ISSN 2710-3056

Grail of Science

Periodical scientific journal

№ 37 March 2024

The issue of journal contains

Proceedings of the VII Correspondence International Scientific and Practical Conference

SCIENTIFIC RESEARCHES AND METHODS OF THEIR CARRYING OUT: WORLD EXPERIENCE AND DOMESTIC REALITIES

held on March 15th, 2024 by

NGO European Scientific Platform (Vinnytsia, Ukraine)
LLC International Centre Corporative Management (Vienna, Austria)

OUCI Open Ukrainian Citation Index

Euro Science Certificate № 22564 dated 12.02.2024
UKRISTEI (Ukraine) Certificate № 69 dated 05.01.2024

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ARTICLE

DOI 10.36074/grail-of-science.15.03.2024.080

STRUCTURE-ACTIVITY RELATIONSHIP OF 5,6-DIHYDRO-TETRAZOLO[1,5-*c*]QUINAZOLINES TARGETING *CANDIDA GLABRATA*

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Summary. *Candida species cause both disseminated and superficial fungal infections in humans. Although azole antifungals have historically proven effective in treating such infections, recent epidemiological studies highlight a crucial concern in the clinical context. Some Candida species exhibit inherent resistance to azoles and echinocandins, and the emergence of high-level resistance poses a significant problem. Consequently, derivatives of 5,6-dihydrotetrazolo[1,5-c]quinazolines have shown potent inhibitory properties against C. glabrata at concentrations range of 0.4 – 55.0 μM, leading to the identification of a structure-activity relationship.*

Keywords. *antifungal activity, C. glabrata, 5,6-dihydrotetrazolo[1,5-c]quinazolines, structure-activity relationship*

Introduction. The contemporary management of *Candida glabrata* poses significant challenges. This is underscored by the prevalence data derived from candidemia cases spanning the years 2008–2013 in metropolitan Atlanta, Georgia, and Baltimore, Maryland, revealing *C. glabrata* as the second most frequently

identified species, accounting for 27% of cases [1]. Furthermore, during this period, there was an observed increase in the proportion of cases exhibiting multidrug-resistant Candida, rising from 1.8% to 2.6%. A parallel trend is evident in a European observational study conducted between 2018 and 2022 across 17 European countries, indicating that France, Czech Republic, and the UK have notably elevated proportions of *C. glabrata* (25–33%, P = 0.2145) [2]. Unlike some other Candida species, it is less susceptible to certain antifungal medications, making it a concern in the context of drug resistance. Within this context, 12% of cases from six European countries exhibited resistance to fluconazole. Individuals with compromised immune systems, such as those with HIV/AIDS, undergoing chemotherapy, or receiving organ transplants, are at a higher risk of developing *C. glabrata* infections. Its genomic characteristics differ from other Candida species: it has a smaller genome size but exhibits a high degree of genetic plasticity, which may contribute to its adaptability and resistance.

Hence, the current exploration of the antifungal properties of 5-phenyl-5,6-dihydrotetrazolo[1,5-*c*]quinazoline derivatives [3, 4] is both relevant and imperative in advancing the understanding of the potential applications and efficacy of these compounds in combating pathogenic fungi. The intricate structure-activity relationship (SAR) investigation not only elucidates the specific molecular features critical for antifungal activity but also provides a roadmap for the rational design of derivatives with enhanced potency and selectivity. SAR empowers researchers to make informed decisions in tailoring the chemical structure, optimizing pharmacological properties, and ultimately contributes to the development of more efficacious antifungal agents.

Materials and methods. The bacteriological laboratory at Zaporizhzhia Regional Clinical Hospital of Zaporizhzhia Regional Council (Ukraine) conducted the method of serial dilutions (0.125–64 mg/L) [5] for 5,6-dihydrotetrazolo[1,5-*c*]quinazoline's derivatives in meat-peptone broth. This evaluation was performed against *Candida glabrata* (*Nakaseomyces glabrata*), isolated from patients' biological material and identified using chromatic Candida media (Liofilchem, Italy). The chosen initial solvent DMSO did not exhibit sensitivity in the tested microorganism strains. Minimum inhibition concentrations of reference drugs amphotericin B and caspofungin was 8 mg/L, micafungin: 4 mg/L. All growth experiments were carried out in duplicate.

Results and discussion. Initial investigations indicated that the antifungal efficacy of 4-(5-methyl-5,6-dihydrotetrazolo[1,5-*c*]quinazolin-5-yl)benzoic acid was observed to be below 2 mg/L against *C. glabrata* [4]. This is noteworthy given the resistance demonstrated by *C. kefyr* (*Kluyveromyces marxianus*) and *C. utilis* (*Cyberlindnera jadinii*). Additionally, earlier for certain other derivatives, the minimum inhibition concentrations ranged between 128-256 mg/L against *C. albicans* ATCC 885-653 [3].

As a result, a strategic choice was made to reduce the concentration range to 0.125-64 mg/L and employ *C. glabrata* as the fungal strain for an extensive screening of the derivative set, aiming to yield more compelling outcomes about a detailed exploration of the SAR.

Notably, the presence of alkyl in the 5th position played a critical role in inhibiting the growth of *C. glabrata*, as evidenced by structural optimization and the

three-dimensional representation of the core derivative (Fig. 1). Prolonging the alkyl chain up to propyl led to a decrease in activity, attributed to the formation of a more cumbersome structure that proved unfavorable for the potential antifungal target, highlighting the importance of molecular size and spatial considerations.

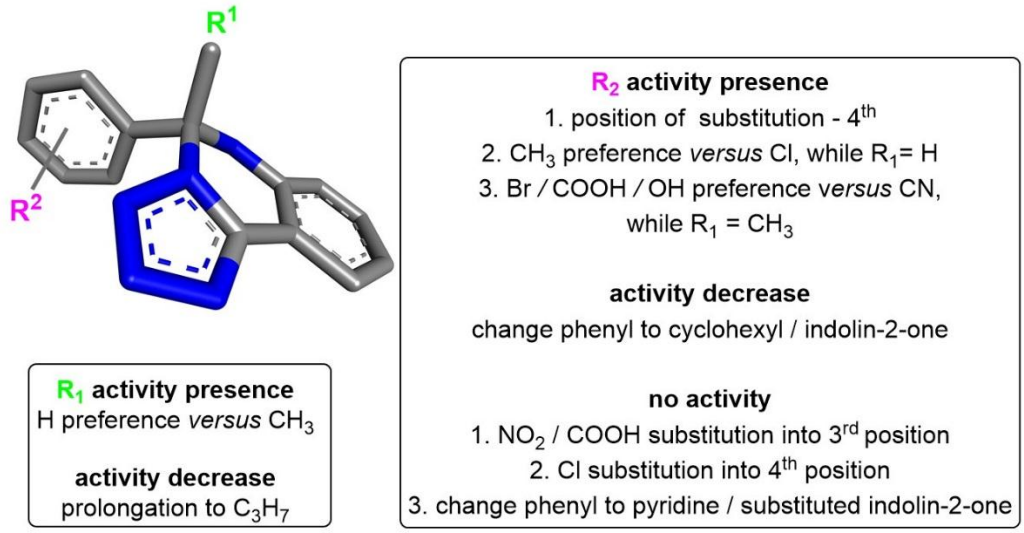

Fig. 1. Structure-activity relationship of 5,6-dihydrotetrazolo[1,5-*c*]quinazolines against *Candida glabrata*. Molecular structure was optimized by HyperChem 8.0.8, and Discovery Studio v21.1.0.20298 was used for 3D visualization.

Besides, it was discerned, that phenyl-substituted compounds, particularly those with a Br, COOH or OH group in the 4th position (Fig. 1), exhibited the most favorable attributes. Moreover, shift of NO_2 and COOH groups to 3rd position lead to the disappearance of *C. glabrata* growth inhibition. The substitution of phenyl with pyridine or indolin-2-one derivatives resulted in a complete absence of activity.

The further used strategy was based on the idea that structurally similar compounds have an affinity for the same proteins in an attempt to find possible binding sites for the chemicals under study. Using the web engine BindingDB [6], which has a large dataset of 2.8 million entries for 1.2 million compounds and 9.2 thousand targets, a thorough search was carried out to find the sdf files that corresponded to the substances under investigation. Surprisingly, no matches were found in the database. This result is especially important when trying to counteract antifungal resistance mechanisms [7], particularly those that affect important processes like ergosterol synthesis, drug uptake mediated by particular permeases, drug efflux mediated by major facilitator superfamilies and ATP-binding cassette, multidrug transporters, chitin and *β*-1,3-glucan synthases, biofilm formation, and cellular stress-response mechanisms mediated by Hsp90, etc.

The lack of matches in BindingDB [6] highlights how unique the chemicals being studied are in relation to the designated antifungal targets. The study's latter stages will focus further on figuring out the complexities of these interactions and how they can affect antifungal resistance mechanisms.

Conclusions. The reported findings provide valuable insights into the design and optimization of antifungal agents targeting Candida species. It is critical to recognize the dynamic nature of antifungal resistance as ongoing research

continually brings new insights. Consequently, several derivatives of 5,6-dihydrotetrazolo[1,5-*c*]quinazolines have demonstrated potent inhibitory properties against *C. glabrata* at concentrations of 0.125 – 16 mg/L (0.4 – 55.0 μM), leading to the establishment of a structure-activity relationship. SAR underscores the importance of a shorter alkyl chain and a phenyl ring substituted at specific positions, as opposed to spiro derivatives with heterocyclic indolin-2-ones in the 5,6-dihydrotetrazolo[1,5-*c*]quinazoline core, for achieving optimal biological activity. These additional facts contribute to a comprehensive understanding of the SAR for compounds against *C. glabrata*, providing nuances related to functional groups and heterocyclic replacements. The next stage of research aims to explore the mechanistic aspects of 5,6-dihydrotetrazolo[1,5-*c*]quinazolines' antifungal activity for further exploration and refinement of antimicrobial agents with improved efficacy and specificity.

Acknowledgements. Authors gratefully acknowledge the Armed Forces of Ukraine and Territorial Defense Forces of the Armed Forces of Ukraine for preparing this paper in the safe conditions of Zaporizhzhia, Ukraine.

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