

# A SERIES OF 2-PYRAZOLINES ENDOWED WITH POTENT ANTICANDIDAL ACTIVITY

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## INTRODUCTION

Invasive fungal infections (IFIs) associated with high morbidity and mortality pose a serious threat to human health across the globe. IFIs are estimated to be responsible for almost 1.5-2 million deaths every year (Liu *et al.*, 2018).

Over the last decades, the incidence of IFIs has dramatically increased due to the growing number of patients at risk for IFIs (Liu *et al.*, 2018), particularly those in intensive care units, those with immune systems compromised by human immunodeficiency virus (HIV), post-transplant immunosuppressive therapy or cancer chemotherapy as well as those with severe viral infections such as influenza virus and COVID-19 (Fisher *et al.*, 2022).

*Candida* species, particularly *Candida albicans*, account for the majority of IFIs (Lopes and Lionakis, 2022). *Candida* spp. are also recognized as the fourth most common cause of nosocomial bloodstream infections (Liu *et al.*, 2018).

Despite tremendous efforts being devoted to the identification of fungal-specific targets and the discovery of selective antifungal drugs, treatment options are rather limited. There are only three classes of antifungal agents (polyenes, azoles, and echinocandins) clinically available for the treatment of IFIs. In addition to these agents, 5-fluorocytosine (5-FC) is frequently used as an adjunctive therapy. Substantial clinical failures associated with these antifungal drugs are attributable to their limited clinical efficacy, narrow antifungal spectrum, significant adverse effects, unfavorable pharmacokinetic features, and drug-drug interactions (Liu *et al.*, 2018). Resistance to antifungal agents, which reduces the clinical success, is also an emerging concern worldwide (Fisher *et al.*, 2022).

Unlike bacteria, eukaryotic fungal cells are similar to mammalian cells (Liu *et al.*, 2018) and therefore the discovery of selective antifungal drugs devoid of severe side effects is an uphill task for researchers.

2-Pyrazoline (also referred to as 4,5-dihydro-1H-pyrazole) is an outstanding and highly versatile scaffold extensively used by many researchers for the design of therapeutically active agents to combat various diseases such as bacterial and fungal infections, central nervous system (CNS) disorders, cancer and so on. In particular, antimicrobial activity of pyrazolines predominates over other reported pharmacological activities (Shaaban *et al.*, 2012).

The vast number of scientific reports appearing in the literature related to 2-pyrazolines exerting pronounced antifungal potency (Nehra *et al.*, 2020) motivated us to evaluate the anticandidal effects of 2-pyrazolines, which were previously synthesized and tested for their inhibitory effects on acetylcholinesterase and butyrylcholinesterase by our research group (Altıntop *et al.*, 2013).

## MATERIALS AND METHODS

### Chemistry

The synthetic method and the spectral data of 1-[[4-(4-alkyl/aryl)piperazin-1-yl]thiocarbamoylthio]acetyl]-3-(2-furyl)-5-(3,4-methylenedioxyphenyl)-2-pyrazolines (**1-10**) were reported previously by our research team (Altıntop *et al.*, 2013).

### Microbiology

The minimum inhibitory concentrations (MICs, µg/mL) of compounds **1-10** were determined by the broth microdilution assay as previously described (Özdemir *et al.*, 2015) to evaluate their antifungal effects on *Candida albicans* (ATCC® 90028™), *Candida glabrata* (ATCC® 90030™), *Candida krusei* (ATCC® 34135™), and *Candida parapsilosis* (ATCC® 22019™). Ketoconazole was used as a positive control.

## RESULTS AND DISCUSSION

Compounds **1-10** were tested for their antifungal effects on *C. albicans* (ATCC® 90028™), *C. glabrata* (ATCC® 90030™), *C. krusei* (ATCC® 34135™), and *C. parapsilosis* (ATCC® 22019™). These compounds were found to be more effective on *C. parapsilosis*. Compounds **1, 3, 4, 7** and **8** showed marked antifungal effects on *C. parapsilosis* with a MIC value of 31.25 µg/mL compared to ketoconazole (MIC= 31.25 µg/mL) (Table 1). Taking into account the *in vitro* cytotoxicity data reported previously by our research team (Altıntop *et al.*, 2013), only 1-[[4-(4-methoxyphenyl)piperazin-1-yl]thiocarbamoylthio]acetyl]-3-(2-furyl)-5-(3,4-methylenedioxyphenyl)-2-pyrazoline (**3**) did not show any cytotoxicity towards NIH/3T3 mouse embryonic fibroblast cells (IC<sub>50</sub>= 191 µg/mL) at its MIC concentration (31.25 µg/mL). Based on this result, it can be concluded that the antifungal activity of compound **3** towards *C. parapsilosis* is selective.

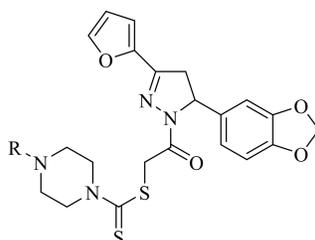


Table 1. Anticandidal activity of compounds **1-10**

Compound	R	MIC values in µg/mL			
		<i>C. albicans</i> <sup>a</sup>	<i>C. glabrata</i> <sup>a</sup>	<i>C. krusei</i> <sup>a</sup>	<i>C. parapsilosis</i> <sup>a</sup>
<b>1</b>	4-Ethylpiperazin-1-yl	125	250	62.50	<b>31.25</b>
<b>2</b>	Morpholin-4-yl	125	250	125	62.50
<b>3</b>	4-(4-Methoxyphenyl)piperazin-1-yl	250	250	125	<b>31.25</b>
<b>4</b>	4-Benzylpiperazin-1-yl	125	250	125	<b>31.25</b>
<b>5</b>	4-Methylpiperazin-1-yl	125	250	125	62.50
<b>6</b>	Thiomorpholin-4-yl	250	250	125	62.50
<b>7</b>	4-(2-Dimethylaminoethyl)piperazin-1-yl	125	250	62.50	<b>31.25</b>
<b>8</b>	4-Phenylpiperazin-1-yl	250	250	125	<b>31.25</b>
<b>9</b>	Pyrrolidin-1-yl	250	250	125	62.50
<b>10</b>	Piperidin-1-yl	125	250	125	62.50
Ketoconazole	-	62.50	125	15.63	<b>31.25</b>

<sup>a</sup> *Candida albicans* (ATCC® 90028™), *Candida glabrata* (ATCC® 90030™), *Candida krusei* (ATCC® 6258™), *Candida parapsilosis* (ATCC® 22019™).

## CONCLUSION

In this paper, we described the *in vitro* evaluation of a series of 2-pyrazolines (**1-10**) as anticandidal agents. Among these compounds, compounds **1, 3, 4, 7** and **8** were the most active compounds against *C. parapsilosis* (MIC= 31.25 µg/mL). However, MTT assay performed previously by our research team (Altıntop *et al.*, 2013) revealed that only compound **3** did not show any cytotoxic activity towards normal (NIH/3T3) cells at its MIC concentration. In the continuation of this work, further studies are required to provide an insight into the mechanism of action underlying its pronounced anticandidal activity. This work could be the first step towards the development of a new generation of antifungal agents for the treatment of IFIs caused by *Candida* species.

## ACKNOWLEDGEMENTS

This study was supported by Anadolu University Scientific Research Projects Commission under the grant no: 2204S033.

## REFERENCES

- Altıntop, M.D., Özdemir, A., Kaplancıklı, Z.A., Turan-Zitouni, G., Temel, H.E., Akalin Çiftçi, G., 2013. Synthesis and biological evaluation of some pyrazoline derivatives bearing a dithiocarbamate moiety as new cholinesterase inhibitors. Arch. Pharm. Chem. Life Sci. 346, 189–199. <https://doi.org/10.1002/ardp.201200384>
- Fisher, M.C., Alastruey-Izquierdo, A., Berman, J., Bicanic, T., Bignell, E.M., Bowyer, P., Bromley, M., Brüggemann, R., Garber, G., Cornely, O.A., Gurr, S.J., Harrison, T.S., Kuijper, E., Rhodes, J., Sheppard, D.C., Warris, A., White, P.L., Xu, J., Zwaan, B., Verweij, P.E., 2022. Tackling the emerging threat of antifungal resistance to human health. Nat. Rev. Microbiol., 1–15. [Epub ahead of print]. <https://doi.org/10.1038/s41579-022-00720-1>
- Liu, N., Tu, J., Dong, G., Wang, Y., Sheng, C., 2018. Emerging new targets for the treatment of resistant fungal infections. J. Med. Chem. 61, 5484–5511. <https://doi.org/10.1021/acs.jmedchem.7b01413>
- Lopes, J.P., Lionakis, M.S., 2022. Pathogenesis and virulence of *Candida albicans*. Virulence 13, 89–121. <https://doi.org/10.1080/21505594.2021.2019950>
- Nehra, B., Rulhania, S., Jaswal, S., Kumar, B., Singh, G., Monga, V., 2020. Recent advancements in the development of bioactive pyrazoline derivatives. Eur. J. Med. Chem. 205, 112666. <https://doi.org/10.1016/j.ejmech.2020.112666>
- Özdemir, A., Altıntop, M.D., Sever, B., Cantürk, Z., Kaplancıklı, Z.A., 2015. Synthesis and evaluation of tetrazole-based hydrazone derivatives bearing a pyridine moiety as antimicrobial agents, Lett. Drug Des. Discov. 12, 687–693. <https://doi.org/10.2174/1570180812666150309235217>
- Shaaban, M.R., Mayhoub, A.S., Farag, A.M., 2012. Recent advances in the therapeutic applications of pyrazolines. Expert Opin. Ther. Pat. 22, 253–291. <https://doi.org/10.1517/13543776.2012.667403>