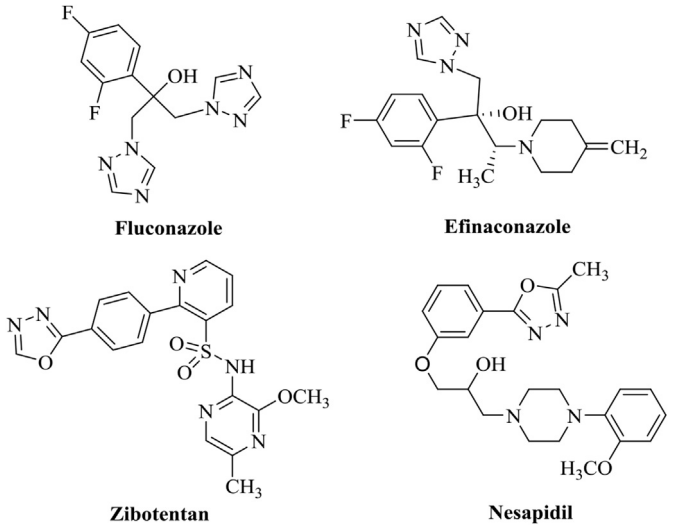


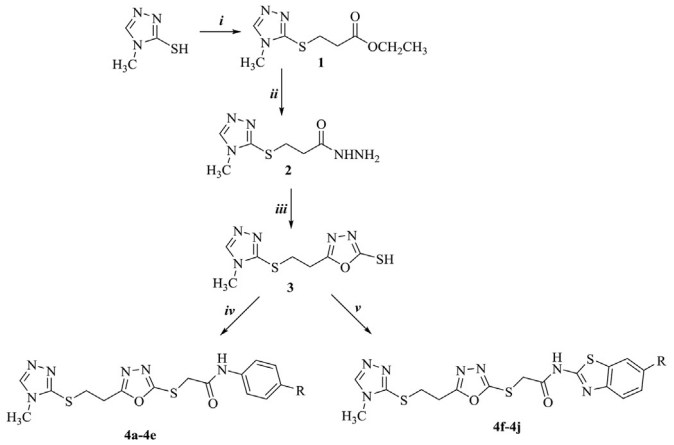
Fig. 1. Structures of some 1,2,4-triazole and 1,3,4-oxadiazole derivatives.

activity of some new hybrid triazole-oxadiazole molecules were planned, along with their mechanism of action was investigated.

2. Results and discussion

2.1. Chemistry

In this study, a multi-step synthesis procedure was performed to reach target compounds (**4a-j**) as outlined in Scheme 1. Commercially available 2-mercapto-4-methyl-1,2,4-triazole was reacted with ethyl 3-chloropropionate to obtain ethyl 3-[(4-methyl-4*H*-1,2,4-triazol)thio]propionate (**1**). The ester group of compound **1** was hyrazinated to obtain 3-[(4-methyl-4*H*-1,2,4-triazol-3-yl)thio] propanehydrazide (**2**). Common intermediate 5-[2-((4-methyl-4*H*-1,2,4-triazol-3-yl)thio)ethyl]-1,3,4-oxadiazole-2-thiol (**3**) was gained via ring closing reaction of compound **2** with carbon disulfide. Using the obtained compound **3**, First part of final compounds, *N*-aryl-2-[(5-(2-((4-methyl-4*H*-1,2,4-triazol-3-yl)thio) ethyl)-1,3,4-oxadiazol-2-yl)thio]acetamide (**4a-4e)** were acquired with the reaction of appropriate phenacyl bromides whereas the second part *N*-(6-substituted benzothiazol-2-yl)-2-[(5-(2-((4-methyl-4*H*-1,2,4-triazol-3-yl)thio)ethyl)-1,3,4-oxadiazol-2-yl)thio] acetamides (**4f-4j**) from 2-chloro-*N*-(6-substituted benzothiazole) acetamides. The accuracy of structures of ten final compounds were proved through ^{1}H NMR, ^{13}C NMR and HRMS spectroscopic data. In ^{1}H NMR spectra of the compounds, methyl proton of imidazole ring was observed at 3.43–3.45 ppm. Three different methylene protons in order of $SCH_2\underline{CH_2}$, $COCH_2$ and $S\underline{CH_2}CH_2$ were resonated at about 3.33, 4.22–4.40 and 4.45 ppm. The hydrogen of amino group was seen at 10.23–10.98 ppm in compounds **4a-4e**, and at 12.60–13.15 ppm in compounds **4f-4j**. In ^{13}C NMR spectra of the compounds, methyl carbon was detected at about 32.72–32.75 whereas the methylene carbon of carbonyl group was observed at 36.02–37.27 ppm. The CH_2 carbons of ethyl bridge were given signals at about 24.26–24.29 ppm and 45.70–45.75 ppm. In the HRMS data, M+1 peaks were detected for all final compounds in accordance with the molecular weights of them.



Scheme 1. The synthetic pathway of the compounds. Reactants and reagents: ***i***: $ClCH_2CH_2COOCH_2CH_3$, K_2CO_3, acetone, 8 h, reflux; ***ii***: $NH_2NH_2.H_2O$, EtOH, 6 h, reflux; ***iii***: CS_2, NaOH, 5 h, reflux; ***iv***: 2-Chloro-*N*-(4-substitutedphenyl)acetamides, K_2CO_3, acetone, 5 h, r.t.; ***v***: 2-Chloro-*N*-(6-substitutedbenzothiazole)acetamides, K_2CO_3, acetone, 4 h, r.t.

2.2. Biology

The synthesized compounds (**4a-4j**) were evaluated for anticandidal activity against various yeast such as *C. albicans* (ATCC 90028), *C. glabrata* (ATCC 90030), *C. krusei* (ATCC 6258), *C. parapsilosis* (ATCC 22019). MIC_{90} values which is defined as minimum inhibitory concentration required to inhibit the growth of 90% of organisms (Table 1) were revealed by fluorometric measurements using MTT solution. Ketoconazole and clotrimazole were used as standard drugs in the activity test. Eight of the compounds were found moderate against tested microbial strains, but compounds namely *N*-(4-nitrophenyl)-2-[(5-(2-((4-methyl-4H-1,2,4-triazol-3-yl)thio)ethyl)-1,3,4-oxadiazol-2-yl)thio]acetamide (**4e)** and *N*-(6-fluorobenzothiazol-2-yl)-2-[(5-(2-((4-methyl-4H-1,2,4-triazol-3-yl)thio)ethyl)-1,3,4-oxadiazol-2-yl)thio]acetamide (**4i)** indicated very strong activity against *C. albicans* and *C. glabrata* (MIC_{90} = 62.5 μg/mL). The anticandidal spectrum of ketoconazole and clotrimazole, shown in Table 1, also supports this suggestion, as a clear similarity between the antimicrobial spectra of ketoconazole, clotrimazole and compounds **4e**, **4i** can be seen. The structures of the compounds belong two main skeletons which are *N*-aryl-2-[(5-(2-((4-methyl-4*H*-1,2,4-triazol-3-yl)thio)ethyl)-1,3,4-oxadiazol-2-yl)thio]acetamide (**4a-4e**) and *N*-(6-substituted benzothiazol-2-yl)-2-[(5-(2-((4-methyl-4*H*-1,2,4-triazol-3-yl)thio) ethyl)-1,3,4-oxadiazol-2-yl)thio]acetamide (**4f-4j**). All compounds contain triazole, oxadiazole rings and they differ from each other due to phenyl and benzothiazole rings bonded to acetamide moiety. An insight into the structure-activity relationship of the compounds revealed that benzothiazole including derivatives (**4f-j**) exhibited higher antimicrobial activity than phenyl including compounds (**4a-4e**). Nevertheless, two compounds from each chemical group, compound **4e** bearing 4-nitrophenyl moiety and compound **4i** bearing 6-fluorobenzothiazole moiety were found as the most active compounds which were subsequently tested in flow cytometry to determine apoptosis and necrosis on aforementioned *Candida species*. Due to the lack of studies on the mechanism of action of antifungal agents [6,25], the determination of apoptosis has recently been a new and important approach in antifungal therapy [26]. Therefore, illustrating the phenomenon of apoptosis in fungi by activating the fungal cells to suicide is beneficial for new antifungal drug discovery strategies [27]. Additionally, yeasts constitute an ideal model organism for studying the interactions and the mechanisms of action of mammalian apoptotic regulators due to their simple eucaryotic structures [28–30]. On this basis, triazole [31–35] and oxadiazole [36–38] containing compounds were identified as apoptosis inducers in many literature. Accordingly, flow cytometric test was applicated to *C. albicans,*