RESEARCH ARTICLE

Design, Synthesis, Molecular Docking and Antifungal Evaluation of Mixed Heterocyclic Moieties Containing Pyridine, 1,3,4-Oxadiazole and 1,2,3-Triazol Rings

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ABSTRACT

In the current study, derivatives containing pyridine, 1,3,4-oxadiazole and 1,2,3-triazole heterocycles in their structures have been synthesized, 1,2,3-triazole moiety was synthesized using click chemistry, a powerful modular synthesis approach, and evaluated for *in-vitro* antifungal activity. When compared to the regular medicine Fluconazole, one derivative (5a) showed superior fungicidal action.

Prior to synthesis, an *in silico* computational analysis was conducted to determine the binding interactions of these synthesized compounds in the active sites of the fungal enzyme sterol 14-demethylase (CYP51) through molecular docking via Genetic Optimization for Ligand Docking (GOLD) suite *v.5.6.2*. Most of the tested compounds in molecular docking showed significant activities compared with fluconazole as reference compound due to their hydrogen bonding interaction with key amino acids in Sterol 14-demethylase enzyme Tyr116 and iron metal, and these results are compatible with their *in vitro* anti-fungal study.

Keywords: Fluconazole, Oxadiazole, Pyridine, Triazole, Sterol TYR116.

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INTRODUCTION

Fungal infection is a prevalent disease that poses a severe threat to mankind, resulting in high morbidity and mortality each year all over the world,[1] particularly among immunocompromised people.[2] As a result, it's necessary to seek new antifungal inhibitors that are both safer and more effective against drug-resistant and sensitive fungi. Recently, the fused heterocyclic compounds that contain bridgehead nitrogen- or oxygen-donor atoms have attracted great attention due to their pharmacological activities and chemical therapeutic qualities. Indeed, pyridine derivatives have recently been found to have therapeutic potential as anti-inflammatory,[3] fungicidal,[4] antiviral,[5] antibacterial,[6] and anticancer agents.[7]

Moreover, 1, 3, 4-Oxadiazole derivatives are the heterocyclic that have attracted considerable attention past couple of decades as they possess wide range of biological properties include antibacterial,[8] anti-inflammatory,[9] antifungal,[10] analgesic,[11] anticancer,[12] antiviral,[13] antidepressant.[14]

Additionally, Triazole derivatives were reported to exhibit exceptional anti-inflammatory properties,[15] antibacterial,[16] antifungal,[17] anti-depressant,[18] analgesic,[19] anticancer.[20] 1,2,3-Triazole moiety was synthesized by using click chemistry approach, click chemistry refers to a set of chemical reactions that meet certain criteria, like being modular, stereospecific, high-yielding and simple experimental procedures.[21] This click reaction has been developed as a functional group tolerant and excellent approach for the synthesis of organic compounds.[22]

In our study, we have reported synthesis of mixed heterocyclic derivatives containing three heterocycles pyridine, 1,3,4-oxadiazole and triazole. These heterocyclic derivatives were thought to be an excellent requirement for antifungal activity. As a result, it was thought to be worthwhile to msynthesize these compounds and test them against a resistant strain of Candida albicans.

Prior to synthesis and in-vitro studies, the new derivatives were screened for their *In silico* sterol 14α-demethylase (CYP51) selectivity using molecular docking via GOLD suite v.5.6.2.[23]

EXPERIMENTAL WORK

Isonicotinoyl hydrazide (Isoniazid) was purchased from Zhejiang Medicine Co. Ltd., Xinchang Pharmaceutical Factory (China). Propagyl bromide, Chloro-acetyl chloride, Aniline

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and p-Nitroaniline were purchased from Sigma-Aldrich (USA).

Chemical Synthesis

The following procedures were used to synthesize intermediates and final heterocyclic compounds, as shown in the diagram. (Scheme 1).

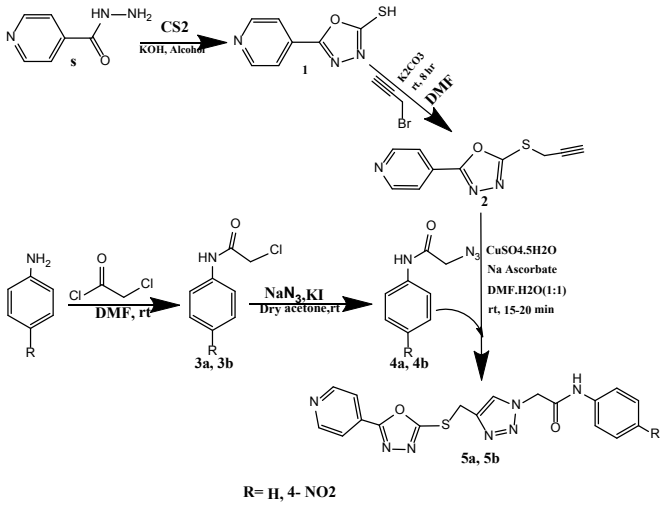
Synthesis of 1,3,4-oxadiazole derivative(1)

Isoniazid (0.01 mol) was dissolved in 100ml ethanol then potassium hydroxide and Carbon Disulfide were added in equimolar amounts to this solution. After that, this mixture was refluxed at 78°C for 24 hours. Distilled water was then added, followed by neutralization with dilute HCl(1N). A solid mass appeared was filtered and recrystallized from methanol yielding bright yellow crystals.[24]

Bright yellow crystal, yield= 82%, M.P.= 263-265°C. IR KBr (cm-1): 2368(S-H str), 1595(C=N str of 1,3,4-oxadiazole), 1008(C-O str of oxadiazole nucleus).

Synthesis of 2-(prop-2-yn-1-ylthio)-5-(pyridin-4-yl)-1,3,4-oxadiazole(2)

8.74 mmol of 5-(pyridin-4-yl)-1,3,4-oxadiazole-2-thiol (Compound **1**) was added to a mixture of 4.91 mmol propargyl bromide and 10.5 mmol K_2CO_3 in 30 ml DMF at room temperature, and stirred for 8 hours. The resulted combination was dissolved in distilled water, then extracted by diethyl ether, the organic layer was dried by using Na_2SO_4 and then was purified. After that, evaporation of filtrate was done to obtain the desired compound.[25]



Scheme 1: Synthesis of the intermediate (2) and final compounds(5a, 5b)

Yellow powder, yield= 66%, M.P= 96-98°C. IR KBr (cm^{-1}): 3151(≡C-H str), 2108(C≡C str). ^{1}H-NMR(DMSO): δ 4.14-4.44 (s, 3H as overlapped two signals; s, 2H, S-CH_2 and s, 1H, ≡C-H), δ 7.75-8.00(d, 2H, CH of pyridine close to the oxadiazole ring), δ 8.65-8.92(d, 2H, CH of pyridine close to N).

Synthesis of 2-chloro-N-(substituted phenyl) acetamide (3a, 3b)

Chloroacetyl chloride (8 mL) was slowly added over a period of 30 minutes to a solution of aromatic amine derivatives (100 mmol) in DMF (50 mL). Stirring continued for an hour. The reaction mixture was quenched with 150 mL cold distilled water. The precipitates appeared collected by filtration, then washed with cold water, dried, and recrystallized using methanol to give pure compound.[26]

- *2-chloro-N-phenylacetamide(3a)*

Physical properties are shown in Table 1. Spectral data: IR KBr (cm^{-1}): 3269(N-H str), 1672(C=O str).

- *2-chloro-N-(4-nitrophenyl) acetamide(3b)*

Physical properties are shown in Table 1. Spectral data: IR KBr (cm^{-1}): 3276(N-H str), 1687(C=O str), 1568(N-O *str, asymmetrical), 1338(*N–O *str symmetrical).*

Synthesis of 2-azido-N-(substituted phenyl) acetamide(4a, 4b)

A mixture of 8 mmol 2-chloro-N-phenylacetamide derivatives(**3a, 3b**) and potassium iodide (10 mmol) in 15 ml acetone(dry) was stirred for 2 hours. Then,the solution of sodium azide (8.8 mmol) dissolved in 10 mL water was added to the residue. After that, the mixture added to crushed ice and extracted by ethyl acetate (3 x 50 mL), followed by washing of the combined organic extract using brine solution, dried by Na2SO4(anhydrous), purified and concentrated in low pressure to give the desired compound.[27]

- *2-azido-N-phenylacetamide(4a)*

Physical properties are shown in Table 1. Spectral data: IR KBr (cm^{-1}): 3257(N-H str), 2102(azide str), 1664(C=O str), 1253(C-N str). ^{1}H-NMR (DMSO): δ 4.04 (s, 2H, CH_2), δ 7.08-7.59 (m, 5H, Ar-H), δ 10.13 (s,1H, N-H).

Table 1: Percent yield, melting point, appearance and percent of yield of the intermediates and final compounds.

Comp.	*Molecular formula*	*Molecular weight*	*Description*	*Percent of yield (%)*	*Melting point (°C)*
S	C6H7N3O	137.14	White crystal	-	171.4
1	C7H5N3OS	179.20	Bright yellow crystal	82	263
2	C10H7N3OS	217.25	Yellow powder	66	96–100
3a	C8H8ClNO	169.61	Orange powder	85	124–126
3b	C8H7ClN2O3	214.61	Off white powder	82	180
4a	C8H8N4O	176.18	Brown	72	82
4b	C8H7N5O3	221.17	Yellow powder	68	91
5a	C18H15N7O2S	393.42	Brown crystal	84	126 dec
5b	C18H14N8O4S	438.42	Yellowish powder	88	150 dec

- *2-azido-N-(4-nitrophenyl) acetamide(4b)*

Physical properties are shown in Table 1. Spectral data: IR KBr (cm^{-1}): 3329(N-H str), 2117(azide str), 1678(C=O str), 1260(C-N str), 1480(N-O *str, asymmetrical), 1257(*N–O *str, symmetrical).* ^{1}H-NMR (DMSO): δ 4.23(s, 2H, CH_2), δ 7.83-8.39(m, 4H, Ar-H), δ 10.69(s,1H, N-H).

Synthesis of final compounds N-(Substituted phenyl)-2-(4-(((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)thio)methyl)-1H-1,2,3-triazol-1-yl)acetamide (5a, 5b)

A mixture of 2 mmol azide derivatives(4a, 4b), 2 mmol terminal alkyne (2), copper sulphate (1-mmol) and sodium ascorbate (1-mmol) in 5 mL DMF was stirred roughly for 5–10 minutes. This mixture was then added into 30g crushed ice. The separated solid has been purified and dried to give the final compounds.[27]

- *N-phenyl-2-(4-(((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl) thio)methyl)-1H-1,2,3-triazol-1-yl)acetamide(5a)*

Physical properties are shown in Table 1. Spectral data: IR KBr (cm^{-1}): 3385(N-H str), 1616(C=O str), 1420*(*triazole ring str*).* ^{1}H-NMR (DMSO): δ 4.63-4.74 (s, 2H, SCH_2), δ 5.21-5.45 (s, 2H, CH_2CO), δ 7.08-7.58(m, 5H, phenyl), δ 7.92-8(d, 4H, pyridine), δ 8.19-8.23(s, 1H, CH of triazole), δ 10.39- 10.47 (s,1H, N-H).

- *N-(4-nitrophenyl)-2-(4-(((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)thio)methyl)-1H-1,2,3-triazol-1-yl) acetamide(5b):*

Physical properties are shown in Table 1. Spectral data: IR KBr (cm^{-1}): 3427(N-H str), 1656(C=O str), 1458*(*triazole ring str*).* ^{1}H-NMR (DMSO): δ 4.71 (s, 2H, SCH_2), δ 5.42(s,2H, CH_2CO), δ 7.79-7.96(m,4H, phenyl), δ 7.96-8.23(d, 4H, pyridine), δ 8.23(s,1H, CH of triazole), δ 11.25(s,1H, N-H).

Antifungal Evaluation

The preliminary antifungal activity of the compounds tested was carried out at the College of Pharmacy, Al-Mustansiriyah University, Iraq. This antifungal test was performed using the Well Diffusion Method.[28] The synthesized compounds were tested against fungi *Candida albicans*. The isolates were collected from different clinical sources. Fluconazole was the reference drug for antifungal testing.

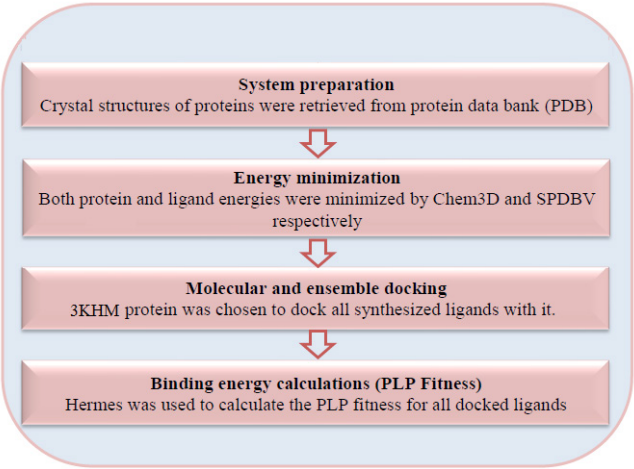


Figure 1: Computational protocol of the desired compounds

Computational Methods

The computational procedures of this work is shown in Figure 1. CCDC GOLD Suite (*v. 5.6.2*) was used to conduct studies of molecular docking for the compounds. CCDC Hermes visualizer program (v. 1.9.2) visualizes: ligands, receptors, hydrogen bonding interactions, short contacts and bond length estimation. Chem BioOffice software (v. 17.1) was used to draw the chemical structures of the desired ligands.

Ligands and Receptor Preparation

The crystal structure of sterol 14*α*-demethylase (CYP51) from *Trypanosoma cruzi* in complex with the inhibitor fluconazole was obtained from the Protein Data Bank (PDB ID: 3KHM), and the missing atoms were introduced through SwissPDB Viewer (SPDBV) (v. 3.7).

The preparation of protein's crystal structure done through 2 processes: eliminating all water molecules and introducing hydrogen atoms to get the right amino acids residues' ionization and tautomeric state. Energy minimization of the synthesized ligands done by using CheBio3D (v. 17.1) with the application of the MM2 force field.

Molecular Docking Protocol

The molecular docking was performed by using the full license version of GOLD (v. 5.6.2).[29] The docking process provided by using the Hermes visualizer program within GOLD. The binding site used in the docking was the protien residues that are within 10 A° of the reference ligand present in the complexes of protein structure.

CCDC Superstar was used to determine the cavity and the active site. The protein reference ligand has been used to determine the active site radius (10 A°). Chemscore kinase was used as a configuration template. The scoring function performed using ChemPLP. All parameters' values used in the GOLD docking process remained default, and all solutions are graded according to fitness function of piecewise linear potential (CHEMPLP).

The ligands' interaction with the protein residues was evaluated using docking outcomes such as docked pose, binding mode, and binding free energy.

RESULTS AND DISCUSSION

Antifungal Activity

Fluconazole was the reference drug and the antifungal activity of the synthesized compounds (2, 5a, 5b) were tested by using Candida Albicans at concentrations of (62.5,125, 250 and 500 µg/mL), while the control DMSO was used in the pure state.

The inhibition zone in (mm) for each concentration of the tested compounds illustrated in Table 2. The tested compounds (5a and 5b) gave an interesting activity against the fungi, these tested derivatives exert significant antifungal activity in comparison to dimethyl sulfoxide (DMSO) as a control group and compound 5a showed better antifungal activity than the reference drug and other synthesized compounds.

Table 2: Antifungal activity of compounds (2, 5a, 5b) and fluconazole against tested fungi

Compound	*Conc. (µg/mL)*	*Inhibition zone(mm)* *Candida albicans*
Fluconazole	500	32
	250	24
	125	16
	62.5	12
DMSO	Pure	-
2	500	8
	250	7
	125	0
	62.2	0
5a	500	34
	250	32
	125	20
	62.5	18
5b	500	25
	250	11
	125	9
	62.5	0

These results are in agreement with many studies showed that pyridine, 1,3,4-oxadiazole and 1,2,3-triazole derivatives have good antifungal action.

Molecular Docking Results

The promising antifungal activity of the heterocyclic analogues (**2**, **5a** and **5b**) explored here encouraged us to conduct molecular docking studies against the important sterol 14-demethylase (CYP51) enzyme (PDB code: 3KHM) to investigate their possible mechanism of action.

In the area of *in silico* structure-based drug design, molecular docking has become an inevitable method for predicting the probable biological target with a high degree of accuracy, especially when there aren't enough resources to conduct an experimental enzyme assay.

Sterol 14-demethylase (CYP51) is a cytochrome P450 superfamily ancestral activity that is required for ergosterol biosynthesis in fungi, where it promotes the formation of ergosterol from lanosterol. Inhibition of CYP51 causes a buildup of 14α-methyl sterols and a depletion of ergosterol in the fungal cell wall, resulted in alterations in cell membrane characteristics and function, increased permeability, and inhibition of cell growth and replication.

The molecular docking methodology was confirmed by removing the co-crystallized Fluconazole and re-docking it utilizing the experimental setup provided in the experimental section of docking.

The binding pocket for the reference fluconazole includes protein residues of the enzyme with Hem moiety (iron metal) of CYP51 which is important for antifungal activity (Figure 2). The resulting docked conformations demonstrated that all synthesized analogues could bind to the active site of CYP51

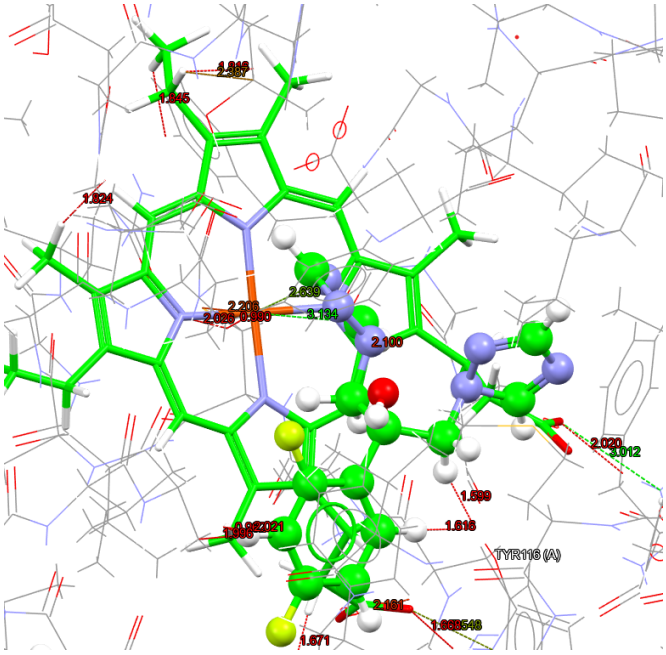


Figure 2: 3 Dimensional (3D) structure image of fluconazole in sterol 14α-demethylase enzyme complex

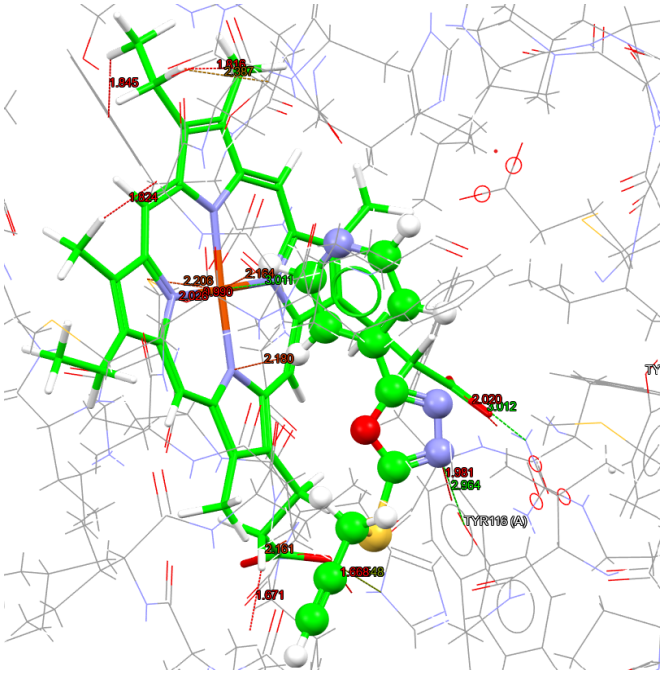


Figure 3: 3 Dimensional (3D) structure image of compound 2 in sterol 14α-demethylase enzyme complex

by forming a network of close interactions using hydrogen bonds and short contacts with varied degrees of affinities at co-ordinates near to the native structure (Figures 3 to 5). Short contacts defined as other binding forces, for example dipole-dipole, van der Waals, steric, electrostatic, pi-pi stacking, and others.

The sterol 14α-demethylase inhibitory activity of the compounds 2, 5a, 5b and fluconazole were ranked based on their PLP fitness implicated in the complex formation in the active sites. The docked compounds' PLP fitness was found

Table 3: The binding energies for desired compounds and fluconazole docked with the sterol 14α-demethylase enzyme complex

Compounds	*Enzyme binding Energy (PLP Fitness)*	*H-bond Interactions*	*Short contact Interactions*
2	59.41	TRY116	HEM500,ALA115,TYR116, LEU365,ALA291
5a	80.17	TYR116, TYR103	HEM500,ALA115,TYR116, LEU365,ALA291
5b	80.87	TYR116, TYR103	HEM500, ALA287, VAL359, MET358,TYR103, ILE105, MET360,LEU356
fluconazole	70.21	TYR116	HEM500,ALA 287, TYR116

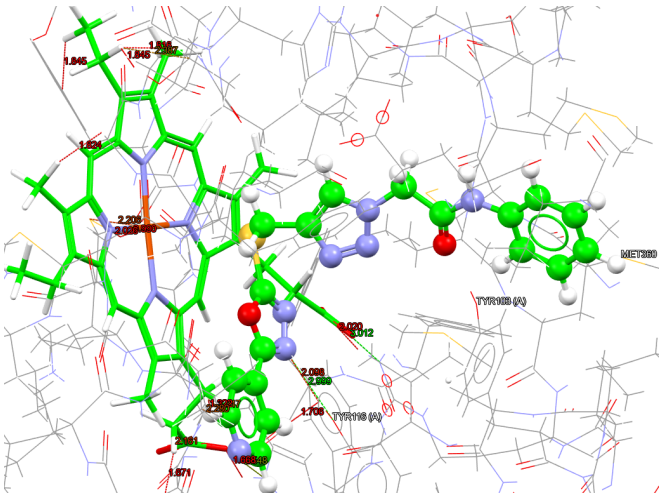


Figure 4: 3 Dimensional (3D) structure image of compound 5a in sterol 14α-demethylase enzyme complex

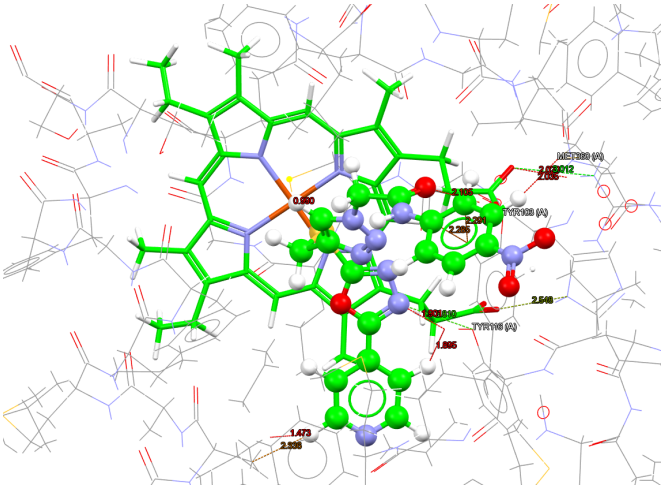


Figure 5: 3 Dimensional (3D) structure image of compound 5b in sterol 14α-demethylase enzyme complex

to be between 59.41–80.87 for the synthesized compounds and 70.21 for fluconazole as shown in Table 3.

Furthermore, Compounds 5a and 5b gave promising docking results with the sterol 14α-demethylase enzyme complex in comparison to fluconazole, with Compound 5b showed the best docking PLP fitness which was 80.87. Finally, there's an excellent correlation between the docking analysis and the experimental result.

CONCLUSIONS

Finally, we synthesized a small, targeted library of heterocyclic derivatives and tested their fungicidal activity in vitro. Most of these derivatives, particularly 5a and 5b, were shown to have excellent fungicidal potential and might be used as lead compounds in the discovery for novel antifungal medicines.

Moreover, a molecular docking investigation could reveal important details about the binding affinity and mechanism of interaction of these substances with the active site of the important fungal enzyme CYP51. The pre-residue interaction study could reveal the bonded and non-bonded interactions affecting the binding affinity to the target.

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