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Original Article

In vitro activity of dihydropyrazole derivatives against *Candida* species

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ABSTRACT

Profound insights for efficient antifungal agents are required for the increased hazardous fungal infections caused by yeast, such as *Candida* species. In this study, we investigate the efficacy of different benzodioxolpyrazoline derivatives against two *Candida* species. All compounds exhibited potent *in vitro* antifungal activity against both species. Compound **5** showed the best inhibition zone in comparison to positive controls for *Candida albicans*. For *Candida parapsilosis*, all the tested compounds were active while the positive controls were inactive. These compounds provide good starting models for the development of new antimicrobial agents which could be useful especially for the treatment of candidiasis.

1. Introduction

Recently, invasive fungal infections have been expressively increased as a result of the inclined number of immune-deficient patients, organ transplantation, tracheal intubation and endoscopic techniques. Moreover, the wide application of broad-spectrum antibiotics, immunesuppressants and corticosteroids has greatly contributed to such increase [1,2]. The development of novel antifungal agents in clinical therapy has lagged behind the increasing incidence of drug resistance. Thus, efforts have been undertaken to overcome the emergence of resistant fungi by using drug combinations. But, the combinations of antifungal drugs faced restrictions due to high costs and serious side effects [3]. As well, inconsistent consequences of either synergistic or antagonistic action of various antifungal combinations have been reported [4,5]. Structural modifications of antimicrobial drugs which experienced microbial resistance are turned out to be an effective and alternative protocol to extend the antifungal agents lifespan in the market such as azoles [6] and nonazoles [7]. However, with the current chemotherapeutics portfolio, it has been agreed that researchers are getting closer to the game end in terms of the structural modification of parent compounds [8]. As a consequence, new candidates have to be introduced that contain chemical features which differ from those of the present drugs.

Candida species, especially *Candida albicans*, has been documented as the most common yeast causing invasive fungal infections [9]. Reduced occurrence of candidaemia with a decrease of infections caused by *C. albicans* has been reported with extensive use of fluconazole, but an increase of infections caused by *C. glabrata*, *C. parapsilosis* and *C. tropicalis* has been stated [10]. Particularly *Candida parapsilosis* was responsible for 21.9% of the incidence [11].

In the present study, the anti-fungal activity of a series of dihydropyrazoles, which in previous work showed potent antibacterial activity [12], will be evaluated for its potentiality as antifungal agents against *Candida albicans* and *Candida parapsilosis*. *C. albicans* remains the main *Candida* species associated with clinical cases, while *C. parapsilosis* is the most common in the southern and eastern parts of the world (including Egypt) [13].

The study is fueled by the fact that these dihydropyrazole derivatives have consisted of non-classical antimicrobial pharmacophores which decrease the possibility of drug resistance as detailed in the above premises. One auxiliary drive is that the ideal antifungal agents should have favorable safety profiles and not only be hepatically metabolized [14]. Fortunately, the new compounds are expected to be metabolized by microsomal hydroxylation and alcohol dehydrogenases which are represented in hepatic and non-hepatic tissue [15].

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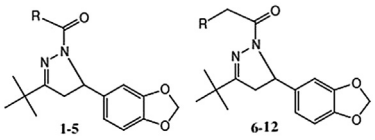

Compound	R
1,6	morpholine
2,7	piperidine
3,8	4-hydroxypiperidine
4,9	4-ethylpiperazine
5,10	pyrrolidine
11	pyrrolidin-2-one
12	piperazine

Fig. 1. The dihydropyrazole candidates tested against *Candida* species.

2. Materials and methods

2.1. Test

Candida parapsilosis (ATCC 22012), *Candida albicans* (clinical isolates).

2.2. Tested compounds

The dihydropyrazoles (**1–12**) (Fig. 1) were synthesized and purified as reported [12].

2.3. Antimicrobial control

Tetracycline (30 mcg), gentamicin (10 mcg), and ofloxacin (5 mcg) commercial discs were used as positive control drugs and dimethyl sulfoxide (DMSO) as a negative control.

2.4. Antimicrobial assay

Antimicrobial activities of compounds were tested using the agar well diffusion method. The microbial suspensions concentration was adjusted to 0.5 McFarland standards. The fungal suspensions were seeded on potato dextrose agar (in duplicates). In each of these plates, many wells were cut out using a sterilized cork borer. The compounds (**1–12**) were dissolved in DMSO (5 mg/ml) then 100 µL of sample was added into the wells. A positive control antimicrobial disc was placed in the plate. Fungal plates were incubated at room temperature for 72 h. The antifungal potentiality was evaluated by measuring the zone of inhibition and the minimum inhibitory concentrations.

3. Results

Zones of inhibition of the twelve compounds (**1–12**) against the two *Candida* species under study are indicated in Table 1. All compounds displayed an antifungal activity against both *C. albicans* and *C. parapsilosis*. The zone of inhibition of the test compounds (**1–12**) was 15–25 mm for *C. albicans* while the positive control drugs were 24–25 mm. Compound **5** considered as the most potent candidate showing a better zone of inhibition than tetracycline and gentamicin and equipotent to ofloxacin. Also, compounds **1**, **2**, **4**, **7**, **8**, **10**, and **11** were moderately active showing zone of inhibition 20–22 mm. For *C. parapsilosis*, zone of inhibition range was 15–22 mm for test compounds while surprisingly the positive control drugs were completely inactive. Compounds **3** and **4** were the best candidates showing the highest zone of inhibition 22 and 20 respectively. While the other candidates' zones of inhibition were 15–19.

Since all compounds (**1–12**) were active against *Candida parapsilosis*, while the positive control drugs were inactive, the minimum inhibitory concentrations (MICs) were estimated for selected compounds (**1**, **2**, **5**, **8** and **10**) based on their activity against *C. albicans* (Table 2). In general, MICs were in the microgram range which reflects the ingenuity of the novel candidates. The best MIC was 166 µg/ml which exhibited for *Candida albicans* by compounds **2** and **5** and for *Candida parapsilosis* by compounds **1** and **2**. 200 µg/ml was the MIC for compound **1** against *Candida albicans* and compounds **5** and **8** against *Candida parapsilosis* (Table 2).

Table 2
Minimum inhibitory concentrations (µg/ml) for selected dihydropyrazole derivatives.

Compound	MICs (µg/ml)	
	C. albicans	*C. parapsilosis*
1	200	166
2	166	166
5	166	200
8	333	200
10	333	250

MICs; minimum inhibitory concentrations, *C. albicans; Candida albicans, C. parapsilosis; Candida parapsilosis.*

4. Discussion

Synthetic medicinal chemistry is considered as the most successful tool for drug discovery. It is mainly depending on the chemical synthesis of novel molecules containing particular pharmacophores related to specific activity and based on rationale design. In this study, the symbiotic approach in medicinal chemistry [16] has been applied to synthesize innovative chemical candidates by the assembly of benzodioxolpyrazoline moiety and different alicyclic amines *via* methanone or ethanone linker in order to test their anticandidal potentials.

1,3-benzodioxole moiety represented a crucial pharmacophore in several chemically synthesized anticandidal compounds [17,18]. Also, several 2-pyrazolines (dihydropyrazoles) showed potent antifungal

Table 1
Zone of inhibition (mm) of test compounds against Gentamicin, Tetracycline, and Ofloxacin as a positive control and DMSO as a negative control.

Compound	Zone of inhibition (mm)		Compound	Zone of inhibition (mm)	
	C. albicans	*C. parapsilosis*		*C. albicans*	*C. parapsilosis*
1	20	15	**9**	19	15
2	20	18	**10**	22	16
3	15	22	**11**	20	15
4	21	20	**12**	19	17
5	25	16	Gentamicin	24	–
6	19	15	Tetracycline	24	–
7	21	15	Ofloxacin	25	–
8	21	18	DMSO	–	–

DMSO; Dimethyl sulfoxide, *C. albicans; Candida albicans, C. parapsilosis; Candida parapsilosis.*

activities [19,20]. In particular, pyrazoline-methanones and pyrazoline-ethanones have displayed an intense antimicrobial potency against several *Candida* species [21]. On the other hand, alicyclic amines such as piperidine and morpholine fostered the antimicrobial activity of chemically synthesized candidates [22,23]. Thus, in the current work, joint structures of these effective anticandidal moieties have been synthesized and evaluated for their anticandidal activity.

As shown in the results, the synthesized candidates (**1–12**) showed potent anticandidal activity, particularly against the resistant *C. parapsilosis*. Great advantages are introduced by such dihydropyrazoles. Its chemical structures are well-characterized thus the structure-activity relationship is very defined and further structural modifications in order to improve the activity are easily achievable in contrast to a variety of natural product extracts which active components and underlying mechanisms of action are unknown [24]. This property is characteristic for all synthetic medicinal chemistry evolved candidates.

We hope the bioactivity results will stimulate further research in this area and provide good starting templates for further structural optimization of these pyrazoline derivatives for development of new antimicrobial agents against the devastating invasive fungal infections, particularly candidiasis.

Conflict of interest statement

Authors declare no conflict.

Author contributions

Both authors have contributed equally.

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