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Original Article

MOLECULAR DOCKING STUDIES AND SYNTHESIS OF 3, 4 - DISUBSTITUTED TRIAZOLES AS *MYCOBACTERIUM TUBERCULOSIS* ENOYL-ACP REDUCTASE AND CYP-51 INHIBITORS

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ABSTRACT

Objective: To design, synthesize and *in vitro* antitubercular, antifungal and antioxidant evaluation of some novel mercapto 1, 2, 4–triazole derivatives.

Methods: New derivatives were designed by using various software like ACD Lab chemsketch, molinspiration and autodock. Designed molecules are obeying Lipinski's rule of five and having highest binding score was selected for the synthesis. The synthesized compounds were subjected to TLC, melting point determination, FTIR, ^{1}H NMR, ^{13}C NMR and mass spectral analysis. The newly synthesized compounds were investigated for *in vitro* antitubercular evaluation by MABA method, antifungal evaluation by cup plate method and antioxidant evaluation by DPPH scavenging assay.

Results: A virtual screening was carried out through docking designed compounds into the InhA and CYP-51 binding site to predict if these compounds have an analogous binding mode of the enoyl ACP reductase (InhA) and CYP-51 inhibitors. Three derivatives (4a1, 4a2 and 4a3) were selected for the synthesis with the help of *in silico* modeling. The selected derivatives were synthesized by a conventional method. All the synthesized compounds showed a characteristic peak in FT IR, ^{1}H and ^{13}C NMR and mass spectroscopic studies. All the selected derivatives showed antitubercular, antifungal and antioxidant activity.

Conclusion: The derivatives were synthesized adopting simple and laboratory friendly reaction conditions to give the target compounds in quantitative yields. Newer derivatives possess good antitubercular, antifungal and antioxidant activity.

Keywords: Triazole, Docking, Antitubercular, Antifungal, Antioxidant

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INTRODUCTION

In the past, most drugs have been discovered either by identifying the active ingredient from traditional remedies or by serendipitous discovery. A new approach has been identified to understand how disease and infections are controlled at the molecular and physiological level and to target specific entities based on this knowledge [1]. Molecular docking is a method, which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation, in turn, may be used to predict the strength of association or binding affinity between two molecules using scoring functions which are used to predict the strength of the non-covalent interaction (also referred to as binding affinity) between two molecules after they have been docked [2]. Molecular docking has become an increasingly important tool for drug discovery.

Mycobacterium tuberculosis (M. tuberculosis) is the fundamental etiologic agent for human tuberculosis and is the biggest killer due to bacterial infection in the world today. Tuberculosis accounts for about 7% of all deaths in developing countries and 26% of avoidable adult deaths [3]. Due to the reduced effectiveness of current drugs resulting from the emergence of multidrug-resistant tuberculosis (MDR TB) and co-infection with human immunodeficiency virus (HIV), there is an urgent need to develop new natural or synthetic antitubercular drugs. The major drawbacks with the therapeutic agents for mycobacterial and fungal infections are prolonged treatment regimen with a combination of drugs associated with significant toxicity and the emergence of multi-drug resistant bacteria and fungi causing morbidity and mortality in immunocompromised hosts. The necessity for effective therapy has stimulated research into the design and synthesis of novel compounds which can treat both mycobacterial and fungal infections. Design of compounds having good anti-tubercular activity is gaining much importance in the field of tuberculosis research due to the resurgence of antibiotic-resistant strains.

Mycobacterium tuberculosis enoyl-acyl carrier protein (ACP) reductase (InhA) has been validated as a promising target for antitubercular agents. InhA was identified as an NADH-dependent enoyl-ACP (CoA) reductase specific for long-chain enoyl thioesters and is a member of the Type II fatty acid biosynthesis system, which elongates acyl fatty acid precursors of mycolic acids which are components of the mycobacterial cell wall [4, 5].

Azoles exert antifungal activity through inhibition of cytochrome P450 14α-demethylase (CYP51), which is crucial in the process of biosynthesis of ergosterol by a mechanism in which the heterocyclic nitrogen atom (N-4 of 1,2,4-triazole) bind to the heme iron atom. Selective inhibition of CYP 51 would cause depletion of ergosterol and accumulation of lanosterol and other 14-methyl sterols resulting in the growth inhibition of fungal cells [6]. When a mercapto group attached to the heterocyclic rings enhance fungicidal activity. It has been reported that structural properties of triazoles, like moderate dipole character, hydrogen bonding capability, rigidity and stability under *in vivo* conditions are the main reasons for their superior pharmacological activities [7].

To date, the prevention of oxidative stress related diseases has been tentatively achieved by the development of antioxidant compounds that are able to scavenge reactive oxygen species and thus avoid radical-induced oxidation damage. Excessive free radical attack can damage DNA, proteins and lipids, resulting in diseases like cancer, neurological degeneration and arthritis, as well as the process of aging [8]. Therefore, considerable speculation has been directed towards the identification of antioxidants for use in preventive medicine.

In recent years, triazole derivatives have acquired conspicuous significance due to their wide spectrum of biological activities such as antibacterial [9], antifungal [10], antiviral [11], anti-inflammatory [12, 13], anthelmintic [14], antitubercular [15], antioxidant [16], antiplasmodial [17] and antileishmanial [18] activity. Nowadays, many triazole containing drugs are available in the market such as fluconazole, itraconazole, voriconazole, posoconazole anastrozole, estazolam,

ribavirin, triazolam [19], etc. The search and evaluation of 1,2,4-triazole compounds and their derivatives with a specific pharmacological activity is a demanding task in the drug discovery process. This study aims to discover potentially active new 1, 2, 4-triazole derivatives through *in silico* drug designing as InhA and cyp 51 inhibitor. Also, we present an evaluation of the antioxidant effect of substituted triazole derivatives.

MATERIALS AND METHODS

Lead optimization

Lead optimization was done through in-silico Lipinski filter. Molinspiration server was used for this purpose [20]. The structure drawn in the JME molecule editor was subjected to analysis of Lipinski rule of five.

Selection of target protein

The 3D crystal structure of InhA receptor (entry code: 4U0J) [21] and CYP 51 receptor/fungal protein (entry code: 1EA1) [22] used for docking was recovered from the Brookhaven Protein Data Bank (http://www. rcsb. org/pdb/home). Reference compounds such as pyrazinamide and fluconazole are directly downloaded from DrugBank 2.5 database [23, 24], and they are potentially competitive inhibitor against target proteins.

Active site prediction

A prediction of the active site and ligand binding sites of 3D protein structure was done by using Computed Atlas of Surface Topography of proteins (CASTp) which is a web server that provides online services for locating, delineating and measuring geometric and topological properties of protein structures [25].

Preparation of ligand files

Ligand files for the molecular docking studies were prepared in Chem Draw Ultra software, Cambridge Soft Corporation, USA. Version-12.0, 1997-2010. It is a Chem Tech tool used for the drawing of ligand molecules. Compound structures were drawn in ChemDraw software and converted to pdb format (. pdb file) with the standard settings and further used for docking studies.

Docking study

Molecular docking studies performed with various targets like enoyl-ACP reductase and cytochrome P450 14α-demethylase receptor with novel compounds were carried out by using AutoDock 4.2.1 and Auto Dock Tools (ADT) v 1.5.4 from the Scripps Research Institute. Firstly, all bound waters, ligands and cofactors were removed from the proteins. Then macromolecule was checked for polar hydrogen. Partial atomic Kollman charges were assigned, and then atomic solvation parameters were allotted. Torsion bonds of the inhibitors were selected and defined. Secondly, the three-dimensional grid box was created by AutoGrid algorithm to evaluate the binding energy on the macromolecule coordinates. Grid maps representing intact ligand in the actual docking target site were calculated with AutoGrid. The three-dimensional grid box with a grid map of size 80x80x80 of x, y and z-axis for different grid parameters with a spacing of 0.300 A ° was created. Eventually, cubic grids encompassed the binding site where the intact ligand was embedded. Finally, resultant compounds were used to compute molecular stimulation parameters like Lamarckian genetic algorithm of population size 150, the mutation rate of 0.02 and crossover rate of 0.8, these simulations were performed up to 2.5 million energy and the evaluations were maximum at 27000 generations. Each simulation was carried about 10 times which ultimately yielded 10 docked conformations. From this, the lowest energy conformations were regarded as the best binding conformations.

Chemistry

Chemicals used were purchased from Merck and Hi-Media, Mumbai, India. Vanillic acid (4-hydroxy-3-methoxy benzoic acid), carbon-disulfide, potassium hydroxide, hydrazine hydrate, ethanol, and anhydrous ether was purchased from Merck. DMSO and aromatic amines (4-nitroaniline, 2-nitroaniline, 4-bromo aniline) were purchased from Hi-Media, India. All the chemicals were used without further purification. Melting points were determined in open capillary tube and were uncorrected. Purity of the synthesized compounds was routinely checked by thin layer chromatography on silica gel G with the solvent system–benzene: methanol (8:2) using iodine vapour for detection. IR spectra of the compounds were recorded using KBr pellets in the range of 4000-500 cm^{-1} on a Jasco FTIR spectrophotometer in Devaki Amma Memorial College of Pharmacy, Malappuram, India. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on Bruker ultra shield DPX 400 spectrometer using tetramethylsilane as an internal standard (chemical shifts in δ, ppm) and mass spectra on LC-MSD Trap-SL 2010 A-Shimadzu spectrometer at Interdisciplinary School of Indian System of Medicine (ISISM), SRM University, Chennai, India.

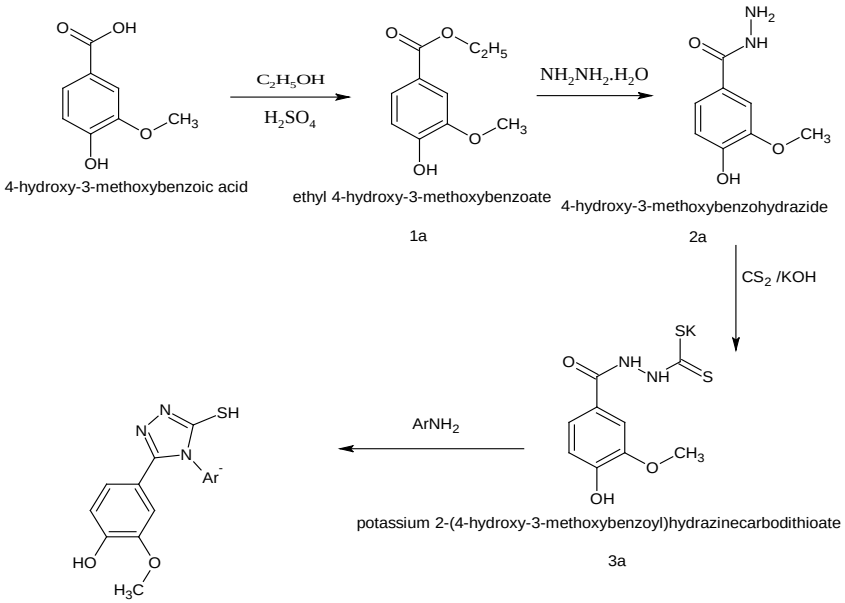

Fig. 1: Schematic presentation of synthesis of 4a1-4a3

Synthesis of ester (1a)

A mixture of 0.01 mol substituted acid, 10 ml of absolute ethanol and 2 ml of concentrated sulphuric acid was refluxed for 4 h. The product was recrystallized from ethanol.

Synthesis of acid hydrazide (2a)

A mixture of 0.01 mol ester and 0.2 mol hydrazine hydrate was refluxed in 50 ml of 95% ethanol for 2 h. The resultant mixture was acidified with concentrated hydrochloric acid. The solid mass thus separated out was filtered, dried and purified by recrystallization from ethanol.

Synthesis of the potassium salt of dithiocarbazinate (3a)

Potassium hydroxide (0.01 mol) was dissolved in absolute ethanol (75 ml). To the above solution, acid hydrazide (0.01 mol) was added with stirring and cooling in ice. To this, carbon disulphide (10 ml) was added in small portions. The reaction mixture was refluxed for 4-6 h. To the resulting solution, anhydrous ether (250 ml) was added and precipitated potassium dithiocarbazinate was collected by filtration, washed with diethyl ether and dried. The dithiocarbamates were obtained in quantitative yield. As most of the potassium salt of dithiocarbazinates were moisture sensitive, they were employed directly for the preparation of aminomercapto triazoles without further purification.

Synthesis of 5-mercapto-1, 2, 4-triazole derivatives ($4a_1$-$4a_3$)

A suspension of potassium salt dithiocarbazinate (0.01 mol), hydrazine hydrate (2 ml), water (80 ml) and a primary aromatic amine (0.01 mol) was refluxed for 3h. The colour of the reaction mixture changed to green, hydrogen sulphide was evolved and a homogenous solution resulted. A white solid was precipitated by dilution with cold water. The product was filtered, washed with cold water and recrystallised from ethanol [26].

Antitubercular activity

Microplate Alamar Blue Assay (MABA) method was used for the study. Prior to the bioassay, a standard stock solution with the concentration of 32 µg/ml was prepared and stored to be used for the positive control. Control wells without tested derivatives and sterility controls were assayed simultaneously. Pyrazinamide was prepared from the stock solution just prior to inoculation time to the concentration of 5 µg/ml in the total volume of 200 µl. The growth inhibition result was explained by MABA using 1 % resazurin. The reagent allows the detection of microbial growth in microtiter plates without the use of a spectrophotometer. The susceptibility test conducted by the MABA was using 96 well microtitre plates to evaluate the susceptibility of $H_{37}Rv$ MTB reference strain to the derivative. The inhibitory concentration of all derivative was evaluated with concentrations of 250 mg/ml, 500 mg/ml and 1000 mg/ml in the total volume of 200 µl. The measured derivatives were mixed with the bacterial suspension and the diluent media (7H9) in the well. The alamar blue oxidation-reduction dye is a general indicator of cellular growth and/or viability; the blue, non-fluorescent, oxidized form becomes pink and fluorescent upon reduction. Growth was therefore determined by a visual color change. The derivatives were considered active (have inhibitory activity) for the well of the plate with unchanged color or the blue, non-fluorescent, oxidized form and if the color of the agent or resazurin is changed to pink (fluorescent) the derivative is inactive or the microorganism is considered resistant strain to the derivative [27].

Antifungal activity

All the synthesized compounds (200 µg/ml) in DMSO solvent were screened for *in vitro* antifungal activity by agar well diffusion method [26]. The activity was evaluated against pathogenic fungal strains such as *Candida albicans* (ATCC. 4563), *Aspergillus niger* (ATCC. 20611) using Sabouraud's dextrose agar medium. The fungal strains were procured from the Department of Microbiology, Devaki Amma Memoria College of Pharmacy. The results were compared with standard fluconazole (25 µg/ml). The plates were incubated for 48 h at 27 °C in an incubator. Plates were read only if the lawn of growth was confluent or nearly confluent. The diameter of the inhibition zone was measured to the nearest whole millimeter by holding the measuring device. The test was carried out in triplicate.

Antioxidant activity

In vitro antioxidant activity was carried out by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay method [28]. Assay was carried out using UV spectrophotometer at 517 nm. To 2 ml solutions of synthesized compounds (50, 100,150 and 200 µg/ml), 2 ml DPPH solutions (400 µmol) in ethanol were added into the test tube. The solution was incubated at 37 °C for 30 min and the absorbance of each solution was measured at 517 nm against a reagent blank solution. Ascorbic acid was used as reference antioxidant. Experimental values summarized for DPPH radical scavenging assays are expressed as the mean±standard error of mean (SEM). The percent free radical scavenging activity was calculated by the formula given by

$$\text{Percentage scavenging} = \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \times 100$$

The experiment was done in triplicate. As opposed to increasing concentration of specimens decline of absorbance is an indication that destroyed DPPH radical. Antioxidant activity results are expressed as IC_{50} value (µg/ml) that reduces by half the effective concentration of DPPH radicals and was calculated by interpolation from the linear regression analysis.

RESULTS AND DISCUSSION

In silico molecular analysis of different triazole derivatives was done. All these compounds obeyed "Lipinski rule of five". These analogues were taken for computing molecular descriptors and which have the best docking score was taken for synthesis. The results are shown in table 1.

Table 1: Analysis of lipinski rule of five

Compound code	Ar	Log p	H donor (nON)	H acceptor (nOHNH)	No. of rotatable bonds (nrotab)	Mol. Wt	No. of violation
4a1	$C_6H_4NO_2$	2.86	8	1	4	344.34	0
4a2	$C_6H_4NO_2$	3.02	8	1	4	344.34	0
4a3	C_6H_4Br	3.71	5	1	3	378.24	0
Fluconazole	-	3.86	4	1	3	362.54	0

Molecular modeling technique was used to explore, predict and understand the protein/enzyme interactions with designed 1, 2, 4-triazole library; and also to visualize the probable binding. Analogues were docked with various receptors using Autodock 4.2.1 and the binding energy obtained are shown in table 2. Docking studies of targeted compounds with CYP51 protein showed good binding interactions and formed various hydrophobic interactions with active site residues. The ligand 4a3 exhibited a docking score of 9.73, with the main hydrophobic interactions with the surrounding residues ALA191A, GLY192A, ILE194A and PRO193A, strongly contributed to the stabilization with 1EA1. The hydrogen bonding interaction of hydroxyl group protons of 4a3 were with the residues SER94A and LYS166A.

Table 2: Docking result with proposed derivatives

Compound code	1EA1			4U0J		
	Binding energy (K. Cal/Mol)	Inhibition constant	No. Of H bonds	Binding energy (K. Cal/Mol)	Inhibition constant	No. Of H bonds
4a1	-8.64	1.1µM	4	-8.47	1.05 µM	4
4a2	-8.75	3.68 µM	3	-8.39	4.06 µM	4
4a3	-9.73	693.55 nM	4	-8.55	1.34 µM	5
Fluconazole	-8.31	258.35 nM	3	-	-	-
Pyrazinamide	-	-	-	-8.1	2.96 µm	3

Binding interactions of derivatives with 1EA1 and 4U0J

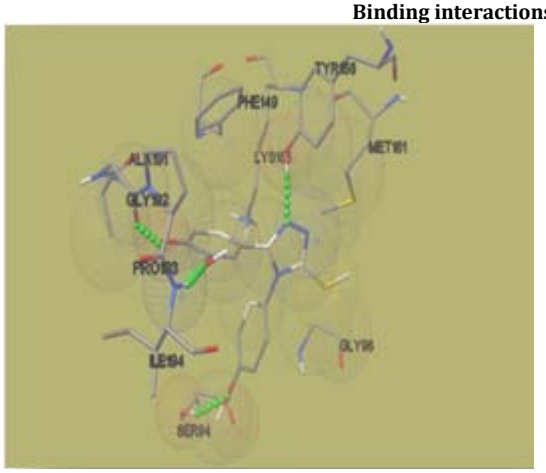

Fig. 2: Interaction of 1EA1 with 4a3

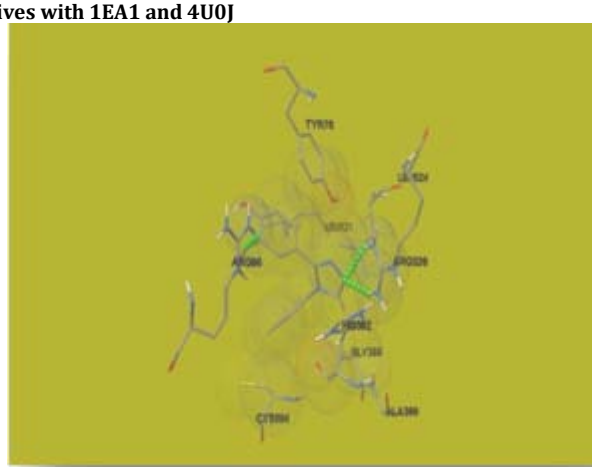

Fig. 3: Interaction of 1EA1 with 4a1

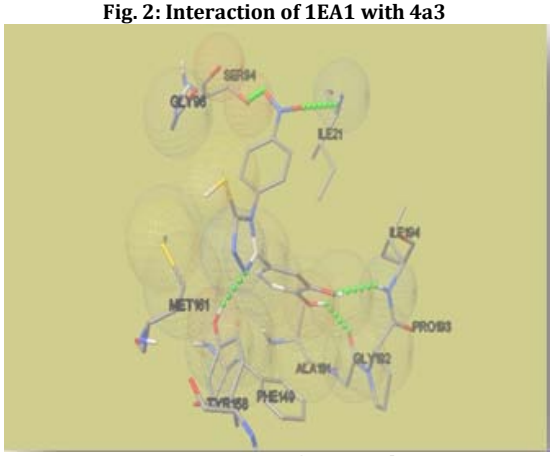

Fig. 4: Interaction of 4U0J with 4a3

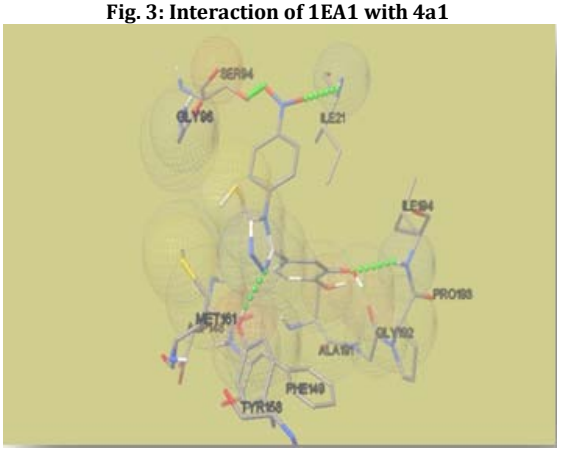

Fig. 5: Interaction of 4U0J with 4a2

Analogues designed by *in-silico* studies were selected for wet lab synthesis based on the good binding energy and availability. Three analogues were synthesized by a conventional method. The reaction sequence leading to the formation of titled compounds is shown on the scheme. First step in synthesis was esterification of carboxylic acids with alcohols which is a kind of nucleophilic acyl substitution. In the second step, an efficient and general process, involving preforming activated ester reacts with hydrazine, for the preparation of hydrazides. This process gives the desired hydrazides in excellent yield and purity under mild conditions [29]. The main synthetic route to dithiocarbamates is based on the interaction between the corresponding amine and CS2 in the presence of a strong base. It was found that upon decreasing of the protoning ability of the solvent, the rate of dithiocarbamate formation increases. Finally, potassium salt of dithio carbazinates was react with corresponding primary aromatic amines like 4-bromo aniline, 4-nitroaniline and 2-nitroaniline, the ring cyclises to form 1,2,4-triazole derivatives (4a1-4a3). All the compounds were obtained in moderate to good yields. The physicochemical data of synthesized derivatives is given in table 3.

Table 3: Physicochemical data of newly synthesized compounds

Compound	Physical appearance	Melting point in °C	R_f value	% yield
1a	Yellowish brown	58	0.84	88
2a	Yellowish orange	198	0.87	88
3a	Yellowish orange	246	0.76	84
4a1	Brown	132	0.80	78
4a2	Red	64	0.79	81
4a3	Pale yellow	86	0.82	67

The structures of all the newly synthesized compounds were confirmed by their IR, ^{1}H and ^{13}C NMR and mass spectral studies. All the newly synthesized compounds exhibited satisfactory spectral data consistent with their molecular structures.

4a1: Molecualr formula: $C_{15}H_{12}N_4O_4S$, IR υ (cm^{-1}) (KBr) 3470 (NH-stretching), 1700 (NO_2-stretching), 2610 (S-H-stretching), ^{1}H NMR ($CDCl_3$) (ppm): δ 5.01 (S, 1H, OH), 3.514 (S, 1H, C-SH), 6.61-6.68 (M, 5H, C_6H_5), 2.507 (S, 3H, CH_3), 7.94-7.96 (D, 2H, CH_2), ^{13}C NMR ($CDCl_3$) ppm: δ 156.08, 136.23, 126.79, 112.85, 56.58, 40.41, 40.25, 40.17, 40.08, 39.91, 39.75, 39.58, 39.41, 18.92, MS m/z (%):132.95, 176.95, 193.05, 239.10 (base peak), 344.34 (molecular ion peak).

4a2: Molecular formula: $C_{15}H_{12}N_4O_4S$, IR υ (cm^{-1}) (KBr) 3470 (NH-stretching), 1700 (NO_2-stretching), 2610 (S-H-stretching), ^{1}H NMR ($CDCl_3$) (ppm): δ 2.51 (S, 1H, C-SH), 3.42 (S, 3H, CH_3) 5.01 (M, 1H, C-OH), 6.6-7.95 (M, 7H, Aromatic H), ^{13}C NMR ($CDCl_3$) ppm: δ 146.65, 136.04, 130.83, 125.80, 119.62, 115.86, 40.47, 40.30, 40.14, 39.97, 39.80, 39.64, 39.47, MS m/z (%):111.05, 132.95, 301.25 (base peak), 344.34 (molecular ion peak).

4a3: Molecular formula: $C_{15}H_{12}BrN_3O_2S$, IR υ (cm^{-1}) (KBr) 2610 (S-H-stretching), 510 (Ar-Br-stretching), 3517 (NH-stretching),^{1}HNMR ($CDCl_3$) (ppm): δ 5.20 (S, 1H, OH), 6.56-7.4 (D, 7H,Aromatic H), 3.53 (S, 3H, CH_3), 2.5 (S, 1H, C-SH), ^{13}C NMR ($CDCl_3$) ppm: δ 148.40, 131.82, 116.37, 106.83, 56.69, 40.50, 40.34, 40.17, 40.00, 39.67, 39.50, 19.06, MS m/z (%): 111.20, 206.10 (base peak), 378.24 (molecular ion peak).

Antitubercular evaluation

Antitubercular study was performed by MABA method using *Mycobacterium tuberculosis* H_{37}Rv strain (ATCC 27294). In table 4 showed the antitubercular effect of synthesized derivatives.

Table 4: Antitubercular screening of synthesized derivatives

Test drugs	MIC in µg/ml
4a1	25
4a2	25
4a3	12.5
Pyrazinamide	3.125

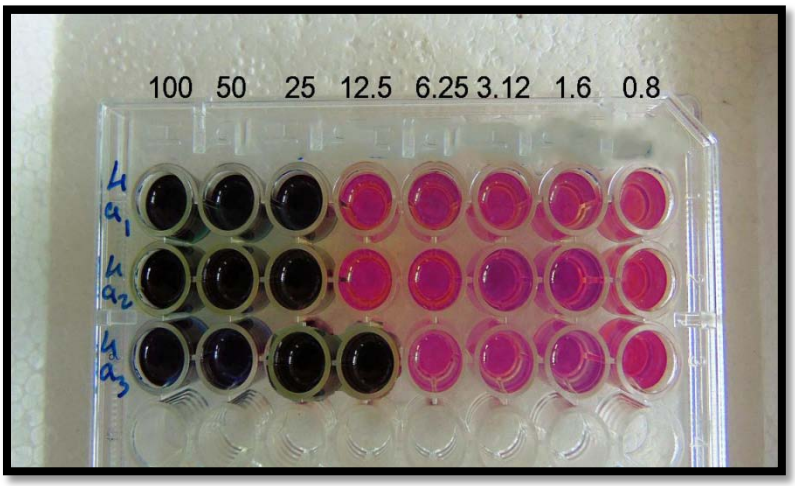

Fig. 6: Mycobacterial sensitivity to synthesized derivatives

Among the compounds evaluated 4-[4-(4-chlorophenyl)-5-sulfanyl-4*H*-1,2,4-triazol-3-yl]-2-methoxyphenol (4a3) showed good binding affinity with Mycobacterial InhA, having minimum inhibitory concentration found to be 12.5µg/ml compared with pyrazinamide as standard drug. The antitubercular data revealed that compounds having an electron withdrawing group like bromo attached to triazole nucleus may prove a template for antitubercular activity for further development.

Antifungal evaluation

The antifungal evaluation was done using 2 microorganisms i.e. *Candida albicans* and *Aspergillus niger*. Growth of inhibition of wells was determined using digital zone reader, and the datas are given in table 5. Azoles perform their antifungal action in two steps: inhibition of ergosterol synthesis, a major component of the fungal membrane and second step involves the blocking of P450-dependent enzyme i.e., lanosterol 14-α-demethylase (CYP 51). The 3D model of CYP 51 obtained on the basis MTCYP51 (*M. Tuberclosis* 14-α-demethylase) showed that triazole ring coordinated to the heme iron of CYP51, a key step in antifungal activity of triazole. Lack of ergosterol and accumulation of 14-α-demethylase disfunctionalize the fluidity of several enzyme located in the membrane which results in inhibition of fungal growth and replication of its DNA [30].

Table 5: Zone of inhibition of 4a1-4a3 in *C. albicans* and *A. niger*

Compound	Diameter of zone of inhibition (mm) in	
	C. albicans	***A. niger***
4a1	10.53±0.251	8.25±0.031
4a2	11.86±0.305	10.85±0.041
4a3	16.63±0.028	15.69±0.015
Fluconazole (25µg/ml)	17.20±0.264	17.86±0.638
DMSO	0	0

*Values are given mean±SD (n=3)

Antioxidant evaluation

Antioxidant evaluation was carried out by DPPH assay using ascorbic acid as a standard. The compounds 4a1-4a3 exhibited significant scavenging activity ranging from 40 to 88% and the data are expressed in mean±SEM in table 6.

Many of them were shown to possess antioxidant properties and inhibit radicals by the effect of electron donating groups or supplying hydrogen atom. Based on the IC50 values of standard ascorbic acid and synthetic derivatives as showed in table 6, it is indicated that 4a3 shows better DPPH scavenging activity.

Table 6: Antioxidant evaluation of synthesized derivatives

Compound	Concentration (µg/ml)	Percentage inhibition	IC_{50} (µg/ml)
Ascorbic acid	12.5	18.23±0.483	54.11
	25	36.99±0.745	
	50	52.48±0.485	
	100	75.01±0.530	
4a1	50	40.05±0.028	114.12
	100	47.41±0.005	
	150	56.43±0.005	
	200	62.89±0.010	
4a2	50	45.37±0.0152	122.46
	100	47.29±0.0115	
	150	53.17±0.0208	
	200	75.01±0.530	
4a3	50	54.21±0.0208	22.32
	100	70.31±0.0057	
	150	80.44±0.0057	
	200	88.9±0.0100	

*Values are given mean±SEM (n=3).

CONCLUSION

An attempt was made to explore the antitubercular potential of triazole scaffold in drug development. In this study, new mercapto1,2,4-triazole derivatives were designed and synthesized from potassium salt dithiocarbazinate and aromatic amines. The data obtained from ^{1}H NMR, ^{13}C NMR and mass spectra confirmed the proposed structures. The products were obtained in good yield. The synthesized compounds have been screened for *in vitro* antifungal activity and showed good to moderate antifungal activity. Results of *in vitro* antifungal, antitubercular activity, and molecular docking study revealed that the synthesized compounds have potential antifungal, antitubercular activity and can be further optimized and developed as a lead compound. 1, 2, 4-triazole derivative compounds were shown to have significant antioxidant properties as measured by DPPH scavenging assay.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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