Journal of Oral Biology and Craniofacial Research 14 (2024) 211–215

Contents lists available at ScienceDirect

Journal of Oral Biology and Craniofacial Research

journal homepage: www.elsevier.com/locate/jobcr

Synthesis and evaluation of the antifungal activity of 5-hydroxy-3-phenyl-1H-pyrazole-1-carbothioamide for use in the oral environment

Hossein Amin Zadeh [a], Ali Asghar Zomorodkia [b], Saeid Hadi [c,**], Iman Mohammad Zadeh [d,*], Seyed Abd Alreza Sabetghadam [e], Vahid Hadi [c]

[a] Department of Chemistry, Shahid Bahonar University of Kerman, 76169, Iran
[b] Department of Chemical Engineering, South Tehran Branch, Islamic Azad University, Tehran, Iran
[c] Department of Health and Nutriotion, Faculty of Medicine, AJA University of Medical Science, Tehran, Iran
[d] Oral and Dental Disease Research Center, Kerman University of Medical Sciences, Kerman, Iran
[e] Department of Polymer Engineering, Science and Research Branch, Islamic Azad University, Tehran, Iran

ARTICLE INFO

Keywords:
Oral health
Antifungal
Pyrazole
Candida albicans
Candida tropicalis
fluconazole

ABSTRACT

Background and aim: Candida albicans and Candida tropicalis, can cause superficial infections of the oral mucosa as well as disseminated bloodstream and deep-tissue infections. The most frequently employed class of antifungals used for Candida infection treatment are the azole antifungals. Their low price, low toxic qualities, and availability for oral use make fluconazole and similar azole antifungals the preferred treatment for various infections caused by Candida. Nevertheless, developed and intrinsic resistance to antifungals of the azole family has been widely documented in association with various species of Candida. Candida infection management requires synthesizing new compounds to improve azole class antifungals, as Candida isolates resistant to azole are increasingly encountered in the clinical setting. This study aimed to synthesize a new azole compound and investigate its antifungal activity.

Methods: In this experimental study, 5-hydroxy-3-phenyl-1H-pyrazole-1-carbothioamide was synthesized by the reaction between thiosemecarbazide and ethylbezoylacetate. The structure of the synthesized compound was characterized by different techniques such as Fourier transform infrared (FT-IR) and nuclear magnetic resonance (NMR) spectra and its antifungal activity against Candida albicans and Candida tropicalis was investigated by the Spread Plat method to determine its minimum fungicidal concentration (MFC) and minimum inhibitory concentration (MIC).

Results and discussion: The Spread Plat test demonstrated that with the increase in 5-hydroxy-3-phenyl-1H-pyrazole-1-carbothioamide concentration, colonies of fungi were increasingly eliminated at a significant level($p < 0.001$). At a concentration of 1000 ppm, all Candida albicans and Candida tropicalis colonies were destroyed.

Conclusions: The results indicate that the synthesized compound showed a promising antifungal effect. On the other hand, it had a suitable spectrum of effect, because it showed antifungal effects on both Candida albicans and Candida tropicalis strains.

1. Introduction

Oral health is not defined as merely having good teeth, but also includes the absence of tooth loss, tooth decay, periodontal disease, birth defects such as cleft palate and cleft lip, oral sores, fungal infections, cancerous growth in the throat and mouth, any kind of chronic orofacial pain, and any other diseases of the oral cavity.[1,2]

The connection between general health and oral health has been abundantly demonstrated. Many systemic diseases such as cardiovascular diseases and diabetes are aggravated by oral diseases, which may themselves be manifestations of general health conditions.[3]

Candida infections of the mouth mostly require a compromised host, and the most common reservoir sites include the tongue's dorsal surface and the palatal and papillated mucosa underlying maxillary dentures.[4]

* Corresponding author. Oral and Dental Disease Research Center, Kerman University of Medical Sciences, Kerman, Iran.
** Corresponding author.
E-mail addresses: haminzadeh77@gmail.com (H. Amin Zadeh), Alikia104343@yahoo.com (A.A. Zomorodkia), s.hadinu@yahoo.com (S. Hadi), i.mohammadzadeh@kmu.ac.ir (I. Mohammad Zadeh), ash.sabbetghadam@gmail.com (S.A.A. Sabetghadam), vahidhadi12@gmail.com (V. Hadi).

https://doi.org/10.1016/j.jobcr.2024.01.008
Received 20 July 2023; Received in revised form 29 August 2023; Accepted 15 January 2024

Intrusive and superficial fungal infections are the specialty of many Candida species. For quite a long time, Candida albicans has been the most commonly implicated species in infections among the 150-plus species of Candida, accounting for over 60% of the overall cases of candidemia.[5,6]

As of late, candidemia or candidiasis patients have been increasingly found to be infected by C. parapsilosis, C. glabrata, and C. tropicalis (NACs or non-albicans Candida species).

The first or second NAC species commonly isolated from those suffering from Candida is C. tropicalis.[7,8]

Among the Candida species capable of causing disease in humans, Candida tropicalis is probably one of the most commonly detected in tropical countries. It has a 3–66% chance of causing candidemia, and the probability of causing invasive disease is different in various geographical areas. With many shared pathogenic traits, C. albicans and C. tropicalis are taxonomically quite close. The highest virulence of C. tropicalis is observed in neutropenic hosts and is usually accompanied with hematogenous peripheral organ seeding. Due to the possibility of gradual resistance to fluconazole, the frequency of C. tropicalis detection in different clinical cases has become a cause of concern.[9,10]

The chemical groups that the numerous antifungals developed for treating Candidiasis belong to are comparatively limited. Moreover, drug resistance and side effects in clinically detached strains have led to failures in and discontent with Candidiasis treatments.[11]

There has been an increase in fluconazole resistance in C. tropicalis clinical isolates, and similar resistance to fluconazole has been also detected in C. albicans. The mechanisms involved in this phenomenon include reduced intracellular drug accumulation associated with membrane transport protein overexpression, reduced 14DM/fluconazole affinity as a result of ERG11 gene mutation, overexpression of ERG11, which encodes 14DM (the drug target enzyme), and sterol biosynthesis pathway modifications. CtERG11 gene overexpression in association with missense mutation may also play a role in the acquired azole resistance in C. tropicalis.[12]

Apart from allergic reactions, many medicines cause estrogen level imbalance and liver-damage as their side-effects.[13]

Basically, most of the clinically-utilized antifungals have disadvantages such as toxicity, side effects, inadequacy of fungicidal effect, and high price. In spite of the emergence of novel antifungal drugs, there are not many of them.[16] As a result, new antifungal drugs have attracted much interest, leading to a search for more successful and less toxic alternatives.[14]

One well-known group of aromatic heterocycles with five-membered rings containing two nitrogen atoms are pyrazoles. This heterocyclic family display many pharmacological, agrochemical, biological, and chemical properties. Their derivatives include a number of highly effective compounds with a huge range of biological functions. Drugs derived from pyrazole have been used to develop a large number of drugs in recent years.[15] Considering the importance of pyrazoles in many fields, especially medicinal chemistry, this review by Sharma and Bhatia focuses on the production and biological activities of various pyrazole derivatives.[16]

This work focused on the evaluation the antifungal activity of 5-hydroxy-3-phenyl-1H-pyrazole-1-carbothioamide against Candida tropicalis and Candida albicans in order to measure the minimal fungicidal concentration and minimal inhibitory concentration of this compound.

2. Methods

2.1. Material and procedure

Thiosemicarbazide (98%), ethyl benzoylacetate (95%), hydrochloric acid (37%), ethanol (96%) Sabouraud dextrose agar, and Sabouraud dextrose broth were purchased from Sigma-Aldrich, and Candida albicans (IBRC-M-30070) and Candida tropicalis (IBRC-M-30420) cells were obtained from the Iranian Biological Resource Center. Melting points were measured on Electrothermal-9200. The open capillary technique was used to measure the melting points and they were then uncorrected. A spectrophotometer (Bruker, Germany) was used to record IR spectra using KBr pellet. A Bruker instrument (^{1}H at 400 MHz and ^{13}C at 100 MHz) was used to record ^{13}C NMR and ^{1}H spectra with tetramethylsilane as the internal standard and DMSOd6 as the solvent. Chemical shifts were reported in ppm.

2.2. Synthesis of 5-hydroxy-3-phenyl-1H-pyrazole-1-carbothioamide

As is shown in Fig. 1, 5 mM of thiosemicarbazide (2) and 5 mM of ethyl benzoylacetate (1) were dissolved in a water and ethanol mixture as solvent. Four to five drops of hydrochloric acid were added to the solution and it was refluxed at 100 °C for 5 hour. The resulting solid was filtered and washed with ethanol and water to finalize the product. Purification of the crude product was done by recrystallizing it in ethanol. Yield: 78 %; m.p:160 °C.

The FT-IR spectra recorded in the range of 400–4000 cm1

IR (KBr):1247,1464, 1491, 1504, 1722, 2981, 3151, 3225, 3408cm-1.

The NMR spectra were recorded on a Bruker 300 AVANCE III NMR magnet (300 MHz for 1H NMRand75MHZfor13CNMR).

1HNMR(300 MHz,DMSOd6)δ(ppm):6.21(H,S,CH),7.52(3H,S,arom),7.97(2H,S.arom),10.06(2H,S,NH2), 12.05(1H,S,OH).

13C NMR (75 MHz, DMSOd6) δ (ppm):93.09, 131.87, 132.38, 132.60, 133.92, 134.38, 135.46, 151.77, 163.77, 181.48.

2.3. Anti-fungous assay and determination of minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) of 5-hydroxy-3-phenyl-1H-pyrazole-1-carbothioamide

First, Candida albicans (IBRC-M-30070) and Candida tropicalis (IBRC-M-30420) cells were purchased from the Iranian Biological Resource Center. In order to prepare a fungal suspension in germ-free conditions, a colony loop was removed from the fungal culture medium and added to 1 mL double-distilled water. Then, 10 μL of the homogenized solution was placed on a Neubauer slide, and the number of spores was counted at × 100 microscope magnification. Then, 10 μL of the counted spore solution, equivalent to 100 on average, were cultured by lawn culture method on the culture medium. Then the concentrations of 1000, 500, 250 and 125 ppm of 5-hydroxy-3-phenyl-1H-pyrazole-1-carbothioamide and fluconazole, as a standard sample, were prepared by dilution method. In brief, first, we prepared a concentration of 1000 ppm from combination of 5-hydroxy-3-phenyl-1H-pyrazole-1-carbothioamide and fluconazole. Then, using the M1V1 = M2V2 formula, we obtained the concentrations of 500, 250, and 125 ppm and added them to the culture medium, specific for the growth of the fungus (Sabouraud dextrose agar). After the agar was set and we ensured that there was no contamination, 10 μL of fungal suspension sample was poured onto the medium. Then the fungal sample was placed on the culture medium using an L-shaped glass cell spreader. After 14 days, the fungal colonies were counted.

2.3.1. Minimal inhibitory concentration (MIC)

The standard broth micro dilution method was used following the Clinical and Laboratory Standards Institute Guidelines (CLSI, 2008) to study how 5-hydroxy-3-phenyl-1H-pyrazole-1-carbothioamide affects the growth of C. tropicalis and C. albicans planktonic cells. Wells with a range of concentrations of 5-hydroxy-3-phenyl-1H-pyrazole-1-carbothioamide (1.56–50 mM) were inoculated with 1 × 103 cells/ml, followed by a 7-day incubation of the microplates at 25 °C. A final volume of 200 μl was maintained in each well of the assay system. Control wells did not contain 5-hydroxy-3-phenyl-1H-pyrazole-1-carbothioamide. Fluconazole concentrations 1–256 μg/ml were employed as a standard antifungal. Using a microplate reader, the spectrophotometrical absorbance was read at 620 nm to analyze the growth. The MIC for growth of

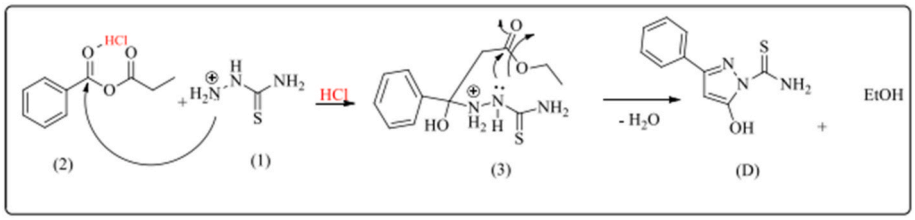

Fig. 1. Synthesis of 5-hydroxy-3-phenyl-1H-pyrazole-1-carbothioamide.

C. tropicalis and C. albicans was the lowest concentration of 5-hydroxy-3-phenyl-1H-pyrazole-1-carbothioamide causing 50% decrease of absorbance compared to control.

2.3.2. Minimum fungicidal concentration (MFC)

Cell samples were selected from MIC concentrations and higher in order to assess the lowest concentrations of 5-hydroxy-3-phenyl-1H-pyrazole-1-carbothioamide with anti-Candida effects. YPD agar was spread with an aliquot of 10 μl cell suspension from these wells, and the plates underwent a 7-day incubation at 25 °C and were assessed for colony formation. If no colonies were observed, the concentration was considered fungicidal.

2.4. Data analysis

Tukey's post-hoc test and one-way analysis of variance (ANOVA) were used for statistical data analysis with SPSS20 (Chicago, Illinois, USA). P-values ≤0.001 were considered statically significant. Probit analysis was used to measure IC50 in SPSS software with the significance level set at 0.05.

3. Results

3.1. Synthesis of 5-hydroxy-3-phenyl-1H-pyrazole-1-carbothioamide

FT-IR spectroscopy is an appropriate analysis to characterize the functional groups of synthesized composite. The FTIR spectrum of the synthesized compound (5-hydroxy-3-phenyl-1H-pyrazole-1-carbothioamide) is shown in Fig. 2. As shown in the spectrum, the absorption band at 1247 cm-1 is related to the stretching vibrations of the thio functional group (C═S), the absorption bands at 11464, 1491, and 1504 cm are related to the vibrations of (C═C) phenyl ring, the absorption band at 11722 cm is related to carbonyl (C═O) in the thioamide group, absorption bands cm-1 2981, 3151, and 3225 are related to (C–H) vibrations, and the stretching vibrations (NH2) of the thioamide group appeared at cm-13408.

3.2. AntiFungal activity of 5-hydroxy-3-phenyl-1H-pyrazole-1-carbothioamide

The antifungal activity of the 5-hydroxy-3-phenyl-1H-pyrazole-1-carbothioamide compound against Candida albicans and Candida tropicalis fungi was investigated by Spread Plate Technique.

As shown in the graphs, the number of fungal colonies was initially 100, but by adding concentrations of 125, 250, 500 and 1000 ppm of fluconazole and compound 5-hydroxy-3-phenyl-1H-pyrazole-1-carbothioamide, the number of colonies decreased. After 14 days, fluconazole in all concentrations destroyed all the colonies of Candida albicans and Candida tropicalis, but compound 5-hydroxy-3-phenyl-1H-pyrazole-1-carbothioamide was most effective at a concentration of 1000 ppm, which, like fluconazole, destroyed all colonies. ($P < 0.001$) which is shown in Fig. 3.

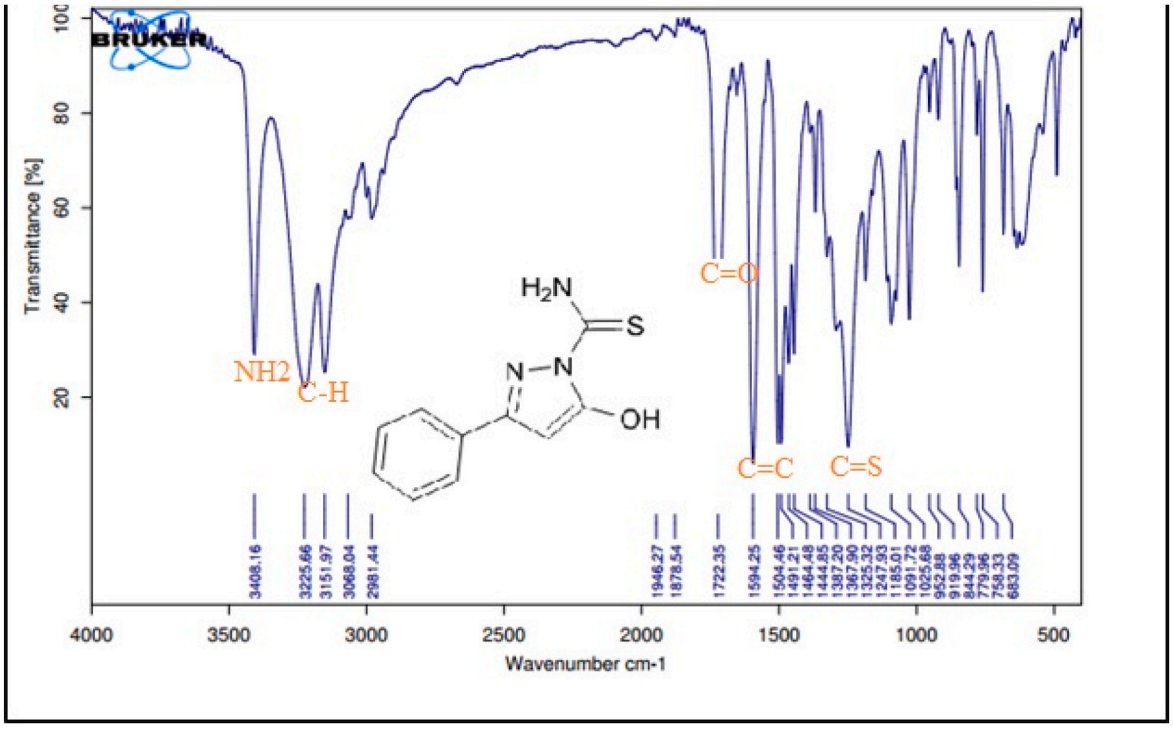

Fig. 2. FT-IR spectra of 5-hydroxy-3-phenyl-1H-pyrazole-1-carbothioamide.

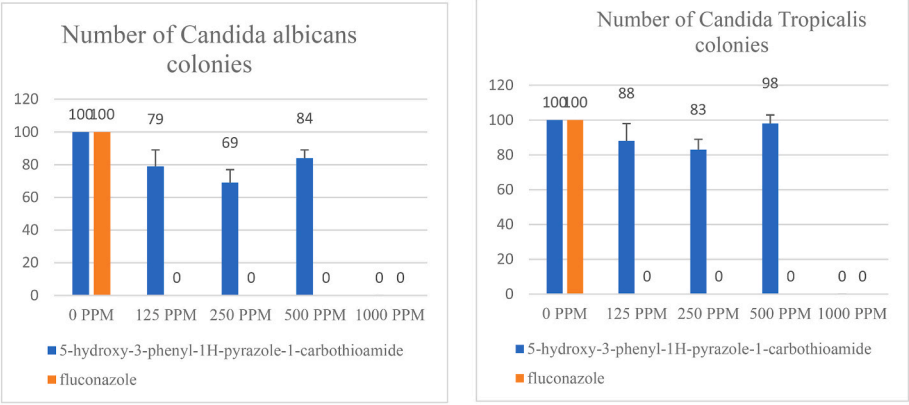

Fig. 3. Antifungal activity of 5-hydroxy-3-phenyl-1H-pyrazole-1-carbothioamide against & Candida albicans Candida tropicalis

The results regarding the minimal inhibitory concentration (MIC) and the minimum fungicidal concentrations (MFC) of this compound are given in Table 1.

The obtained results showed that the MIC of 5-hydroxy-3-phenyl-1H-pyrazole-1-carbothioamide compound was 62.5 μg.ml-1 against Candida albicans and 125 μg.ml-1 against Candida tropicalis and the MIC of fluconazole was 15.62 μg.ml-1 against Candida albicans and 31.25 μg.ml-1 against Candida tropicalis. The MFC of 5-hydroxy-3-phenyl-1H-pyrazole-1-carbothioamide compound was 1000 μg.ml-1 for Candida albicans and 1000 g.ml-1μ for Candida tropicalis, and the MFC level for fluconazole was 62.5 μg.ml-1 against Candida albicans and 62.5 μg.ml-1 against Candida tropicalis ($P > 0.001$).

4. Discussion

There has been a recent increase in Candida-related systemic fungal infections due to the increase in immunosuppressive diseases such as AIDS, hematogenous diseases, malignancies, and the emergence of antibiotic resistance to commonly used drugs. For this reason, finding compounds that can be suitable substitutes for common antibiotics and antifungal drugs is one of the topics discussed in this field.[17]

According to the research of Whaley et al., considering their low toxicity, cheapness, and availability, azoles and their synthesized derivatives, such as fluconazole, are the first choice of doctors for managing infections caused by Candida. Nevertheless, developed and intrinsic resistance to antifungals of the azole family have been documented in the treatment of multiple species of Candida.[18]

As important nitrogen heterocycles, azole compounds have electron-rich properties, which enables their derivatives to bond with organism receptors and enzymes by noncovalent reactions such as Van der Waals force, cation-π,π-π stacking, and hydrophobic effect, ion-dipole, coordination bonds, hydrogen bonds, etc. This makes them extremely useful in medicinal chemistry, especially in their triazole and imidazole forms, against fungal infections. There have been many investigations focusing on their synthesis, design, and antimicrobial activity in recent years, and they have made quite rapid progress as a result. Therefore, a large number of azole-based agents have been extensively studied as antifungal and antibacterial candidates, showing great potential and value in clinical experiments.[19]

In 2014, Samet Mert et al. synthesized a series of compounds associated with pyrazole-3,4-dicarboxylic acid and pyrazole-3-carboxylic acid with structures tested by elemental analysis and FT-IR and NMR spectra. Modified agar well diffusion assay was used to screen the antifungal and antibacterial activities of the compounds against five fungal and five bacterial pathogens. The majority of the compounds were able to inhibit Candida glabrata, Candida albicans, Candida parapsilosis, and Candida tropicalis strains. Based on this research, the derivatives of pyrazole assessed in this research showed stronger inhibition of C. albicans, but these compounds showed only some inhibitory effects on C. glabrata, C. tropicalis, and C. parapsilosis strains.[20]

The drug ketoconazole and posaconazole, voriconazole, itraconazole, and fluconazole (triazoles) are the most widely used azole antifungals. They bind to the heme cofactor in the active lanosterol 14a-demethylase site, inhibiting ergosterol synthesis. Depletion of ergosterol and methylated sterol precursor accumulation are believed to influence fungal cell proliferation and growth through affecting the function of some membrane-bound accumulation proteins and membrane integrity. Compared to echinocandin and polyene antifungals, azoles have many advantages, including their availability for oral administration, wider tissue distribution, lower toxicity, and higher solubility. However, the rise in resistant strains has limited the clinical use of azoles, particularly in long-term administration. Thus, finding new azole anti-fungal agents that strains have not developed resistance to is a priority.[21]

The obtained results show that the compound 5-hydroxy-3-phenyl-1H-pyrazole-1-carbothioamide destroyed all Candida albicans and Candida tropicalis colonies in the spread plate method at a concentration of 1000 ppm, and the minimum inhibitory concentration against Candida albicans and Candida tropicalis was 62.5 and 125 μg.ml-1.

The advantage of 5-hydroxy-3-phenyl-1H-pyrazole-1-carbthioamide is that, while it has the same mechanism of action as other azole compounds, it has not yet developed resistance to fungi.

Table 1
MIC and MFC of 5-hydroxy-3-phenyl-1H-pyrazole-1-carbothioamide and fluconazole.

	5-hydroxy-3-phenyl-1H-pyrazole-1-carbothioamide		Fluconazole	
	$g.ml^{-1}\mu$ **MIC**	$g.ml^{-1}\mu$ **MFC**	$g.ml^{-1}\mu$ **MIC**	$g.ml^{-1}\mu$ **MFC**
Candida albicans	62.5	1000	15.62	62.5
Candida tropicalis	125	1000	31.25	62.5

5. Conclusion

The search for effective and commercially feasible drugs is a complex interdisciplinary process. It involves the identification of a compound able to bind, both chemically and geometrically, to a specific site on a target protein. The compound can be recognized as a drug only after it has passed the animal and human trials. The compounds screened for their medical properties, are either identified in nature or synthesized in laboratories.

This study aimed to synthesize a new pyrazole compound and investigate its antifungal activity in an attempt to discover a new

antimicrobial agent. The physical properties of the newly synthesized pyrazole derivative were determined, and its biological activity was evaluated by Spread Plat method against Candida albicans and Candida tropicalis.

Based on the obtained results, it can be stated that compound 5-hydroxy-3-phenyl-1H-pyrazole-1-carbothioamide has a favorable effect against Candida tropicalis and Candida albicans, and considering resistance these to common drugs such as fluconazole, this compound can be considered a suitable alternative. Of course, due to the new structure of this compound, more studies are needed to increase the power and effect spectrum of this compound.

Declaration of competing interest

The authors whose names are listed immediately below certify that they have NO affi liations with or involvement in any organization or entity with any fi nancial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-fi nancial interest (such as personal or professional relationships, affi liations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

References

1. Hammond L, Conroy T, Murray J. Exploring oral care practices, barriers, and facilitators in an inpatient stroke unit: a thematic analysis. *Disabil Rehabil*. 2023 Feb 27;45(5):796–804. https://doi.org/10.1080/09638288.2022.2040616.
2. Zhu Y, Tao C, Goh C, Shrestha A. Innovative biomaterials for the treatment of periodontal disease. *Frontiers in Dental Medicine*. 2023 May 30;4, 1163562. https://doi.org/10.3389/fdmed.2023.1163562.
3. Henni SH, Skudutyte-Rysstad R, Ansteinsson V, Hellesø R, Hovden EA. Oral health and oral health-related quality of life among older adults receiving home health care services: a scoping review. *Gerodontology*. 2023 Jun;40(2):161–171. https://doi.org/10.1111/ger.12649.
4. Al-Obaidi MM, Ostrosky-Zeichner L, Nix DE. Trends of azole antifungal prescription in the United States: medicare Part D provider utilization and payment data analysis. *InOpen Forum Infectious Diseases*. 2023 Aug;10(8). ofad345). US: Oxford University Press.
5. Colombo RE, Vazquez JA. An evaluation of ibrexafungerp for the treatment of invasive candidiasis: the evidence to date. *Expet Opin Pharmacother*. 2021 May 3;22 (7):797–807. https://doi.org/10.1080/14656566.2021.1890026.
6. Ghojoghi A, Erfaninejad M, Ahmadpour E, Nazar E, Barac A, Fatahinia M. Non-albicans candidemia in cancer patients as an increasing health problem: a comprehensive review and meta-analysis. *Asian Pac J Tropical Med*. 2022 Sep 1;15 (9):387. https://doi.org/10.4103/1995-7645.356992.
7. El-Kholy MA, Helaly GF, El Ghazzawi EF, El-Sawaf G, Shawky SM. Virulence factors and antifungal susceptibility profile of C. tropicalis isolated from various clinical specimens in Alexandria, Egypt. *Journal of Fungi*. 2021 Apr 29;7(5):351. https://doi.org/10.3390/jof7050351.
8. Rokas A. Evolution of the human pathogenic lifestyle in fungi. *Nature Microbiology*. 2022 May;7(5):607–619. https://doi.org/10.1038/s41564-022-01112-0.
9. Spruijtenburg B, Meijer EF, Xiao M, et al. Genotyping and susceptibility testing uncovers large azole-resistant Candida tropicalis clade in Alexandria, Egypt. *Journal of Global Antimicrobial Resistance*. 2023 Sep 1;34:99–105. https://doi.org/10.1016/j.jgar.2023.06.012.
10. Françoise U, Desnos-Ollivier M, Le Govic Y, et al. Candida haemulonii complex, an emerging threat from tropical regions? *PLoS Neglected Trop Dis*. 2023 Jul 31;17(7), e0011453. https://doi.org/10.1371/journal.pntd.0011453.
11. Song YD, Hsu CC, Lew SQ, Lin CH. Candida tropicalis RON1 is required for hyphal formation, biofilm development, and virulence but is dispensable for N-acetylglucosamine catabolism. *Med Mycol*. 2021 Apr;59(4):379–391. https://doi.org/10.1093/mmy/myaa063.
12. Fan X, Tsui CK, Chen X, Wang P, Liu ZJ, Yang CX. High prevalence of fluconazole resistant Candida tropicalis among candiduria samples in China: an ignored matter of concern. *Front Microbiol*. 2023 Mar 2;14, 1125241. https://doi.org/10.3389/fmicb.2023.1125241.
13. Ulanowska M, Olas B. Biological properties and prospects for the application of eugenol—a review. *Int J Mol Sci*. 2021 Apr 1;22(7):3671. https://doi.org/10.3390/ijms22073671.
14. Montoya MC, Moye-Rowley WS, Krysan DJ. Candida auris: the canary in the mine of antifungal drug resistance. *ACS Infect Dis*. 2019 Aug 1;5(9):1487–1492. https://doi.org/10.1021/acsinfecdis.9b00239.
15. Bangia R, Sharma G, Dogra S, Katare OP. Nanotechnological interventions in dermatophytosis: from oral to topical, a fresh perspective. *Expet Opin Drug Deliv*. 2019 Apr 3;16(4):377–396. https://doi.org/10.1080/17425247.2019.1593962.
16. Boniche C, Rossi SA, Kischkel B, et al. Immunotherapy against systemic fungal infections based on monoclonal antibodies. *Journal of Fungi*. 2020 Feb 29;6(1):31. https://doi.org/10.3390/jof6010031.
17. Brilhante RS, Lopes RG, de Aguiar L, et al. Inhibitory effect of proteinase K against dermatophyte biofilms: an alternative for increasing the antifungal effects of terbinafine and griseofulvin. *Biofouling*. 2022 Mar 16;38(3):286–297. https://doi.org/10.1080/08927014.2022.2063720.
18. Yet L. Five-membered ring systems: with more than one N atom. *InProgress in Heterocyclic Chemistry*. 2020 Jan 1;31:325–361. https://doi.org/10.1016/B978-0-12-819962-6.00008-7. Elsevier.
19. Whaley SG, Berkow EL, Rybak JM, Nishimoto AT, Barker KS, Rogers PD. Azole antifungal resistance in Candida albicans and emerging non-albicans Candida species. *Front Microbiol*. 2017 Jan 12;7:2173. https://doi.org/10.3389/fmicb.2016.02173.
20. Verma C, Thakur A, Ganjoo R, et al. Coordination bonding and corrosion inhibition potential of nitrogen-rich heterocycles: azoles and triazines as specific examples. *Coord Chem Rev*. 2023 Aug 1:488. https://doi.org/10.1016/j.ccr.2023.215177, 215177.
21. Mert S, Kasımoğulları R, Iça T, Çolak F, Altun A, Ok S. Synthesis, structure–activity relationships, and in vitro antibacterial and antifungal activity evaluations of novel pyrazole carboxylic and dicarboxylic acid derivatives. *Eur J Med Chem*. 2014 May 6; 78:86–96. https://doi.org/10.1016/j.ejmech.2014.03.033.