Design, synthesis and evaluation of antifungal activity of novel triazole-3-thiol derivatives containing substituted phenyl moiety as inhibitors of enolase 1

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KEYWORDS

Triazole-3-thiol derivatives, Antifungal, Minimal Inhibitory Concentrations, Molecular docking

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ABSTRACT

The emergence of azole resistant candida species is a significant clinical problem and indicates an urgent need for novel medications and treatment approaches. This research outlines the design, synthesis, and assessment of novel triazole-3-thiol derivatives that act as inhibitors of enolase 1 (Eno1). A total of seven triazole-3-thiol derivatives were designed to target Candida albicans by interfering with glycolysis through the inhibition of enolase 1 (Eno1). The molecular docking of these compounds into the active site of the crystal structure of Enolase1 from Candida albicans that is complexed with 2'-phosphoglyceric acid sodium (PDB ID: 7vrd) demonstrated similar binding modes and docking scores compared to those of Fluconazole. The compound (E)-4-(((3-mercapto-5-phenyl-4H-1,2,4-triazol-4-yl)imino)methyl)-2-methoxyphenol exhibits the greatest binding affinity to the target. Derivatives were synthesized by the reaction of thiocarbohydrazide, different carboxylic acid and vanillin. The structures of the newly created compounds were determined through their spectral data. The antifungal properties were assessed in vitro by determining the minimal inhibitory concentrations (MICs) using a microdilution assay. All synthesized derivatives exhibit notable antifungal efficacy against candida albicans.

INTRODUCTION

Candidiasis, caused predominantly by *Candida albicans*, is a significant health concern worldwide, including in India**[1].** Globally, *C. albicans* remains the leading cause of both mucosal and invasive candidiasis, accounting for approximately 50–70% of cases[2]. Approximately 75% of fungal infections worldwide are attributed to C. *albicans*[3]. In the United States, candidemia—a bloodstream infection caused by Candida species—is among the most frequently occurring

bloodstream infections, with an estimated annual incidence of 25,000 cases.[4]. The incidence of systemic candidiasis varies globally, typically ranging from 2 to 21 per 100,000 individuals, influenced by regional healthcare practices and patient demographics[5]. The prevalence is particularly high in immunocompromised populations, such as those with HIV/AIDS, cancer, or diabetes, and among hospitalized patients, especially in intensive care units[6]. In India, the burden of candidiasis is notable due to high rates of diabetes and the increasing use of invasive medical devices, with studies indicating *C. albicans* as the most prevalent species[7].

Treatment for *Candida albicans* infections is contingent upon the infection's location and intensity. Topical antifungal medications like clotrimazole, miconazole, or nystatin are commonly used to treat superficial infections including vaginal candidiasis and oral thrush[8]. Systemic antifungal treatment is necessary for systemic infections, such as invasive candidiasis and candidemia. Echinocandins, such as caspofungin, micafungin, or anidulafungin, are first-line treatments because they prevent the formation of fungal cell walls[9]. As an alternative, azole antifungals like voriconazole or fluconazole are frequently utilized, particularly in stable individuals who have never been exposed to azoles before[10]. Because of its increased toxicity, amphotericin B, a polyene antifungal, is only used in severe or refractory instances[11]. In high-risk patients, such as those receiving chemotherapy or organ transplantation, prophylactic antifungal medication may be taken into consideration[12]. A growing issue is drug resistance, which emphasizes the necessity of customized treatment regimens and susceptibility testing[13].

The increasing prevalence of azole-resistant *Candida* species has become a significant clinical challenge in recent years. Candida infections are among the most common fungal infections, with *Candida albicans* being a leading causative agent[14]. The resistance to azole-based antifungal medications, such as fluconazole, is alarming due to its widespread use and the limited number of alternative antifungal agents. Recent studies indicate that up to 10% of *Candida albicans* isolates and an even higher percentage of non-albicans *Candida* species exhibit resistance to azoles[15]. This rising resistance highlights the urgent need for novel therapeutic strategies to address this critical health issue.The rising occurrence of resistant Candida species highlights the shortcomings of existing antifungal treatments and emphasizes the necessity for novel therapeutic strategies.

Enolase is found in all tissues and organisms that can perform glycolysis or fermentation. In mammals, α-enolase (Eno1) is widely expressed across different tissue types and has been implicated in numerous biological and pathophysiological processes. Eno1 is distributed throughout the cytosol and is also located on the cell surface as a receptor that binds plasminogen. Null mutants of C. albicans Eno1 (CaEno1) show changes in drug susceptibility, hyphal development, and virulence, strongly indicating that the presence of CaEno1 is essential for the growth of *C. albicans* cells. Mutations in CaEno1 result in reduced cell growth. The extracellular Eno of C. albicans plays a role in the colonization of the small intestine, an activity that can be inhibited by anti-Eno antibodies[16].

Enolase 1 (Eno1) plays a crucial role in the glycolysis process by facilitating the transformation of 2-phosphoglycerate into phosphoenolpyruvate, which is a vital step in energy metabolism[17]. In Candida albicans, Eno1 plays a dual role: as a metabolic enzyme and as a surface protein involved in fungal adhesion and invasion. This makes Eno1 an attractive target for antifungal drug development, particularly against azole-resistant strains[17].The Protein Data Bank (PDB) entry 7VRD contains the crystal structure of enolase 1 from Candida albicans, which is complexed with sodium 2'-phosphoglyceric acid[18]. This structure provides a detailed view of the enzyme's active site, facilitating the design of small-molecule inhibitors. The crystal structure of enolase 1 from Candida albicans (PDB ID: 7VRD) is a pivotal resource in the development of novel antifungal therapies. The detailed understanding of its active site and its role in fungal physiology enables the

rational design of targeted inhibitors, such as triazole-3-thiol derivatives, offering hope for combating azole-resistant Candida infections.

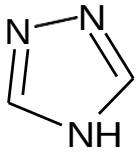


Fig.1- Structure of 1,2,4-triazole

A triazole, characterized by the molecular formula $C_2H_3N_3$, is a heterocyclic compound that consists of a five-membered ring containing two carbon atoms and three nitrogen atoms. Triazoles show significant isomerism, depending on where the nitrogen atoms are located in the ring[19]. 1,2,4-triazole (Fig.1) is one of the important heterocycles with a wide range of biological activities, including antimicrobial[20], analgesic[21], anti-inflammatory[21], anticonvulsant[22], anticancer[23], antimalarial[24], antiviral[25], and antiproliferative[26]properties. Azole antifungal medications, including Fluconazole, Itraconazole, Voriconazole, and Posaconazole (Fig.2), play a crucial role in combating invasive fungal infections (IFIs)[27].

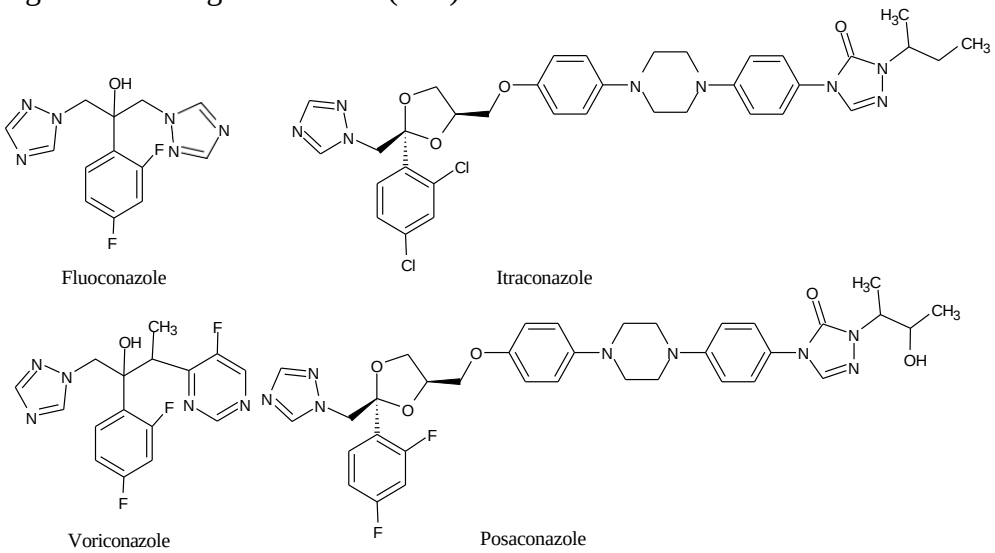


Fig.2.- Triazole antifungal agents

Triazole-based compounds have demonstrated broad-spectrum antifungal properties by interfering with ergosterol biosynthesis[28]. Incorporating a thiol group (-SH) into the triazole scaffold enhances binding interactions with enzyme active sites, particularly through covalent and hydrogen bonding. The triazole-3-thiol framework also allows for versatile functionalization, facilitating the optimization of physicochemical and pharmacokinetic properties[29]. In silico docking studies using the crystal structure of Candida albicans enolase 1 guided the design of triazole-3-thiol derivatives. Molecular docking and dynamic simulations identified key residues within the active site, such as Lys343 and His157, that could form interactions with triazole and thiol moieties[30].

The rise of azole-resistant Candida species poses a significant clinical challenge, highlighting the urgent need for new treatment options and medications. Enolase 1 (Eno1) is a crucial marker for invasive candidiasis. Candida albicans enolase 1 (CaEno1) is considered a vital target for the creation of therapeutic agents. This research outlines the design, synthesis, and assessment of novel triazole-3-thiol derivatives that act as inhibitors of enolase 1 (Eno1). The focus of this study is on designing, synthesizing, and evaluating new triazole-3-thiol derivatives as inhibitors of enolase 1 (Eno1), an essential enzyme in the glycolytic pathway of *Candida albicans*. Eno1 is crucial for

fungal metabolism, making it a promising target for antifungal drug development. By inhibiting Eno1, these derivatives seek to disrupt glycolysis and threaten fungal survival.

MATERIALS AND METHODS

Chemistry

The melting points were obtained using a digital melting point apparatus and are reported without corrections. The synthesis process and the purity of the compounds were evaluated using thin layer chromatography. Infrared spectra were obtained on a Shimadzu FT-IR spectrometer utilizing KBr pellets. Proton NMR spectra were recorded using a digital NMR spectrometer (Jeol, JNM-ECZ400S/L1) in a DMSO-d6 solution with TMS as the internal standard. Liquid chromatography-mass spectrometry (LC-MS) analyses were conducted using a liquid chromatography-mass spectrometer.

In silico studies

Molecular docking studies

The three-dimensional conformation of the antifungal medication bound to its target protein was obtained from the Protein Data Bank (PDB ID: 7vrd; accessible at www.rcsb.org). This structure provided the basis for computational docking studies to explore the binding interactions between the synthesized antifungal compounds and the target enzyme. The chemical structures of the ligands (1a-1g) were prepared using ChemSketch freeware software, which facilitated the accurate representation of the molecular geometry and ensured compatibility with the docking tools Table 1. The docking process utilized a comprehensive set of advanced software tools, including DisGeNET and SwissTargetPrediction, to predict potential targets and pathways. The RCSB Protein Data Bank served as the repository for obtaining the protein structure, while Venny 2.1.0 was used for data visualization and analysis. Docking studies were performed using PyRx Virtual Screening Tool, AutoDock, and MGLTools, allowing precise ligand-receptor interaction modeling.

A three-dimensional affinity grid box was carefully designed to cover the entire active site as well as peripheral binding regions of the target enzyme. This ensured a comprehensive evaluation of potential binding modes and interaction hotspots. The results of the docking experiments were analyzed more thoroughly with the help of BioDiscovery Studio Visualizer, which produced intricate graphical illustrations of the interactions between ligands and enzymes, emphasizing critical molecular connections like hydrogen bonds, hydrophobic interactions, and pi-pi stacking. This analysis yielded essential insights regarding the binding effectiveness and possible mechanisms of action of the antifungal medications, assisting in the selection of promising compounds for further experimental and clinical investigations.

In silico ADME evaluation

All designed compounds (1a–1f) were subjected to a thorough evaluation of their toxicity and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) characteristics to assess their potential as lead candidates for clinical trials and therapeutic development. The SwissADME online platform (http://www.swissadme.ch) was utilized for this analysis, providing an in-depth review of essential pharmacokinetic parameters such as oral bioavailability, solubility, permeability, and lipophilicity. Additionally, this platform assessed the compounds' compliance with recognized drug-likeness criteria, including Lipinski's, Veber's, and Egan's rules, which are crucial for evaluating their viability as drug candidates. Simultaneously, the toxicity profiles of the compounds were estimated using the ProTox-II chemical toxicity prediction tool (http://tox.charite.de/protox_II/), which offered insights into possible toxic effects like mutagenicity, carcinogenicity, and hepatotoxicity—factors that are vital for the safety evaluation of drug candidates.

After the initial assessment of their biological activities, the compounds were further analyzed for their physicochemical properties, including molecular weight, topological polar surface area (TPSA), the count of hydrogen bond donors and acceptors, and the number of rotatable bonds. These properties play a vital role in determining the compounds' pharmacokinetic behavior and their ability to interact effectively with biological targets. The combined analyses using SwissADME and ProTox-II highlighted the potential of the synthesized compounds as viable drug candidates. By identifying molecules with favorable pharmacokinetic profiles, drug-likeness, and acceptable toxicity levels, this study laid the groundwork for advancing the most promising compounds to experimental validation and further drug development processes.

Procedure for Synthesis

General procedure for synthesis of *Thiocarbohydrazide* (TCH)

A round-bottom flask was filled with 1.0 mol of hydrazine hydrate, which was set up with a thermometer, a highly effective mixer, and a reflux condenser. The temperature was decreased to 1°C, and 0.2 mol of carbon disulfide (15.2 g, 12.1 mL) was slowly added to the flask while keeping the temperature below 15°C, then the temperature was gradually increased to 85°C over a period of 1.5 hours. After cooling the reaction mixture to 10°C, the resulting precipitate was filtered and rinsed with water[31].

Synthesis of *4-Amino-5-(substituted-phenyl)-4H- [1,2,4]-triazole-3-thiol*

A round-bottomed flask was filled with a blend of substituted benzoic acid (0.01 mol) and thiocarbohydrazide (0.015 mol), which was then heated on a mantle until the contents melted. After cooling, the resulting product is treated with a sodium bicarbonate solution to neutralize any residual carboxylic acid. The product was rinsed with water and collected via filtration. The obtained products were recrystallized using ethanol to yield the desired compounds (Fig.3) (Scheme)[31].

Synthesis of *4-(4-substituted benzylieneamino -5-(substituted phenyl)-4H-[1,2,4]triazole-3-thiol*

To the 4-Amino-5-(substituted-phenyl)-4H- [1,2,4]-triazole-3-thiol add suitable amount of methanol and vanillin and condensed for 2 hours. Filter and dried the product[32] (Fig. 3) (Scheme) (Table 1).

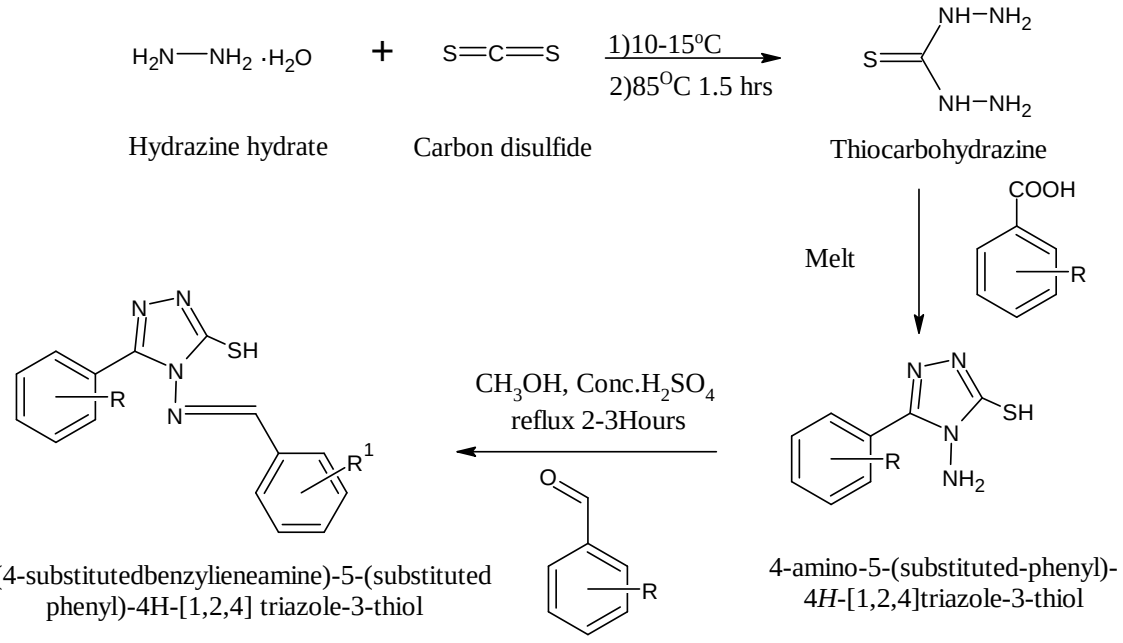


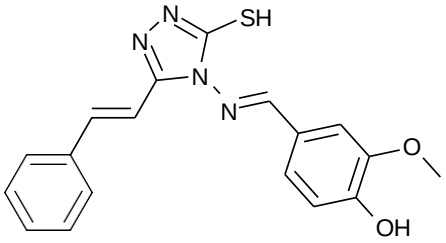
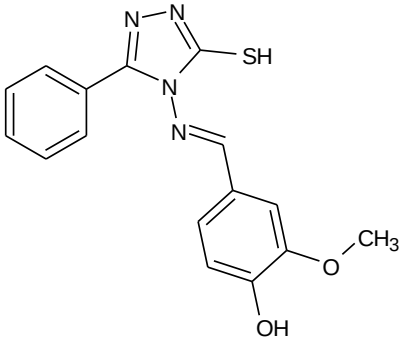
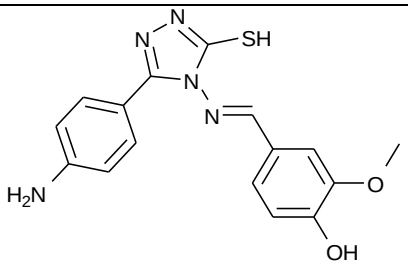
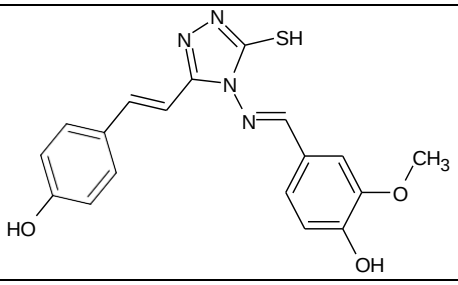
Fig. 3. Scheme for Synthesis of 4-(4-substituted benzylieneamino -5-(substitutes phenyl)-4H-[1,2,4]triazole-3-thiol

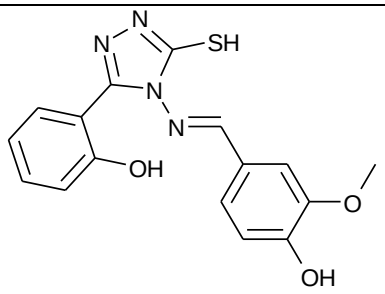
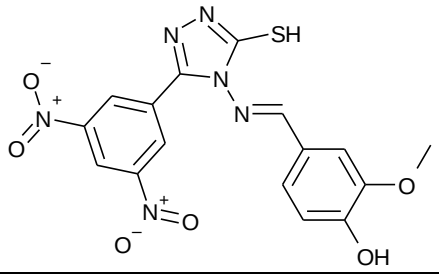
Invitro anti-fungal activity

The antifungal activity of synthesized derivative was determined against *Candida albicans* (ATCC:90028) by MIC method[33]. Different concentration i.e., 200 mg/mL, 100 mg/mL, 50 mg/mL and 25 mg/mL of samples were incubated with sabouraud dextrose broth for 24 hours. Fluconazole was used as standard drug and broth without sample was considered as control. The turbidity was measure at 600nm using multimode microplate reader (Fluostar Omega, BMG Labtech). Percentage inhibition of growth was calculated by using the formula:

% Inhibition of growth = O.D value of control - O.D value of growth inhibition/ O.D value of control (O.D -Optical density)

Table 1: Triazole-3-thiole derivatives

Compounds	IUPAC Name	Structure
1a	*4-((E)-((3-mercapto-5-((E)-styryl)-4H-1,2,4-triazol-4-yl)imino)methyl)-2-methoxyphenol*	
1b	*(E)-4-(((3-mercapto-5-phenyl-4H-1,2,4-triazol-4-yl)imino)methyl)-2-methoxyphenol*	
1c	*(E)-4-(((3-(4-aminophenyl)-5-mercapto-4H-1,2,4-triazol-4-yl)imino)methyl)-2-methoxyphenol*	
1d	*4-((E)-((3-mercapto-5-((E)-styryl)-4H-1,2,4-triazol-4-yl)imino)methyl)-2-methoxyphenol*	

1e	*(E)-4-(((3-(2-hydroxyphenyl)-5-mercapto-4H-1,2,4-triazol-4-yl)imino)methyl)-2-methoxyphenol*	
1f	*(E)-4-(((3-(3,5-dinitrophenyl)-5-mercapto-4H-1,2,4-triazol-4-yl)imino)methyl)-2-methoxyphenol*	

RESULTS AND DISCUSSIONS

In silico Molecular Docking Analysis

The novel Triazol-3-thiol derivatives were computationally evaluated for their binding affinity to the enolase1 protein target, with the structural information sourced from the Protein Data Bank (PDB ID: 7vrd). Molecular docking studies were performed to predict the interaction of these derivatives with the active site of enolase1, a critical enzyme implicated in various biological pathways and considered a potential therapeutic target (Fig.4 & Fig.5). The docking scores obtained, which reflect the affinity of each compound for enolase1, are presented in Table 2. These scores act as numerical indicators, where lower (more negative) values typically suggest a stronger binding affinity and potentially greater biological effectiveness. The docking analysis offered valuable insights into the binding interactions of Triazol-3-thiol derivatives and aided in pinpointing promising candidates for additional experimental testing. The derivatives with the highest docking scores (suggesting the strongest interactions) are likely to demonstrate increased inhibitory activity against enolase1 and may represent viable lead compounds for antifungal or therapeutic uses.

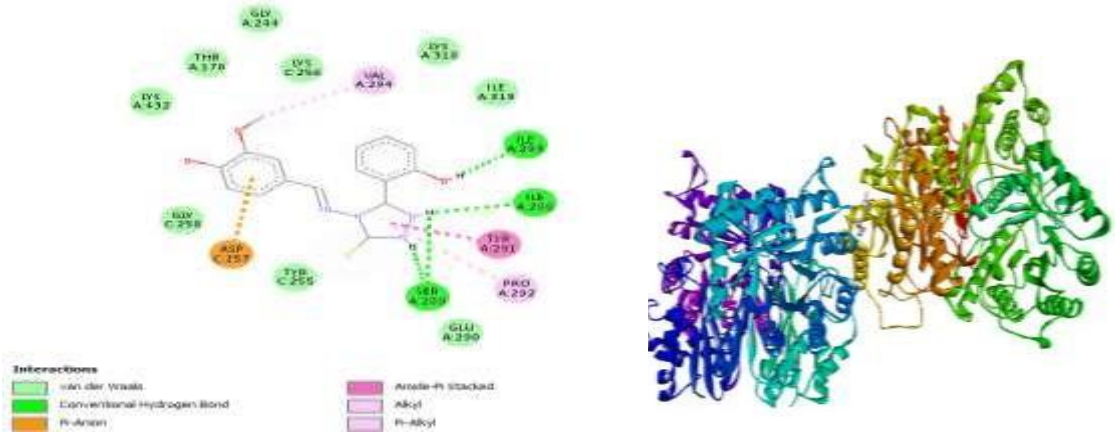


Fig.4- 2D and 3D interaction of *1b* on Crystal structure of Enolase1 from Candida albicans

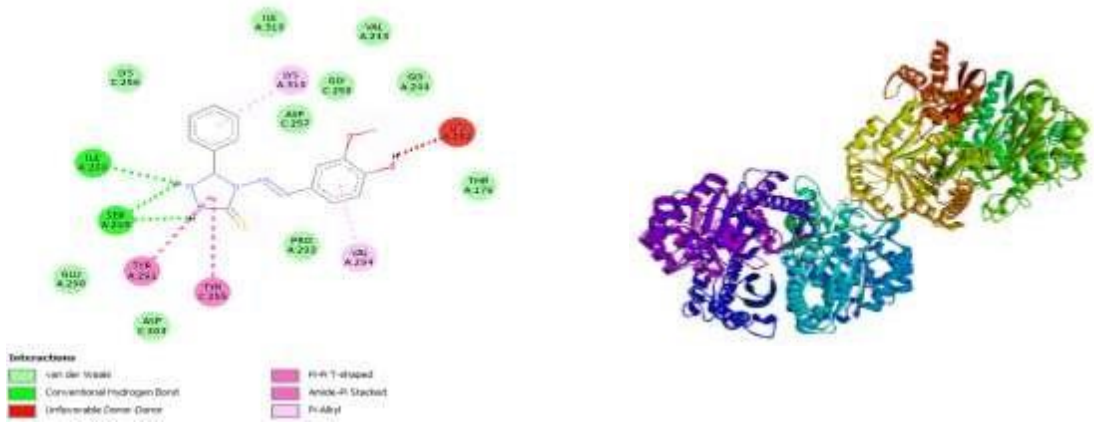


Fig.5- 2D and 3D interaction of *1c* on Crystal structure of Enolase1 from *Candida albicans*

Table 2: Docking scores of novel Triazol-3-thiol derivatives against 7vrd

Derivative	**Docking score** (kcal/mol) (PDB ID: 7vrd)
1a	-7.8
1b	-7.8
1c	-8.1
1d	-7.9
1e	-7.7
1f	-7.1
Standard (Fluconazole)	-7.0

***In silico* ADME evaluation**

As highlighted in the model study, the compounds demonstrated potent bioavailability (oral) and satisfactory performance. Additionally, the compounds exhibited favorable drug-likeness when considering factors such as topological surface area (TPSA), lipophilicity, molecular weight, flexibility, solubility, and saturation. All synthesized compounds showed acceptable ranges for these parameters, which are visually represented in radar plots (Fig. 6). Drug likeness was further assessed based on the number of free rotatable bonds and compliance with Lipinski's, Egan's, and Veber's rules. Consequently, these compounds were found to possess favorable pharmacokinetic profiles that meet the criteria for drug-likeness. Among the synthesized compounds, specific molecules such as 1**a**, 1**b**, 1**c**, and 1**d** emerged as prominent antifungal agents and were selected for further pharmacokinetic analysis to support future drug development.

1a

1b

1c

1d

Fig.6- Drug likeness in 1a, 1b, 1c, and 1d

The radar charts depict the oral bioavailability and drug-like characteristics of the compounds. The pink region in the radar diagram signifies the optimal range of physicochemical parameters for achieving good oral bioavailability, which includes molecular weight (MWt), lipophilicity (LIPO), solubility (INSOLU), topological polar surface area (TPSA), unsaturation (INSATU), and flexibility (FLEX). The red line in the figure represents the range of properties for the compounds being tested. The accompanying table presents the results of these properties, indicating that there are no major breaches of Lipinski's rule. The assessed physicochemical and pharmacokinetic characteristics lie within the acceptable limits for drug-likeness, with no violations observed (Table 3 &4).

Table 3: Physicochemical characteristics utilizing SwissADME

Comp	MW[a] (g/mol)	$ClogP_{o/w}$[b]	MlogP[c]	nHBA[d]	nHBD[e]	nRB[f]	TPSA[g] ($Å^2$)	logS[h]
1a	352.41	3.17	2.8	5	1	5	111.33	-5.58
1b	326.37	2.67	2.39	5	1	4	111.33	-4.92
1c	341.39	2.19	1.86	5	2	4	137.35	-4.76
1d	368.41	2.75	2.27	6	2	5	131.56	-5.64
1e	342.37	2.36	1.86	6	2	4	131.56	-4.98
1f	416.37	1.26	0.7	9	1	6	202.97	-6.49
1g	416.37	1.26	0.7	9	1	6	202.97	-6.49

a-Molar mass; b, c-Hydrophobicity; d-Count of hydrogen bond acceptors; e-Count of hydrogen bond donors; f-Count of rotatable bonds; g-Topological surface area; h-Dissolution capacity.

Table 4: Assessment of Drug-likeness compliance, Number of Violations.

Comp	Lipinski	Veber	Egan	Bio-availability score	GI absorption	BBB permeant	CYP 1A2	CYP 2C19	CYP 2C9	CYP 2D6	CYP 3A4
1a	Yes	Yes	Yes	0.55	High	No	No	Yes	Yes	No	No
1b	Yes	Yes	Yes	0.55	High	No	Yes	No	Yes	No	No
1c	Yes	Yes	No (1)	0.55	High	No	No	No	No	No	No
1d	Yes	Yes	Yes	0.55	High	No	No	No	Yes	No	No
1e	Yes	Yes	Yes	0.55	High	No	Yes	No	No	No	No
1f	Yes (1)	No (1)	No (1)	0.55	Low	No	No	Yes	Yes	No	Yes
1g	Yes (1)	No (1)	No (1)	0.55	Low	No	No	Yes	Yes	No	Yes

***In vitro* anti-fungal activity**

The antifungal efficacy of the synthesized derivatives was evaluated against *Candida albicans* using the Minimum Inhibitory Concentration (MIC) method, a standard approach to determine the lowest concentration of a compound required to inhibit visible fungal growth. The results demonstrated that all synthesized derivatives exhibited significant antifungal activity against *Candida albicans*.Among the tested samples, compounds 1**a**, 1**b**, and 1**c** showed an MIC of 100 mg/mL, indicating potent inhibitory activity. In contrast, the compound 1**d** displayed a slightly higher MIC value of 200 mg/mL, suggesting comparatively lower antifungal potency. Further analysis of the percentage inhibition of fungal growth revealed that compound 1**b** exhibited the highest inhibition, outperforming the other derivatives. It was followed by compounds 1**c**, 1**d**, and 1**a**, which also demonstrated notable but comparatively lesser antifungal activity. These findings highlight the potential of the synthesized derivatives, particularly 1**b**, as strong candidates for antifungal therapy. The superior activity of 1**b** suggests it could serve as a lead compound for further optimization and development in antifungal drug research (Table 5)(Fig.7 & Fig.8).

Table 5: The percentage inhibition of fungal growth at different concentration

S. No	Concentration (mg/mL)	Percentage of growth inhibition in 96 well plate (%)				
		1a	1b	1c	1d	Fluconazole
1	200	90.8	95.4	93.5	93.5	90.1
2	100	90.4	90.2	93.4	56.5	52.4
3	50	16.9	12.3	32	32	33
4	25	17.2	3.9	31.9	19.5	20

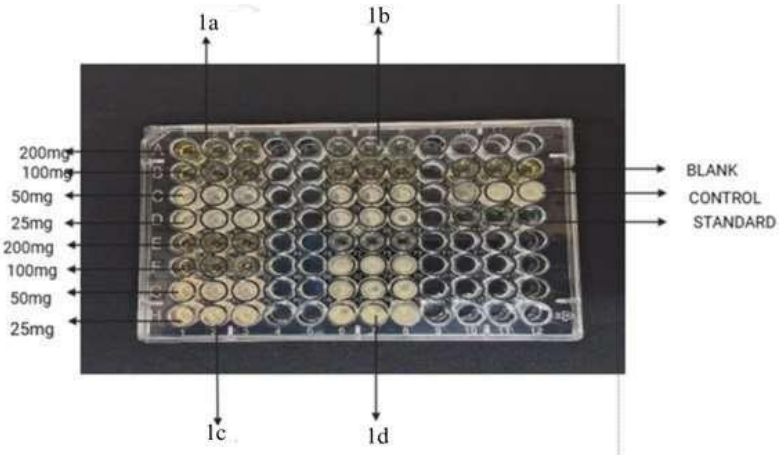


Fig. 7: The MIC of novel derivatives at different concentration (mg/mL) showed in 96 well plate method

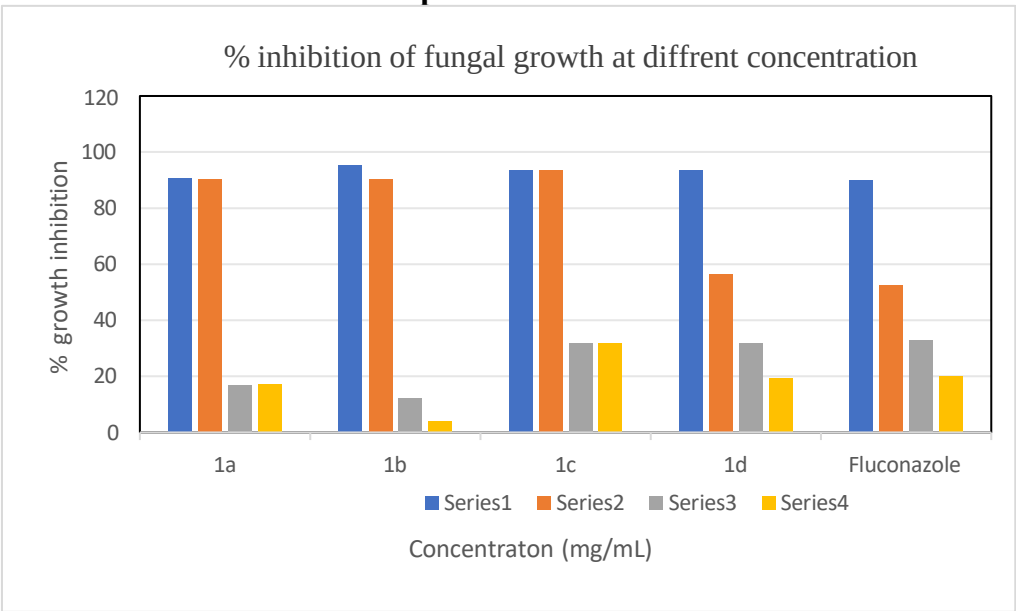


Fig. 8: Antifungal activity of synthesized derivatives

Procedure for synthesis of *4-((E)-((3-mercapto-5-((E)-styryl)-4H-1,2,4-triazol-4-yl)imino)methyl)-2-methoxyphenol* (1a)

An equivalent amount of TCH and Cinnamic Acid (1 g) and 5 ml of ethanolic KOH were taken in a round bottom flask and then reflexed on a condenser for 2 hours. The mixture was then filtered and dried. An equivalent amount of the above reaction mixture, vanillin (0.73 g), and 15 ml of methanol are taken in a round bottom flask and then reflexed on a condenser for 2 hours. After the mixture cooled, 1 ml of sulfuric acid was added dropwise, and the mixture was filtered and dried. Pale yellow crystalline powder, (72%), mp. 224-226 °C; IR (KBr) 3432 (OH), 3220 (NH), 1552 (C=C), 1590 (C=N), 2574 (S-H)cm^{-1}. The ^1H NMR spectrum reveals signals characteristic of the compound's structure. A singlet around δ 9.0–10.0 ppm corresponds to the phenolic O-H proton, while aromatic protons of the styryl group resonate between δ 6.5–8.0 ppm. The imine proton typically appears as a singlet near δ 8.5 ppm. Methoxy (-OCH_3) protons are observed as a singlet around δ 3.7–4.0 ppm. MS: m/z 353 (M+/2).

Procedure for synthesis of *(E)-4-(((3-mercapto-5-phenyl-4H-1,2,4-triazol-4-yl)imino)methyl)-2-methoxyphenol* (1b)

An equivalent amount of TCH and Benzoic Acid (1 g) and 5 ml of ethanolic KOH were taken in a round bottom flask and then reflexed on a condenser for 2 hours. The mixture was then filtered

and dried. An equivalent amount of the above reaction mixture, vanillin (0.73 g), and 15 ml of methanol are taken in a round bottom flask and then reflexed on a condenser for 2 hours. After the mixture cooled, 1 ml of sulfuric acid was added dropwise, and the mixture was filtered and dried. Light yellow crystalline powder, (65%), mp. 165-168 °C; IR (KBr) 3320 (NH), 3425 (0H), 1555 (C=C), 1610 (C=N), 2554 (S-H)cm^{-1}. In the ^{1}H NMR spectrum, a singlet at δ 9.5ppm represents the phenolic O-H proton, while the imine proton resonates as a singlet at δ 8.4ppm. Aromatic protons from the phenyl group appear as multiplets ATδ 7.0 ppm, and the methoxy (-OCH_3) group is observed as a singlet at δ 3.7 ppm. A weak singlet near δ 3.0ppm corresponds to the S-H proton. MS: m/z 326 (M+/2).

Procedure for synthesis of *4-((E)-((3-mercapto-5-((E)-styryl)-4H-1,2,4-triazol-4-yl) imino)methyl)-2-methoxyphenol* (1c)

An equivalent amount of TCH and p- Coumaric Acid (1 g) and 5 ml of ethanolic KOH were taken in a round bottom flask and then reflexed on a condenser for 2 hours. The mixture was then filtered and dried. An equivalent amount of the above reaction mixture, vanillin (0.73 g), and 15 ml of methanol are taken in a round bottom flask and then reflexed on a condenser for 2 hours. After the mixture cooled, 1 ml of sulfuric acid was added dropwise, and the mixture was filtered and dried. Yellow crystalline powder, (78%), mp. 156-158 °C; IR (KBr) 3345 (NH), 3525 (0H), 1565 (C=C), 1623 (C=N), 2572 (S-H)cm^{-1}. In the ^{1}H NMR spectrum, methoxy (–OCH_3) group as a singlet around δ 3.7 ppm, aromatic and styryl protons at δ 6.5 ppm, and the phenolic –OH as a broad singlet at δ 11 ppm. The imino (C=N) proton appeared as a singlet at δ 8.5ppm. MS: m/z 341 (M+/2).

Procedure for synthesis of *(E)-4-(((3-(2-hydroxyphenyl)-5-mercapto-4H-1,2,4-triazol-4-yl)imino)methyl)-2-methoxyphenol* (1d)

An equivalent amount of TCH and Salicylic acid (1 g) and 5 ml of ethanolic KOH were taken in a round bottom flask and then reflexed on a condenser for 2 hours. The mixture was then filtered and dried. An equivalent amount of the above reaction mixture, vanillin (0.73 g), and 15 ml of methanol are taken in a round bottom flask and then reflexed on a condenser for 2 hours. After the mixture cooled, 1 ml of sulfuric acid was added dropwise, and the mixture was filtered and dried. Orange crystalline powder, (78%), mp. 176-180 °C; IR (KBr) 3325 (NH), 3550 (0H), 1623 (C=N), 2590 (S-H)cm^{-1}. In the ^{1}H NMR spectrum the methoxy (–OCH_3) group appears as a singlet at δ 3.5 ppm, while aromatic and phenolic protons are observed in the δ 6.5–7.5 ppm range. The phenolic –OH groups typically resonate as broad singlets at δ 12 ppm, and the imino proton (HC=N) is observed around δ 9.5 ppm. MS: m/z 369 (M+/2).

CONCLUSION

The molecular docking assessment reveals the promising potential of new Triazol-3-thiol derivatives as potent enolase1 inhibitors (PDB ID :7VRD), with all tested compounds demonstrating superior binding affinities compared to the standard antifungal drug Fluconazole. Among these, compound 1c achieved the highest docking score (-8.1 kcal/mol), suggesting it could serve as a lead for antifungal drug development. These findings highlight the potential of these derivatives as viable candidates for additional experimental testing and therapeutic use. The synthesized Triazol-3-thiol derivatives exhibited encouraging antifungal activity against Candida albicans in comparison to fluconazole, a widely used antifungal agent. Data on Minimum Inhibitory Concentration (MIC) and percentage growth inhibition reveal that compounds 1a, 1b, 1c, and 1d show significant inhibitory effects, with 1b emerging as the most potent derivative. At a concentration of 200 mg/mL, compound 1b demonstrated an impressive 95.4% inhibition of fungal growth, outperforming fluconazole's effectiveness of 90.1%. Even at a lower concentration of 100 mg/mL, compounds 1b and 1c maintained higher inhibition rates (90.2% and 93.4%, respectively) when compared to fluconazole (52.4%). This indicates that these derivatives, particularly 1b, may possess enhanced efficacy in combating fungal growth. These findings imply

that the synthesized derivatives, especially 1b, could serve as effective alternatives or complementary options to current antifungal therapies, with the potential for further optimization to enhance their therapeutic effectiveness.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

MIC: Minimal Inhibitory Concentrations; **TPSA**: Topological surface area; **IFIs**: Invasive Fungal Infections; **HIV**: Human Immunodeficiency Virus; **NMR**: Nuclear Magnetic Resonance

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