RESEARCH PAPER

EXPLORING THE ANTIBACTERIAL EFFICACY OF SYNTHESIZED NOVEL TRIAZOLE DERIVATIVES

Paramita Das [1*], Gokulraj M [1], Tejaswini [1], Apurwa Rahul Salunkhe [2], Povindhar C [3], Dhanasekar M K [4]
[1]Department of Pharmaceutical Chemistry, Krupanidhi College of Pharmacy, Bengaluru, Karnataka 560035, India
[2]Department of Pharmaceutical Quality Assurance, Krupanidhi College of Pharmacy, Bengaluru, Karnataka, India
[3]Department of Pharmacology, The Oxford College of Pharmacy, Bengaluru, Karnataka 560068
[4]B. Pharm graduate, The Oxford College of Pharmacy, Bengaluru, Karnataka, 560068
[*]Corresponding author Email: paramitadas04@gmail.com

ABSTRACT
The World Health Organization found that out of 32 antibiotics under clinical research, just six of them were deemed novel. There is currently more than 70% documented resistance to the broad-spectrum antibiotics, fluoroquinolones and third-generation cephalosporins. Triazole, often referred to as pyrrodiazole, is a five-membered saturated heterocyclic compound that serves as a fundamental structural element in a variety of pharmacological classes with a diverse array of biological activities. The study mainly focused on synthesis of novel analogues of 1,2,4-triazole derivatives and their antibacterial efficacy. The *In-silico* studies for 10 triazole derivatives were performed against *Escherichia coli* (4Y6I) and *Staphylococcus aureus* (4JE0). The best 10 docked compounds were synthesized and characterized by IR, NMR, and MASS. The newly synthesized compounds, compared with the standard antibacterial drug (ofloxacin), were evaluated *in vitro* for their antibacterial activity against *Pseudomonas aeruginosa, Escherichia coli, Micrococcus lutes* and *Staphylococcus aureus* by Disk Diffusion Method and Cytotoxicity studies by MTT assay. The findings indicated that, of the developed derivatives, compound TA04 had the best binding energy of -9.5 kcal/mol. It also had the best activity and the highest zone of inhibition of 6 mm, with IC50 value of 1.42µg/ml. When compared to the reference drug of loxacin, TA04 had the strongest activity against *P. aeruginosa*, with an IC50 value of 0.42 µg/ml. It has also demonstrated greater action against gram-negative bacteria.

Keywords: 1,2,4-Triazole, Molecular Docking, Antibacterial activity, *Pseudomonas aeruginosa*, Ofloxacin.
How to cite this article: Paramita Das, Gokulraj M, Tejaswini, Apurwa Rahul Salunkhe, Povindhar C, Dhanasekar M K. Exploring The Antibacterial Efficacy Of Synthesized Novel Triazole Derivatives. Int J Drug Deliv Technol. 2026;16(7):456-465. DOI: 10.25258/ijddt.16.7.49.

INTRODUCTION

Bacterial infections and the effects associated with them are a major concern for researchers across the globe. It is crucial to develop new compounds to combat bacterial infections in humans, as bacterial resistance to antibiotics continues to evolve [1]. *Staphylococcus aureus* belongs to class of gram-positive bacteria that endures as part of the human flora [2] and causes moderately severe skin infections and could cause fatal infections of the blood, lungs and bones [3]. *Micrococcus luteus* also belongs to gram-positive bacterium and is widely found in natural environments and is typically seen as a resident of human skin and pharyngeal mucosa [4]. Likewise, *Escherichia coli* and *Pseudomonas aeruginosa* are gram-negative pathogens which are well-known zoonotic and anthropogenic. These infections sometimes could be fatal [5]. In the exploration for novel derivatives with potential antibacterial efficacy, multiple investigations focus on activity of triazoles due to their intrinsic bioactivities and prevalence in biological molecules [6]. Triazole also named as pyrrodiazole is a five membered organic heterocyclic saturated ring [7] is a core structure with numerous biological activities such as antimicrobial [8], anti-inflammatory, analgesic [9], antiviral [10] and antifungal activities [11]. Therefore, continuing to explore their synthesis is commendable.

MATERIALS AND METHODS

Materials

For the experiment, analytical-grade chemicals were acquired from TCI and they were all utilized without additional purification. Precoated TLC plates were used to determine the compound's purity (Merck 60F 254). With Brucker Alpha II, infrared spectra were recorded. Tetramethyl silane (TMS) served as the standard for the 1H NMR, which were recorded at 360 MHz on a Brucker W.M. 400 Spectrometer.

Molecular Docking Studies:

The investigation entailed employing Auto Dock Tools 1.5.7 to scrutinize the binding energies interactions between 10 distinct triazole derivatives and two protein variants (PDB ID: 4Y6I and 6LNW). The protein structures are downloaded from the RCSB Protein Data Bank, the downloaded protein prepared using PyMOL and the ligands were designed via Chem Sketch. The compound which is having lowest energy was considered as optimal conformation. The result output provided detailed information about encompassing binding energies, the precise conformers used for each ligand, and

inhibition constants. Visualization of the intricate ligand-protein complexes post-docking was executed utilizing Biovia Discovery Studio. Additionally, a comprehensive analysis of the pharmacokinetic and pharmacodynamic properties, colloquially termed ADMET, was conducted via Swiss ADME and OSIRIS Property Explorer [12,13].

Conventional approach for the synthesis of Potassium {[2-(4 -nitro benzoyl) hydrazinecarbothioyl] amino} sulfanide [14]:

Mixture of 1mmol (0.68g) of 4-nitrobenzhydrazide, 3 mmol (0.68g) of ethanolic potassium hydroxide solution were stirred for 15 hours constantly at 10 - 15°C and Carbon disulfide was gradually added with stirring. After stirring for 15 hours, anhydrous ether was introduced to induce precipitation of the potassium salt of 4-nitrobenzhydrazide. This precipitate was subsequently rinsed with anhydrous ether, dried, and filtered using a vacuum filtration system.

Conventional approach for the synthesis of 4-amino-5-(4-nitrophenyl)-4H-1,2,4- triazole-3-thiol [15]:

To potassium salt of 4-nitrobenhydrazide, 1 mmol (0.27g) in water (5ml) and hydrazine hydrate 3 mmol (0.4ml) was incorporated and refluxed for 6-8hrs with occasional shaking. Following the conclusion of the reaction, the solution was gradually cooled to match room temperature. Subsequently, 100 ml of water were introduced to the mixture in order to achieve the desired level of dilution. It was further acidified with concentrated HCl which precipitated out the required triazole, filtered and recrystallized with ethanol.

Conventional approach for the synthesis of 4-amino-5-(4-aminophenyl)-4H-1,2,4- triazole-3-thiol [16]:

To a round bottom flask set up with reflux condenser 0.09 mol i.e., 1.5 g of p-nitro-5-(4-aminophenyl)-4H-1,2,4-triazole-3-thiol and 0.295 mol which is 3.5 g of powdered tin and 7.5 ml of concentrated hydrochloric acid was introduced and this mixture was subjected to gentle heating for 20 mins with frequent shaking in order to solubilize insoluble material to get the clear solution, further it was cooled and the liquid was decanted, tin was again washed with 15 ml of water and the washed water was added to the decanted solution. To this concentrated ammonia solution was introduced until solution became alkaline to litmus. Then it was kept on water bath for 5 mins to digest the suspension. To the above solution 2.5g of Bentonite was added, stirred and filtered by vacuum filtration. It was washed with hot water and the cake was transferred to a beaker and heated on water bath with 50 ml of water and re-filtered combining both filtrates was heated to concentrate, solid which separates out were filtered off and removed which is further acidified with glacial acetic acid until the liquid turns blue litmus to red and it was evaporated on a water bath until crystals commence to separate cooled on ice bath, and crystals are filtered using the pump and dried in the steam oven.

Conventional approach for the synthesis of Aromatic acid and aldehyde derivatives of 4-amino-5-(4-aminophenyl)-4H-1,2,4-triazole-3-thiol [17]:

Equimolar quantity of 4-amino-5-(4-aminophenyl)-4H-1,2,4-triazole-3-thiol and the respective aromatic acid was taken in a beaker and mixed thoroughly. To the mixture 2 drops of acetic acid was added and sufficient quantity of dimethyl formamide was introduced and the mixture was agitated at ambient temperature for 4 hours. After completion of the reaction, the reacted mixture was heated until it started boiling, then hot ethanol was added in order to dissolve the catalyst and filtered off to obtain a crystallized product. The crystallized product was recrystallized with ethanol.

Antibacterial Activity:

The antibacterial efficacy was evaluated using the Disc Diffusion Method, where nutrient agar petri plates were prepared and inoculated with four distinct bacterial strains for each sample. These plates were divided into six equal sections, each with six wells created using a cork borer. The wells were filled with various agents: one with the solvent (DMSO), another with the respective standard, and the remaining with samples of increasing concentrations. After refrigerating the plates for thirty minutes to aid in sample diffusion, they were then incubated for 24 hours at 37 °C. After incubation, the diameter of the inhibition zone of each sample was measured and the average values were calculated and recorded [18].

Cytotoxicity Studies:

The *in-vitro* cytotoxicity of different concentration of synthesized hybrid compounds was evaluated using the MTT assay against selected cell lines [19]. This procedure is based upon the principle of MTT uptake of cells which are metabolically active, subsequently metabolized by the active mitochondria. It turns into a blue-colored formazan product, which is then quantified spectrophotometrically. In brief, bacterial cells were seeded into 96-welled culture plates at 37 °C within a CO_2 incubator, exposing them to varying concentrations of synthetic hybrid analogues spanning from 0.8 to 100 µg/ml. Relevant controls were maintained in triplicate wells. Following a 72-hour incubation period, the assay was terminated by the addition of 25 ml of MTT solution (5 mg/ml) into each well.

The IC_{50} values, indicating of the concentration at which a compound shows half-maximal inhibitory effects, were derived via nonlinear regression analysis using Prism software version 4.01[20]. The synthesized compounds were obtained through the respective synthetic steps as illustrated in Fig.1.

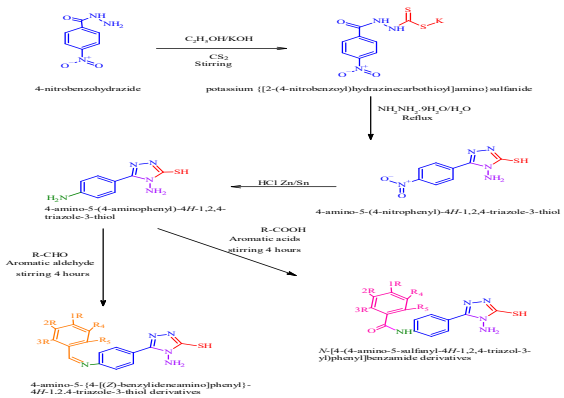


Fig. 1: Scheme of the synthesis

RESULTS AND DISCUSSION:

Table 1: Docking Results

Compound Code	Docking score (kcal/mol) (4Y6I)	Interacted residue and atoms.	Docking score (kcal/mol) (4JEO)	Interacted residue and atoms.
TA01	-9.1	ALAF33, LYSF35, SERF110, ALAF109, TYRE51, TYRB60	-8.1	THRA183, VALA238, TYRA236, ASPA237, ALAA120, GLNA58, PROA26, GLYA61, LYSA181, THRA165
TA02	-9.2	SERF110, ALAF109, ALAF33, TYRE51, VALB61, GLYB45,	-8.8	ASNB263, ASNA233, ASNA239, ALAB258, PROA124, THRB255
TA03	-9.2	SERF110, ALAF109, TYRE51, LYSF35, GLYB45	-8.1	TYRA230, GLNA242, ASNB259, ALAB258, LYSB199, PROA124, THRB225, ASNB263
TA04	-9.5	SERF110, LYSF35, LEUF32, LEUE56, GLUB2, VALB61, TYRE51, ALAF33	-8.4	ASNB263, PROA124, ALAB258, LYSB199, ASNB259
TA05	-9.2	ALAF109, TYRE51, LYSF35, ALAF33	-8.1	TYRA230, GLNA242, ASNB259, ALAB258, LYSB199, PROA124, ASNB263, THRB255
TA06	-9.0	ALFF105, LYSF35, TYRE51	-7.9	GLNA58, GYLA61,THRA165, ASPA237, PROA26, ALAA27, LEUA62, ALAA120
TA07	-9.3	TYRE51, ASYB20, VALB61, ALAF33, LYSF35, ALAF109, ARGF112	-8.2	ASPB265, ASPA121, ALAB258, PROA124, LYSB199, ASNB263
TA08	-9.3	PROB19, GLYE54 ASPB20, TRPF106, SERF110, TRYE51, ALAF35, LYSF35,	-8.4	ALAB258, ASNB259, THRB255, GLYB257, ASNB263

		GLU B21		
TA09	-9.2	SERF 110, ALA F109, GLY B45, ALA F33, TRY E51, LYSF 35	-8.6	ALAB258, LYSB199, PROA124, GLNA242, TRYA230, ASPA121, ASNA233, THRB255, GLYB257
TA10	-8.9	ALA F33, ASPB 20, LYSF 35, TYR E51, ARG F112, ALA F109	-8.6	ASPA237, ASPA121, ASNA239, GLNA242, TYRA230, LYSB199, PROA124

Table 2: Physicochemical Characterization of Synthesized Compounds

Compound Code	Compound Name	Molecular Formula	MW (g/mol)	M.P. (°C)	Yield (%)
TA01	N-[4-(4-amino-5-mercapto-1,2,4-triazol-3-yl) phenyl]-3-methoxybenzamide	$C_{16}H_{15}N_5O_2S$	341	196±2	80
TA02	N-[4-(4-amino-5-mercapto-1,2,4-triazol-3-yl)phenyl]-4-nitrobenzamide	$C_{15}H_{12}N_6O_3S$	356	198±2	74
TA03	N-[4-(4-amino-5-mercapto-1,2,4-triazol-3-yl) phenyl]-4-hydroxybenzamide	$C_{15}H_{13}N_5O_2S$	311	196±2	76
TA04	2-amino-N-[4-(4-amino-5-mercapto-1,2,4-triazol-3-yl)phenyl]-4-chlorobenzamide	$C_{13}H_{13}ClN_6OS$	360	200±2	90
TA05	4-[(E)-{[4-(4-amino-5-mercapto-1,2,4-triazol-3-yl)phenyl]imino}methyl]phenol	$C_{15}H_{13}N_5OS$	311	198±2	76
TA06	N-[4-(4-amino-5-mercapto-1,2,4-triazol-3-yl) phenyl]-2-hydroxybenzamide	$C_{15}H_{13}N_5O_2S$	327	198±2	78
TA07	N-[4-(4-amino-5-mercapto-1,2,4-triazol-3-yl) phenyl]-3-hydroxybenzamide	$C_{15}H_{13}N_5O_2S$	327	198±2	78
TA08	N-[4-(4-amino-5-mercapto-1,2,4-triazol-3-yl)phenyl]-2,3-dihydroxybenzamide	$C_{15}H_{13}N_5O_3S$	343	196±2	81
TA09	N-[4-(4-amino-5-mercapto-1,2,4-triazol-3-yl) phenyl]-2,4,5-trihydroxybenzamide	$C_{15}H_{13}N_5O_4S$	359	198±2	76
TA10	N-[4-(4-amino-5-mercapto-1,2,4-triazol-3-yl)phenyl]benzamide	$C_{15}H_{13}N_5OS$	311	196±2	74

The synthesized 1,2,4-triazole derivatives were evaluated through molecular docking, physicochemical and spectral characterization, antibacterial activity, cytotoxicity assessment, and *in-silico* toxicity prediction. Molecular docking

studies against the selected target proteins 4Y6I and 4JE0 demonstrated favorable binding affinities for all ten derivatives, with docking scores ranging from -8.9 to -9.5 kcal/mol against 4Y6I and -7.9 to -8.8 kcal/mol against 4JE0 (Table 1). Among the synthesized compounds, TA04 exhibited the most favorable binding affinity toward 4Y6I, with a docking score of -9.5 kcal/mol, and showed interactions with residues including SERF110, LEUF32, VALB61, GLUB21, TYRE51, and ALAF33, indicating favorable stabilization within the binding pocket (Fig. 2). The physicochemical characterization of the synthesized derivatives revealed molecular weights ranging from 311 to 360 g/mol, melting points ranging from 196 ± 2ºC to 200 ± 2ºC, and percentage yields ranging from 74% to 90%, with TA04 showing the highest yield of 90% and the highest melting point of 200 ± 2ºC (Table 2). Structural characterization by IR, ^{1}HNMR, and mass spectrometry further supported the formation of the proposed derivatives, with characteristic functional group absorption bands, appropriate proton signals, and observed molecular masses corresponding closely to the calculated values (Table 3). The antibacterial activity of the synthesized compounds was evaluated against *Escherichia coli, Pseudomonas aeruginosa, Micrococcus luteus,* and *Staphylococcus aureus,* using ofloxacin as the reference drug. variable antibacterial potency among the synthesized compounds, with reported MIC values ranging from 0.2 to 1.0 µg/mL (Fig. 4). The selectivity index values also varied among the synthesized derivatives, indicating differences in their relative antibacterial activity and cytotoxic response (Fig. 5). Cytotoxicity evaluation demonstrated IC_{50} values ranging from 1.42 to 5.20 µg/mL, with TA04 showing an IC_{50} value of 1.42 µg/mL (Fig. 6). In addition, the *in-silico* toxicity assessment evaluated parameters indicating mutagenicity, tumorigenicity, irritation, and reproductive effects, revealing variable predicted toxicity profiles among the synthesized derivatives (Fig. 7). Overall, the result indicates that the synthesized 1,2,4-triazole derivatives possess promising biological activity, with TA04 emerging as the most prominent derivative based on its favorable docking affinity, highest synthetic yield, and notable antibacterial activity against *P.aeruginosa* (Tables 1-3; Figs. 2-7).

Table 3: Spectral Data of synthesized compounds (IR, 1H NMR, and MASS)

Sl No.	Compound Name	Key IR Peaks (υ/cm-1)	^{1}H NMR (500 MHz, CH Cl3- d) δ ppm	Mass (m/z) [M+H^{+}]
a	Methoxy benzamide derivative ($C_{16}H_{15}N_5O_2S$)	3075-3062cm^{-1} NH_2 Stretching, 1682cm^{-1} -C=O- amides Stretching, 1603cm^{-1} C=C in ring Stretching, 1581cm^{-1} -NH (20amide) Bending	8.50 (s, 1H), 7.88 – 7.83 (m, 2H), 7.57 – 7.51 (m, 2H), 7.44 – 7.38 (m, 2H), 6.94 – 6.88 (m, 2H), 5.67 (s, 2H), 5.22 (s, 1H)	Calculated 342.10; Found 341.08
b	Nitro benzamide derivative ($C_{15}H_{12}N_6O_3S$)	3080-3062cm^{-1} NH_2Streching, 1689cm^{-1} -C=O- amides Streching, 1605cm^{-1} C=C in ring Stretching, 1539cm^{-1} -NH (20amide) Bending	9.35 (s, 1H), 8.07 – 8.01 (m, 2H), 7.82 (d, J = 8.7 Hz, 1H), 7.72 – 7.66 (m, 2H), 7.26 (s, 0H), 7.12 (dd, J = 8.7, 2.1 Hz, 1H), 6.78	Calculated 357.076; Found 356.06

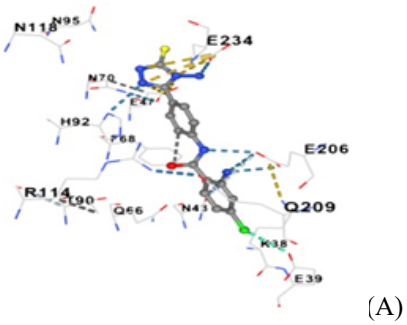
			(s, 2H), 5.67 (s, 2H), 5.22 (s, 1H)	
c	4-Hydroxy benzamide derivative ($C_{15}H_{13}N_5O_2S$)	3441-3015cm^{-1} NH_2 stretching, 1661cm^{-1} - C=O-amides stretching 1610cm^{-1} C=C in ring stretching, 1585cm^{-1} - NH (20amide) Bending	8.50 (s, 1H), 7.88 – 7.83 (m, 2H), 7.57 – 7.51 (m, 2H), 7.44 – 7.38 (m, 2H), 6.94 – 6.88 (m, 2H), 5.67 (s, 2H), 5.22 (s, 1H)	Calculated 312.09; Found 311.07
d	Chloro benzamide derivative ($C_{13}H_{13}ClN_6OS$)	757 (C–Cl), 1687 (C757cm^{-1} C-Cl Bending, 1687 cm^{-1} - C=O amides Stretching, 1633cm^{-1} C=C in ring Stretching, 1574 cm-1 - NH (20amide) Bending, 3280NH_2 Stretching cm^{-1}	9.35 (s, 1H), 8.07 – 8.01 (m, 2H), 7.82 (d, J = 8.7 Hz, 1H), 7.72 – 7.66 (m, 2H), 7.26 (s, 0H), 7.12 (dd,	Calculated 361.06; Found 360.05

			J = 8.7, 2.1 Hz, 1H), 6.78(s, 2H), 5.67 (s, 2H), 5.22 (s, 1H)	
e	Schiff base phenol derivative ($C_{15}H_{13}N_5OS$)	3387cm^{-1}- OH Stretching, 1673cm^{-1} C=N Stretching, 1656cm^{-1} C=C in ring Stretching, 3387cm^{-1} - NH_2 Stretching, 2341cm^{-1} - SH Stretching	8.50 (s, 1H), 7.88 – 7.83 (m, 2H), 7.57 – 7.51 (m, 2H), 7.44 – 7.38 (m, 2H), 6.94 – 6.88 (m, 2H), 5.67 (s, 2H), 5.22 (s, 1H)	Calculated 312.09; Found 311.07
f	2-Hydroxy benzamide derivative ($C_{15}H_{13}N_5O_2S$)	3508cm^{-1} - OH Stretching, 1741cm^{-1} C=O amide Stretching, 1597cm^{-1} C=C in ring Stretching, 3308cm^{-1} - NH_2 Stretching, 2483cm^{-1} - SH Stretching, 1568cm^{-1} -	9.72 (s, 1H), 8.07 – 8.01 (m, 2H), 7.95 (dd, J = 8.0, 1.6 Hz, 1H), 7.72 –	Calculated 328.08; Found 327.07

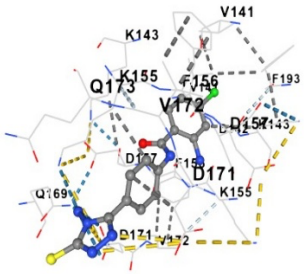
		NH (20 Bending)	7.66 (m, 2H), 7.45 – 7.38 (m, 1H), 7.03 – 6.94 (m, 2H), 5.67 (s, 2H), 5.22 (s, 1H)	
g	3-Hydroxy benzamide derivative ($C_{15}H_{13}N_5O_2S$)	3508cm^{-1} - OH Stretching, 1708cm^{-1} C=O amide Stretching, 1596cm^{-1} C=C in ring Stretching, 3318cm^{-1} - NH_2 Stretching,2489cm^{-1}-SH Stretching, 1569cm^{-1} - NH (20 Bending)	8.69 (s, 1H), 8.51 (s, 1H), 8.07 – 8.01 (m, 2H), 7.73 – 7.67 (m, 2H), 7.45 (ddd, J = 7.9, 2.1, 1.2 Hz, 1H), 7.37 – 7.30 (m, 1H), 6.96 (ddd, J = 8.4, 2.1, 1.1 Hz, 1H), 5.67 (s, 2H), 5.22	Calculated 328.08; Found 327.07

			(s, 1H)	
h	Dihydroxy benzamide derivative ($C_{15}H_{13}N_5O_3S$)	3553cm^{-1} and 3485cm^{-1} - OH Stretching, 1738cm^{-1} C=O amide Stretching, 1592cm^{-1} C=C in ring Stretching, 3309cm^{-1} - NH_2 Stretching, 2480cm^{-1} - SH Stretching, 1568cm^{-1} - NH (20 Bending)	9.01 (s, 1H), 8.07 – 8.01 (m, 2H), 7.72 – 7.66 (m, 2H), 7.38 – 7.31 (m, 1H), 6.90 – 6.82 (m, 2H), 5.67 (s, 2H), 5.22 (s, 1H)	Calculated 344.08; Found 343.39
i	Trihydroxy benzamide derivative ($C_{15}H_{13}N_5O_4S$)	3553cm^{-1}, 3515cm^{-1} and 3480cm^{-1} - OH Stretching, 1739cm^{-1} C=O amide Stretching, 1592cm^{-1} C=C in ring Stretching, 3308cm^{-1}- NH_2 Stretching, 2482cm^{-1} - SH Stretching, 1566cm^{-1} - NH (20 Bending)	8.33 (s, 1H), 8.07 – 8.01 (m, 2H), 7.72 – 7.66 (m, 2H), 6.53 (s, 1H), 5.67 (s, 2H), 5.22 (s, 1H)	Calculated 360.07; Found 359.06
J	Benzamide derivative ($C_{15}H_{13}N_5OS$)	1725cm^{-1} C=O amide Stretching, 1596cm^{-1} C=C in ring Stretching, 3324cm^{-1} -	8.72 (s, 1H), 8.07 – 8.01 (m,	Calculated 312.09; Found 311.08

		NH_2 Stretching, 2507cm^{-1} - SH Stretching, 1570cm^{-1} - NH (20 Bending)	2H), 7.95 (dt, J = 7.8, 1.1 Hz, 2H), 7.73 – 7.67 (m, 2H), 7.54 – 7.40 (m, 3H), 5.67 (s, 2H), 5.22 (s, 1H)	



(A)



(B)

Fig.2: 2D interaction of ligand TA04 with protein 4Y6I(A) and 4JE0(B)

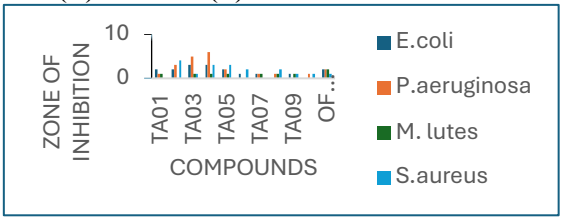


Fig. 3: Antibacterial efficacy of the synthesized compounds-Diameter of Zone of Inhibition (in mm)

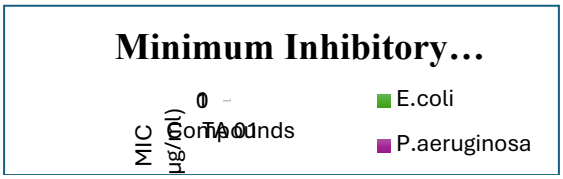


Fig. 4: Results of Cytotoxicity studies- Minimum Inhibitory Concentration (MIC)

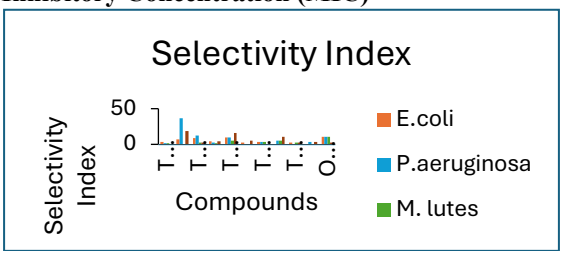


Fig. 5: Results of Cytotoxicity studies- Selectivity Index

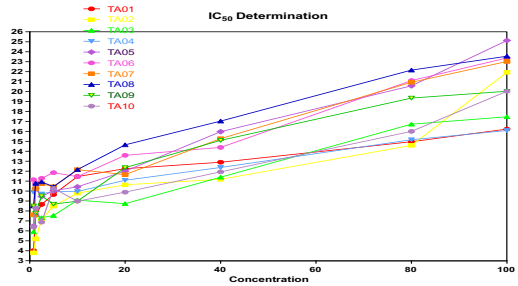


Fig. 6: Results of Cytotoxicity studies- IC50 Determination

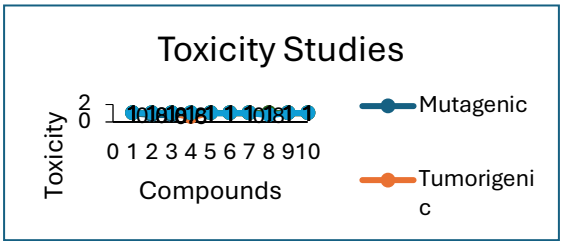


Fig. 7: Toxicity studies of the synthesized compound

CONCLUSION

A comprehensive analysis of various conducted studies reveals that amino phenyl-containing 1,2,4-triazoles, along with select aromatic acids and aldehyde derivatives, exhibited promising binding energies. A total of Ten compounds were synthesized, characterized, and evaluated for antibacterial and cytotoxicity activities. Compound TA04 exhibited the highest docking score and strong binding affinity toward the target protein (PDB ID: 4Y6I), indicating its potential as an effective antibacterial agent against Gram-negative bacteria compared with the other synthesized compounds. These findings suggest that amino phenyl-containing 1,2,4-triazole nucleoside derivatives, particularly TA04, represent a promising class of target-specific antibacterial agents with improved efficacy and the potential to minimize adverse effects.

ACKNOWLEDGEMENT

We express our sincere gratitude to the principal and management of Krupanidhi College of Pharmacy, Bengaluru, for their encouragement and invaluable support throughout this endeavor. Additionally, we would like to extend our appreciation to SAIF Punjab for their assistance with IR and NMR analyses, VIT for their contribution to MASS spectroscopy, and CDRI-CSIR Lucknow for their collaboration on antibacterial activity and cytotoxicity studies. Their expertise and assistance have been instrumental in the successful execution of this research.

REFERENCES

1. Figueroa LP, Santos NA, Santiago PH, Romão W, Lacerda Junior V, Ellena J, Micheletti AC, Borges WS. Synthesis and evaluation of antibacterial activity of 1,2,3-triazole and ether derivatives of phenol. Journal of the Brazilian Chemical Society. 2023;34:25-35.
2. El Malah T, Farag H, Hemdan BA, Mageid RE, Abdelrahman MT, El-Manawaty MA, Nour HF. Synthesis, in vitro antimicrobial evaluation, and molecular docking studies of new isatin-1,2,3-triazole hybrids. Journal of Molecular Structure. 2022;1250:131855.
3. Otto M. Critical assessment of the prospects of quorum-quenching therapy for Staphylococcus aureus infection. International Journal of Molecular Sciences. 2023;24(4):4025. DOI: 10.3390/ijms24044025.
4. Zhu M, Zhu Q, Yang Z, Liang Z. Clinical characteristics of patients with Micrococcus luteus bloodstream infection in a Chinese tertiary-care hospital. Polish Journal of Microbiology. 2021;70(3):321-327.
5. Andrade L, Chique C, Hynds P, Weatherill J, O'Dwyer J. Antimicrobial resistance profiles of Escherichia coli and Pseudomonas aeruginosa from private groundwater wells. Environmental Pollution. 2023;317:120817.
6. Tian G, Song Q, Liu Z, Guo J, Cao S, Long S. Advances in triazole hybrids as antimicrobials and their SAR. European Journal of Medicinal Chemistry. 2023;259:115603.
7. Matin MM, Matin P, Rahman MR, Ben Hadda T, Almalki FA, Mahmud S, Ghoneim MM, Alruwaily M, Alshehri S. Triazoles and their derivatives: Chemistry, synthesis, and therapeutic applications. Frontiers in Molecular Biosciences. 2022;9:864286. DOI: 10.3389/fmolb.2022.864286.
8. Shireen S, Kumar BRP. Synthesis, characterization and antimicrobial evaluation of 3,5-disubstituted triazoles bearing 5-chloro-2-methylindole. Asian Journal of Pharmaceutical and Clinical Research. 2019;12(10):184-187.
9. Azim T, Wasim M, Akhtar MS, Akram I. In vivo evaluation of anti-inflammatory and analgesic activities of triazole derivatives. BMC Complementary Medicine and Therapies. 2021;21(1):1-5.
10. Viegas DJ, da Silva VD, Buarque CD, Bloom DC, Abreu PA. Antiviral activity of triazoles against HSV-1. Antiviral Therapy. 2020;25(8):399-410.
11. Kazeminejad Z, Marzi M, Shiroudi A, Kouhpayeh SA, Farjam M, Zarenezhad E. Novel 1,2,4-triazoles as antifungal agents. BioMed Research International. 2022;2022:4584846. DOI: 10.1155/2022/4584846.
12. Yang R, Zha X, Gao X, Wang K, Cheng B, Yan B. Virtual screening against p38α kinase using machine learning and docking. Heliyon. 2022;8(9). DOI: 10.1016/j.heliyon.2022.e10495.
13. Das K, Das P, Orfali R, Ramya A, Joseph A, Imran M, Asdaq SM, Alshehri S, Rabaan AA, Aljeldah M, AlShehail BM. Synthesis and anti-tubercular evaluation of oxadiazole derivatives. Journal of King Saud University - Science. 2023;35(6):102737.
14. Jalihal PC, Rajoriya V, Kashaw V. Design, synthesis and evaluation of new derivatives of 1,2,4-triazoles for antimicrobial and anti-inflammatory activity. International Journal of Current Pharmaceutical Research. 2018;10(4):29-35.
15. Vogel A. Vogel's Textbook of Practical Organic Chemistry. 5th ed. Harlow: Pearson India; 1989. p. 896-897.
16. Abdelli A, Azzouni S, Plais R, Gaucher A, Efrit ML, Prim D. Advances in the chemistry of 1,2,4-triazoles. Tetrahedron Letters. 2021;86:153518. DOI: 10.1016/j.tetlet.2021.153518.
17. Das P, Lal VK, Saxena V, Himaja M. Synthesis of imidazolyl benzoyl amino acids. Indian Journal of Heterocyclic Chemistry. 2007;17(1):71-74.
18. Das M, Das B, De A, Banerjee S, Samanta A. Antimicrobial investigation and binding mode analysis of newly synthesized 4-amino-5-[(aryl-substituted)-4H-1,2,4-triazole-3-yl] thio-linked hydroxamic acid derivatives. Asian Journal of Pharmaceutical and Clinical Research. 2019;12(3):469-476.
19. Ghasemi M, Turnbull T, Sebastian S, Kempson I. The MTT assay: Utility and limitations. International Journal of

Molecular Sciences. 2021;22(23):12827. DOI: 10.3390/ijms222312827.

20. Cho K, Choi ES, Kim JH, Son JW, Kim E. Deep learning to calculate IC50 from cell images. Scientific Reports. 2022;12(1):6610. DOI: 10.1038/s41598-022-10531-3.