Synthesis, Characterization, Molecular Docking And Biological Screening Of Novel 5-((2,5-Dichlorophenoxy) Methyl)-4-Phenyl-4h-1,2,4-Triazole-3-Thiol

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Keywords:
1,2,4triazole, thiosemicarbazide, biological activity, molecular Docking

Abstract:
In the present investigation, we report synthesis of 1,2,4 triazole derivatives from a base catalysed condensation of substituted thiosemicarbazide followed by a cyclization to target product substituted-1,2,4-triazole compounds. synthesized derivatives were confirmed by adequate analytical techniques such as FTIR, ^{1}H NMR and MASS. Synthesized derivatives were tested experimentally to evaluate their therapeutic potential against pathogenic strains of Klebsiella, Salmonella, Serratia marcescescens, and S. aureus. Biological screening study revealed that the triazole derivatives 4a, 4d, and 4i show potent antibacterial activity, while other derivatives show moderate activity. Molecular docking of 1,2,4 triazole derivatives have been carried out to put forth the structural rationale behind the observed antibacterial effect of the substituted-1,2,4-triazole compounds. In silico data revealed binding affinity towards the O-Acetylserine Sulfhydrylase enzyme.

1. Introduction:

1,2,4-Triazole is a five-membered aromatic heterocycle composed of two carbons and three nitrogen atoms at the 1, 2, and 4 positions of the ring[1–4]. The 1,2,4 Triazoles have attracted great attention in biology and chemistry in recent decades due to their facile synthetic techniques as well as its versatile interaction with biological systems[5]. This system is stable to metabolic degradation[6] and it is capable of interacting with biological receptors or enzymes with high affinity due its dipolar character, rigidity, hydrogen bonding capacity as a hydrogen bond acceptor or donor, and it shows broad biological activities like antibacterial[7–10], antiviral[11-12], anticoagulant[13], anti-inflammatory[14], anticancer[15], and antioxidant properties[16]. Other interactions with biological systems involve ion-dipole, cation-π, -π–π and Van der Waals forces. Furthermore, its polar nature can increase the solubility of the drug resulting in the improvement of pharmacological profile of the drug. A large number of representatives of this class of compounds are in clinical trials for the treatment of various diseases[17–19]. Examples of 1,2,4-triazoles commercialized as important fungicides are shown in Figure 1 and include fluconazole 1, voriconazole 2, isavuconazole 3, itraconazole 4, ravuconazole 5 and posaconazole 6[20]. These compounds inhibit the lanosterol-14-alpha-demethylase activity of enzyme CYP51, which is an essential step in ergosterol biosynthesis[21].

Triazoles, sterol 14-alpha demethylase inhibitors, such as triadimefon (TDF) 7, triadimenol (TDN) 8, flusilazole 9, difenoconazole 10, tebuconazole 11 and flutriafol 12 are widely used in agriculture for protecting crops against fungal diseases (Figure 2) [22-24]. These fungicides are highly effective against a broad spectrum of fungal pathogens and are used on various crops including cereals, fruits, vegetables and ornamental plants. The synthesis of several innovative 2, 5-disubstituted-1,2,4- triazole derivatives was done in light of the aforementioned facts and as part of our ongoing work on the synthesis of effective treatments for infectious diseases. Scheme 1 describes the chemical sequence that produces

the necessary heterocyclic compounds.

Figure 1. Examples of commercialized antifungal drugs 1–6 containing the 1,2,4-triazole nucleus

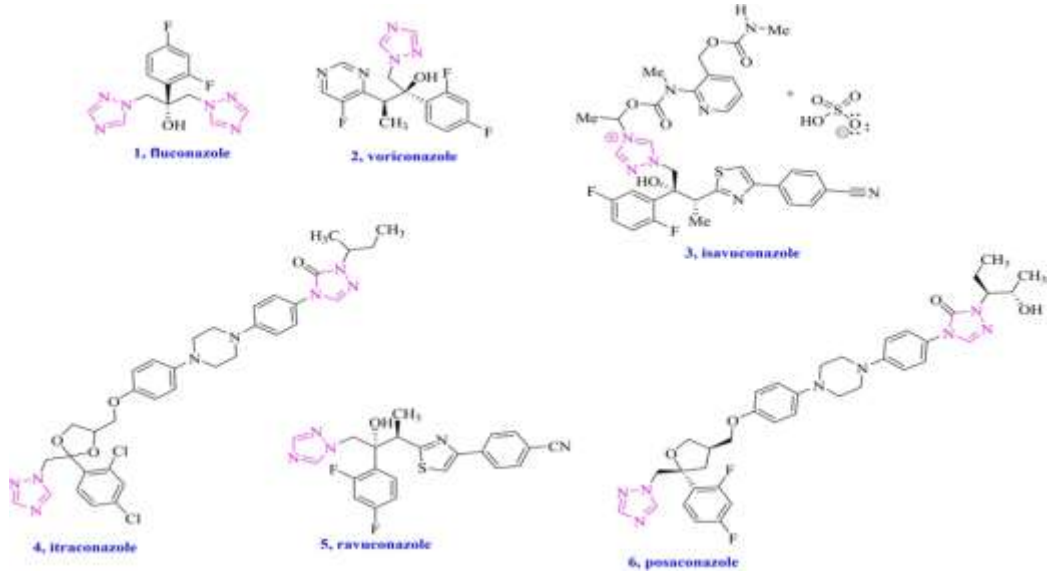


Figure 2. Some examples of 1,2,4-triazole antifungals 7--12 used in agriculture.

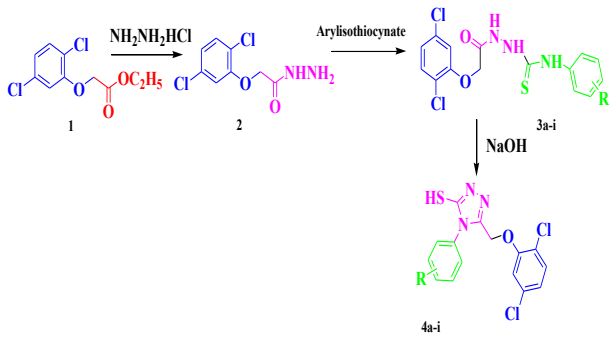
In the present study, we investigated themolecular docking and antibacterial activity of the novel triazole compound, 5-((2,5-dichlorophenoxy) methyl)-4-phenyl-4H-1,2,4-triazole-3-thiol by the minimum inhibitory concentration (MIC), which was determined before the well diffusion assay.

2. Material and Methods:

2.1 Experimental:

Uncorrected melting points were measured in open capillaries. TLC was used to verify the compounds' purity. A SHIMADZU spectrophotometer in KBr disk was used to record infrared spectra. Using DMSO as a solvent and TMS as an internal standard, ^{1}H NMR spectra were captured on a Bruker Advance Neo 500 MHz spectrometer δ (ppm) is used to display peak values. A SYNAPT-XS mass spectrometer was used to record the mass spectra. CHNS analysis were done by Thermo Scientific (Flash 2000) elemental analyser.

Scheme: Synthesis of 1,2 4 Triazole derivatives



2.2 Synthesis of 5-((2,5-dichlorophenoxy) methyl)-4-phenyl-4H-1,2,4-triazole-3-thiol (4a-i)
In a 50 ml round-bottom flask, 1-(2-(2,5-dichlorophenoxy)acetyl)-4-phenylthiosemicarbazide 3 (0.001 mol) was dissolved in 15 ml of 1N NaOH and heated for two hours under gentle reflux. TLC technique is used for monitor the reaction's development. glacial acetic acid was used to acidify the contents after they had cooled and been poured into crushed ice. Triazole 4 was produced by filtering and recrystallizing the solid from a 1:1 combination of DMF and water. Table 1 lists the chemicals that were produced using the aforementioned approach.

2.3 Spectral Analysis 4a: IR (KBr) cm-1: 2920 (S, -SH), 1579 (C=N of triazole),1479 (C=C in Aromatic), 1138 (Ar-O), 1H NMR (DMSO): 5.39 (s, 2H, O-CH2), 7.02-7.88 (m, 6H, Ar-H), 14.21 (S, 1H - SH), Mass: (M+); m/z- 421,
4c: 1H NMR (DMSO): 5.10 (s, 2H, O-CH2), 7.02-7.55 (m, 8H, Ar-H), 14.02 (S, 1H - SH);
Mass: (M+); m/z- 352.
4e: 1H NMR (DMSO): 5.15 (s, 2H, O-CH2), 7.03-7.58 (m, 7H, Ar-H), 14.14 (S, 1H - SH),
Mass: (M+); m/z- 387.

Table 1: The Physical data of Synthesized compounds (4a-i)

Comp.	R Group	Melting point (0^0C)	Yield (%)
4a	2,4 Dichloro	256	86
4b	2,3 Dichloro	240	85
4c	H	140	80
4d	2 Methyl	170	82
4e	4 Chloro	196	85
4f	3,5 Dichloro	210	84
4g	3 methoxy	201	76
4h	4 methyl	202	78
4i	4 fluoro	124	80

2.4 Antimicrobial Activity:
1,2,4-triazole derivatives was dissolved in DMSO and evaluated at concentrations of 100, 50, 25 and 12.5 µg/ml. Amoxycillin a common antibiotic was used as reference antibiotic to test compounds for antibacterial activity against the microorganisms S. aureus, Klebsiella pneumoniae, Salmonella typhi and Serratia marcescens. Antimicrobial study was assessed by Minimum Inhibitory Concentration (MIC) by serial dilution method[17]. The Agar well diffusion method has been used to assess these substances' biological activity. 50 µL of the sample was added into each well. The results are displayed in Table 2, and the zone of inhibition is measured in millimeters. Against both bacteria, the majority of

the compounds exhibited moderate bactericidal activity.

Table 2: Antibacterial activity data in MIC (µg/mL) of the compounds (4a-i)

Comp. No.	Conc. µg/mL	klebsiella pneumoniae	Salmonella typhi	Serratia marcescens	S. aureus	A. Niger	C. Albicans
4a	100	22mm	22mm	23mm	20mm	12mm	10mm
	50	18mm	18mm	18mm	18mm	10mm	-
	25	16mm	15mm	15mm	15mm	-	-
	12.5	-	-	10mm	-	-	-
4b	100	10mm	12mm	10mm	12mm	12mm	15mm
	50	-	10mm	-	-	10mm	12mm
	25	-	-	-	-	-	-
	12.5	-	-	-	-	-	-
4c	100	12mm	12mm	15mm	15mm	10mm	22mm
	50	10mm	10mm	12mm	12mm	-	20mm
	25	-	-	-	10mm	-	18mm
	12.5	-	-	-	-	-	16mm
4d	100	22mm	10mm	22mm	24mm	12mm	10mm
	50	18mm	-	20mm	20mm	10mm	-
	25	16mm	-	18mm	16mm	-	-
	12.5	12mm	-	16mm	12mm	-	-
4e	100	18mm	12mm	18mm	-	12mm	15mm
	50	16mm	10mm	14mm	-	10mm	12mm
	25	12mm	8mm	10mm	-	-	-
	12.5	10mm	12mm	10mm	12mm	-	-
4f	100	12mm	12mm	15mm	15mm	10mm	22mm
	50	10mm	10mm	12mm	12mm	-	20mm
	25	-	-	-	10mm	-	18mm
	12.5	-	-	-	-	-	16mm
4g	100	12mm	12mm	15mm	15mm	12mm	15mm
	50	10mm	10mm	12mm	12mm	10mm	12mm
	25	-	-	-	10mm	-	-
	12.5	-	-	-	-	-	-
4h	100	20mm	10mm	22mm	22mm	12mm	15mm
	50	18mm	-	20mm	20mm	10mm	12mm
	25	16mm	-	18mm	16mm	-	-
	12.5	12mm	-	16mm	10mm	-	-
4i	100	22mm	23mm	20mm	24mm	23mm	20mm
	50	18mm	18mm	15mm	20mm	18mm	15mm
	25	14mm	16mm	12mm	16mm	16mm	12mm
	12.5	10mm	10mm	10mm	12mm	10mm	10mm
Amoxicillin	100	22mm	18mm	20mm	22mm	18mm	10mm

Rationale of target selection:
The selection of a biologically relevant target is a critical step in any structure-based drug design study. In the present investigation, the enzyme O-Acetylserine Sulfhydrylase (OASS) from Salmonella enterica serovar Typhimurium was chosen as the molecular target for docking studies involving the synthesized triazole derivatives. This choice is supported by well-established biochemical evidence demonstrating that triazole-based compounds can interfere with the L-cysteine biosynthetic pathway in Salmonella species.

A classic study provided the foundational insight into the inhibitory action of triazoles on Salmonella typhimurium growth28. The authors reported that triazole acts by inhibiting O-Acetylserine Sulfhydrylase A (OASS-A), the terminal enzyme in the L-cysteine biosynthetic pathway responsible for catalyzing the conversion of O-acetyl-L-serine and sulfide to L-cysteine. Instead of forming cysteine, OASS was found to catalyze an alternate reaction with triazole, yielding triazole-1-alanine as a byproduct. This side reaction depletes the pool of O-acetyl-L-serine, which in turn suppresses the synthesis of enzymes required for sulfate reduction, ultimately leading to L-cysteine starvation and growth inhibition of the bacterial cells. Furthermore, cysK mutant strains deficient in OASS exhibited resistance to triazole, directly linking the enzyme to triazole-mediated inhibition.
Given this biochemical precedent, the OASS enzyme represents a validated and mechanistically relevant target for assessing the antimicrobial potential of triazole and thiadiazole analogs. The triazole nucleus is known to engage in key hydrogen bonding and coordination interactions with active-site residues, making it a promising scaffold for enzyme inhibition. Therefore, molecular docking of the synthesized compounds with OASS from Salmonella enterica serovar Typhimurium was performed to explore their binding affinities and possible interaction patterns at the enzyme's active site. This approach provides a rational framework to correlate the computational binding predictions with observed antimicrobial activity, thereby elucidating the potential molecular basis of inhibition.

System Preparation
The macromolecular model of OASS-A from Salmonella enterica serovar Typhimurium was obtained from PDB with identifier 1D6S25. The model contained two identical chains of the enzyme. Since the binding sites were independent and do not contain the overlapping residues from the other chain, A single chain was separated and utilized for molecular docking and virtual screening. The co-crystallized PLP-Methionine complex was separated and used for the validation process. The Apo-form of the enzyme was saved in a separate file and subsequently converted to pdbqt format as per the requirement of AutoDock. The Compound library of the test compound was prepared by first obtaining the SMILES of the triazole compounds. The 3D conformers of these SMILES were obtained in SDF format using OpenBabel software32. Both the compound libraries were converted to the pdbqt format for subsequent docking procedure

System validation and Virtual Screening
Molecular docking and virtual screening were conducted following a well-established protocol from our laboratory[27,29-30,33]. Briefly, AutoDock 4.231 implemented in PyRx GUI26 was implemented in this study. Initially, the grid of 50×50×50 points was set around the centre of gravity of the co-crystallized ligand to ensure the entire active site coverage. The grid was generated using AutoGrid program. AutoDock program implementing the lamarkian genetic algorithm was used for molecular docking and virtual screening of the test compounds. In order to test the reliability of the docking system, the co-crystallized ligand was re-docked in the binding site and the ability of the software to re-generate the experimental binding pose was evaluated. The system is said to be validated if the RMSD between the predicted pose and the co-crystallized pose is below 2 Å [34-35]. The RMSD value was observed to be 1.80 Å during the validation procedure and hence the system was assumed to be validated. The same settings were used for docking the test ligands.

3.0 Results and Discussion

3.1 Chemical synthesis
Cyclization of thiosemicarbazides 3a–i under alkaline conditions (1N NaOH) followed by acidification with glacial acetic acid furnished the corresponding 5-((2,5-dichlorophenoxy)methyl)-4-aryl-4H-1,2,4-triazole-3-thiols (4a–i). The IR spectrum of 4a displayed a broad absorption at 3400 cm^{-1}, characteristic of the thiol (–SH) group, along with a band at 1546 cm^{-1} corresponding to the triazole C=N stretch, confirming successful ring closure. The ^1H NMR spectrum further supported cyclization, showing a distinct singlet at δ 3.31 ppm for the –SH proton, a singlet at δ 5.39 ppm for the O–CH_2 group, and multiplets at δ 7.02–7.88 ppm for aromatic protons. The molecular ion peak at m/z 421 (M^+) was in excellent agreement with the calculated molecular weight of the triazole derivative. All synthesized triazoles (4b–i) displayed comparable spectroscopic trends, confirming the general applicability and

efficiency of the cyclization strategy.

3.2 Antimicrobial Activity

The in vitro antimicrobial screening of compounds (4a–i) was performed using the agar well diffusion method at four concentrations (12.5–100 µg/mL) (Table 2), and the results are expressed as zones of inhibition (mm). Compounds 4a, 4b, 4c, 4f, and 4i demonstrated significant and concentration-dependent antimicrobial activity, with the maximum zone of inhibition (23 mm) observed at 100 µg/mL, which is notably higher than the standard drug amoxicillin (18 mm at 100 µg/mL). These compounds maintained appreciable activity even at lower concentrations (16–12 mm at 25–12.5 µg/mL), indicating strong intrinsic antimicrobial potency. In contrast, compounds 4d, 4e, 4g, and 4h exhibited only weak to moderate activity, with inhibition observed mainly at the highest concentration and complete loss of activity at lower doses. Among them, 4h was the least active, showing a zone of only 10 mm at 100 µg/mL and no detectable inhibition at lower concentrations. The superior performance of compounds 4a, 4b, 4c, 4f, and 4i suggests that specific structural features present in these molecules may strongly favor microbial growth inhibition. Overall, the results clearly differentiate the highly active compounds from weakly active ones and identify 4a, 4c, 4f, and 4i as promising leads for further mechanistic and MIC-based evaluation.

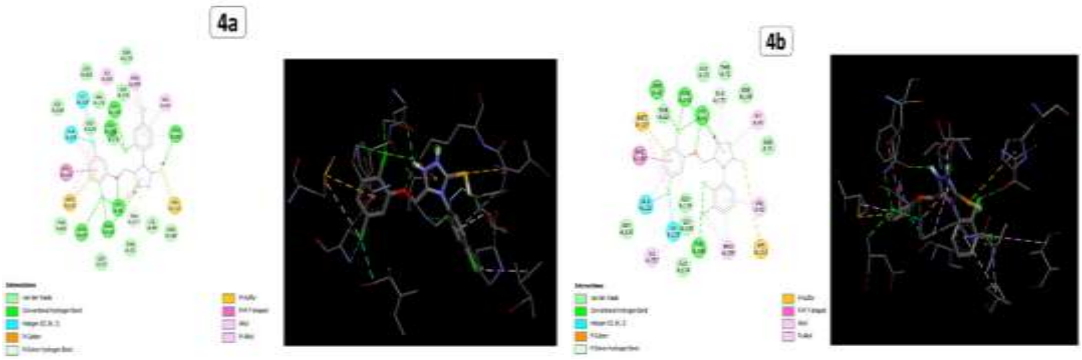
Computational Studies

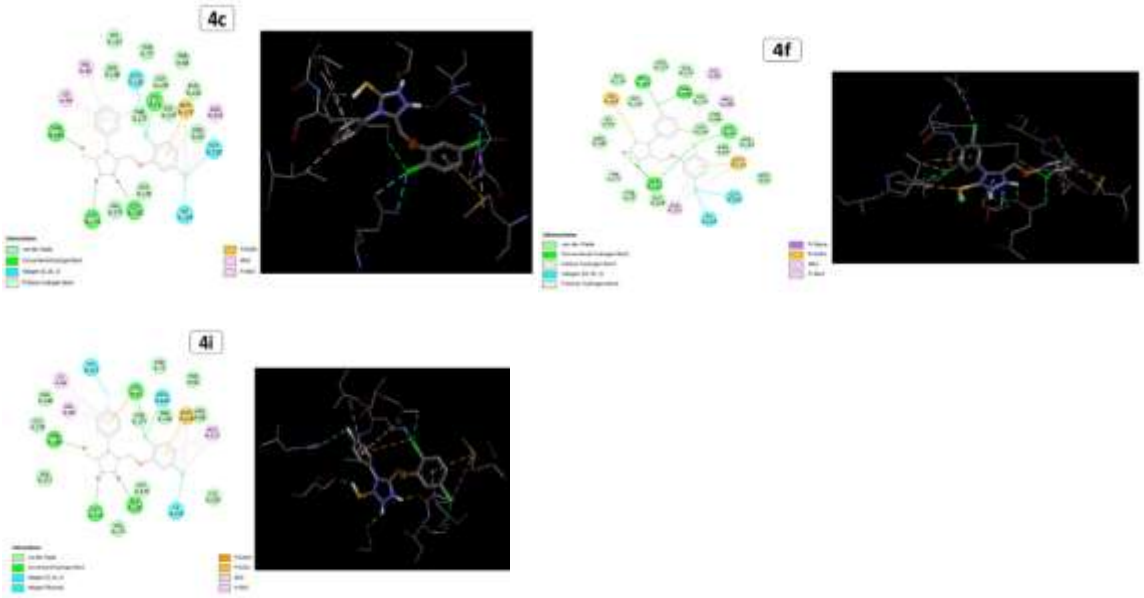
The results of the binding free energy obtained from molecular docking are tabulated in comparison to the experimental zone of inhibition data in table 03 below.

Table 3: Summary of the molecular docking results (Binding free energies) compared to the experimental assay data

Compound	Binding free energy
4a	-6.4
4b	-6.04
4c	-6.88
4d	-6.54
4e	-5.96
4f	-6.54
4g	-6.6
4h	-6.19
4i	-5.74
Crystallographic inhibitor	-7.05

Interaction analysis study





Conclusion:
In the present study, a new series of 2,5-dichlorophenoxy-substituted thiosemicarbazide intermediates (3a–i) and their corresponding 1,2,4-triazole-3-thiol derivatives (4a–i) were successfully synthesized using a simple, efficient, and reproducible two-step synthetic protocol. The structures of synthesized compounds were unambiguously confirmed by IR, ^{1}H NMR, and mass spectral analyses. Subsequent in vitro antimicrobial screening revealed that several triazole derivatives, particularly 4a, 4b, 4c, 4f, and 4i, exhibited pronounced and concentration-dependent inhibitory activity, with zones of inhibition surpassing or comparable to the standard drug amoxicillin. In contrast, a few analogues displayed only marginal or no activity, highlighting the influence of aryl substitution on biological performance. Collectively, these findings demonstrate that the newly synthesized triazole scaffold represents a promising lead for further optimization and detailed mechanistic and MIC-based investigations. In summary, the docking studies provide valuable preliminary insights into the binding potential of the synthesized compounds, and in most cases, the computational predictions correlate reasonably well with experimental zone of inhibition values. Nevertheless, variations between theoretical and experimental results emphasize the importance of integrating in silico findings with empirical assays. Together, these complementary approaches enable a more comprehensive understanding of the structure–activity relationships governing the antibacterial efficacy of the synthesized triazole derivatives. All the active compounds demonstrated stable binding conformations within the enzyme's catalytic pocket, primarily mediated through hydrogen bonding, p–sulfur, p–alkyl, and halogen interactions with catalytically important residues such as LYS41, THR177, GLY176, MET119, and HIS152. These residues are known to play a critical role in substrate recognition and catalysis, suggesting that the ligands may competitively interfere with the enzyme's normal activity.

Acknowledgements:
Authors thankful to the Savitribai Phule Pune University Pune and management, Principal, Padmashri Vikhe Patil College of Arts, Science and Commerce Pravaranagar, Maharashtra, India for their invaluable support and availability of resources for this work.

Ethical Approval: This has not been published elsewhere and is not currently under consideration for publication elsewhere. This study does not involve experiments on animals or human subjects.

Conflict of interest: The authors declare that they have no conflict of interest.

Informed consent: Written informed consent was obtained from all individual participants included in the study.

Data and materials availability: All data associated with this study are present in the paper.

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