RESEARCH PAPER

Development of Novel 1,2,4-Triazole Derivatives: Synthesis, In Silico Evaluation, and Biological Activity Studies

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ABSTRACT

The present study describes the synthesis, characterization, molecular docking, toxicity prediction, and biological evaluation of novel substituted 1,2,4-triazole derivative with potential anti-inflammatory and antimicrobial activity. The compound were synthesized by both conventional reflux and microwave assisted methods, where the microwave approach demonstrated improved reaction efficiency, reduced reaction time, and higher product yield (78-95%) compared to the conventional method (55-70%). The synthesized derivative were structurally characterized using 1HNMR and mass spectroscopic techniques, confirming the successful formation of the triazole scaffold and the presence of characteristic functional groups.

Molecular docking studies were carried out against cyclooxygenase protein (6COX) for anti-inflammatory activity and antibacterial target protein (1TH4) for for antimicrobial activity using AutoDock vina. Comparable to the standard drug celecoxib, while compound e showed the strongest interaction against 1TH4 (-8.6kcal/mol), exceeding that of amoxicillin. In silico toxicity prediction indicated acceptable safety profile for most compounds particularly compound b and e which demonstrated inactive cardiotoxicity and nepgotoxicity profile with moderate LD50 values.

Biological evaluation revealed that compound e exhibited promising concentration dependent anti-inflammatory activity in the protein denaturation assay, showing 80.62% inhibition at 1000µg/ml, comparable to diclofenac sodium. However, antimicrobial study against staphylococcus aureus demonstrate only weak antibacterial activity. Overall, the finding suggest that substituted 1,2,4-triazole derivative possess significant anti-inflammatory potential and may serve as promising lead molecules for further structural optimization and pharmacological development.

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INTRODUCTION

1,2,4-Triazole derivatives have emerged as an important class of nitrogen-containing heterocyclic compounds in medicinal chemistry due to their broad spectrum of biological activities. The 1,2,4-triazole ring system is widely recognized as a privileged scaffold present in numerous therapeutically active molecules, exhibiting antifungal, antibacterial, anti-inflammatory, anticancer, antiviral, and antidiabetic properties. Their structural versatility, stability, and ability to act as bioisosteres of functional groups such as amides and esters make them highly suitable for drug design and development(1).

The biological significance of 1,2,4-triazoles is largely attributed to their unique electronic properties and capacity to form hydrogen bonds and other noncovalent interactions with biological targets, including enzymes, receptors, and nucleic acids. These interactions enable triazole derivatives to modulate key biochemical pathways. For instance, many triazole-based compounds exhibit antimicrobial activity by inhibiting essential enzymes such as lanosterol 14α-demethylase (CYP51), which plays a critical role in fungal ergosterol biosynthesis. Similarly, their anti-inflammatory effects are associated with the inhibition of enzymes such as cyclooxygenase (COX) and lipoxygenase (LOX), along with the suppression of pro-inflammatory mediators(2,3).

The growing prevalence of antimicrobial resistance (AMR) and the increasing incidence of inflammatory and infectious diseases have intensified the demand for new therapeutic agents. In this context, 1,2,4-triazole

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**Author for Correspondence:Pankaj Shankar Kore* derivatives have gained considerable attention due to their potent activity, favorable pharmacokinetic properties, and relatively low toxicity. Structural modifications, including the incorporation of functional groups such as amides, Schiff bases, and heterocyclic moieties, have been shown to enhance their biological efficacy and selectivity.

Recent advances in drug discovery have highlighted the importance of computational approaches in accelerating the development of new bioactive molecules. In silico techniques such as molecular docking, quantitative structure-activity relationship (QSAR) modeling, pharmacophore mapping, and ADMET prediction play a crucial role in predicting biological activity, optimizing lead compounds, and reducing the cost and time associated with experimental studies(4,5).

Therefore, the present study focuses on the design and synthesis of novel 1,2,4-triazole derivatives with potential antimicrobial and anti-inflammatory activities, supported by computational and experimental evaluation. This approach aims to contribute to the development of safer and more effective therapeutic agents to address current challenges associated with drug resistance and inflammation(6).

Materials and methods

All required chemicals and solvents were sourced from standard commercial suppliers. Before use, they were purified and dried according to established procedures available in the literature. The reagents were procured from research laboratories located in Islampur and Loba Research Lab Mumbai, and made available through the store section of Rajarambapu College of Pharmacy, Kasegaon.

A. Synthesis of 1,2,4 triazole derivatives by conventional method

The synthesis of substituted 1,2,4-triazole derivatives was carried out by the conventional reflux method through a three-step reaction sequence. In the first step, substituted aroyl hydrazide acids were synthesized by refluxing 0.02 mole of aromatic acid with 0.04 mole of hydrazine hydrate in 0.2 mole ethanol in the presence of a few drops of concentrated sulfuric acid as a catalyst for 4-5 hours. In the second step, the obtained hydrazides were reacted with 0.04 mole of thiourea and 0.04 mole of sodium hydroxide in 10 mL of water under reflux for 2-4 hours to produce aroyl-amino-guanidines, and the progress of the reaction was monitored by thin-layer chromatography (TLC). In the final step, 0.02 mole of arylaminoguanidine was refluxed in 10 mL of water for 3-5 hours for cyclization to form 5-amino-3-aryl-1,2,4-triazoles. After completion of the reaction as confirmed by TLC, the reaction mixture was cooled, and the precipitated triazole derivatives were filtered, washed, and dried to obtain the final product(7).

B. Synthesis of 1,2,4-triazole derivatives by microwave method

The synthesis of substituted 1,2,4-triazole derivatives was carried out using a microwave-assisted method through a three-step reaction sequence. In the first step, substituted aroyl hydrazide acids were synthesized by reacting 0.01 mole of aromatic acid with 0.02 mole of hydrazine hydrate in 0.1 mole of ethanol in the presence of a few drops of concentrated sulfuric acid as a catalyst. The reaction mixture was subjected to microwave irradiation under reflux conditions at 340 W for 20-25 minutes. The progress of the reaction was monitored by TLC, and after completion, the precipitated solid was collected, dried, and recrystallized from ethanol. In the second step, substituted hydrazide (0.01 mole) was reacted with thiourea (0.76 g) in 10 mL of distilled water to obtain a uniform suspension, which was irradiated in a microwave reactor at 300-400 W and 80-90 °C for 5-7 minutes. The reaction progress was monitored using TLC with ethyl acetate : hexane (7:3) as the mobile phase. After completion, the reaction mixture was cooled to room temperature, and the product was washed with cold ethanol. In the final step, 0.01 mole of substituted amino-guanidine was mixed with 10 mL of distilled water and irradiated in a microwave reactor at 400-500 W and 100-110 °C for 6-10 minutes. The reaction progress was monitored by TLC, and upon completion, the mixture was cooled to allow separation of the solid product. The obtained product was filtered, washed with cold water, recrystallized using ethanol, and dried under vacuum to obtain the final substituted 5-amino-3-aryl-1,2,4-triazole derivatives(8,9).

Characterization of 1,2,4-triazole derivatives

Fourier Transform infrared (FTIR):-The synthesized substituted 1,2,4-triazole derivative were characterized by FTIR spectroscopy for identification of characteristic functional groups and structural confirmation. FTIR spectra were recorded using a shimadzu FTIR spectrophotometer in the range of 4000-400cm^{-1} employing the potassium bromide pellet method.

NMR Spectra :- the structural elucidation of the synthesized compound was further carried out using proton nuclear magnetic resonance (H1 NMR) spectroscopy. The spectra were recorded on brukeravance NMR spectrometer operating at 400 MHz using Dimethyl sulfoxide-d6 (DMSO-d6) as the solvent and tetramethylsilane (TMS) as the internal reference standard. Chemical shift were expressed in parts per million (ppm) the H1 NMR spectra were interpreted on the basis of chemical shift values, multiplicity, coupling patterns, and integration of proton signals. Characteristic signals corresponding to aromatic protons, amino protons, triazole N-H protons and other substituent groups were analyzed to confirm the molecular structure of the synthesized derivatives. Residual solvent peaks and exchangeable proton signals were also identified during spectral interpretation.

Mass spectroscopy:- Mass spectrometric analysis of the synthesized substituted 1,2,4-triazole derivatives was performed to determine their molecular weights and fragmentation patterns. The spectra were recorded using an LC-MS/MS mass spectrometer equipped with an electrospray ionization source operating in positive ion mode.

Molecular Docking studies:-Molecular docking studies were performed using Autodock vina integrated with PyRx software. Thecrystal structures of cyclooxygenase Protein (6COX) and antibacterial target protein (1TH4) were retrieved from the Protein Data Bank. Protein preparation involved removal of water molecules and addition of hydrogen atoms. Ligands were energy minimized before docking. Binding affinity were expressed in kcal/mol(10,11).

Toxicity Prediction :- Toxicity prediction was performed using the ProTox-II online server. Hepatotoxicity, neurotoxicity, nephrotoxicity, respiratory toxicity, cardiotoxicity, and LD50 values were evaluated for all synthesized compounds(12).

Anti-inflammatory:-The in vitro anti-inflammatory activity of compounds was evaluated using the protein denaturation assay. Briefly, 0.5 ml of test sample at different concentration (200/1000µg/ml) was mixed with 0.5ml of 1% bovine serum albumin prepared in phosphate buffer (pH6.4). Diclofenac sodium was used as the reference standard. The reaction mixture were incubated at 37^0C for 20min followed by heating at 70^0C for 10 min to induce protein denaturation. Aftercooling to room temperature, absorbance was measured at 660nm using a UV-Visible spectrophotometer. Percentage inhibition of protein denaturation was calculated relative to the control(13,14).

Antimicrobial Activity:-The antimicrobial activity of compounds was evaluated against *staphylococcus aureus* ATCC6538using the agar well diffusion method. Sterile antibiotic assay medium no.19 was incubated with the microbial suspension and poured into sterile petri plates. Well of 6mm diameter were prepared aseptically and 100µl of the test and standard solution were introduced into the respective well. Streptomycin (1mg/ml) was used as the standard drug, while DMSO served as the negative control. The plates were incubated at 30-35^0C for 24-48 h, and the antibacterial activity was determined by measuring the diameter of the zone of inhibition in millimetres(15).

Result and discussion:-

Physicochemical Properties:- The synthesized substituted 1,2,4-triazole derivative were obtained with moderate to excellent yields. Microwave assisted synthesis resulted in higher percentage yield (78-95%) compared to the conventional reflux method (55-70%) as shown in table 1 indicating improved reaction efficiency and reduced reaction time

Table 1:- The physical data of substituted aroyl hydrazide acid By Conventional and microwave method.

Sr. no.	Compound code	Molecular formula	Molecular weight (g/mol)	M.P ^{0}C	Percentage yield %		Rf value	Mobile phase
					Conven-tional	Micro-wave		
1	a	C8H7CLN4	194.62	198-202	65-70	88-92	0.70	Ethyl acetate: N-hexane
2	b	C8H5N604	250.17	225-230	63-69	86-92	0.76	Ethyl acetate: N-hexane
3	c	C8H6N5O2	175.19	212-216	61-67	83-89	0.75	Ethyl acetate: N-hexane
4	d	C8H8N5	188.23	205-209	57-62	80-87	0.68	Ethyl acetate: N-hexane
5	**e**	C8H6N5O2	205.17	212-216	65-70	87-90	0.84	Ethyl acetate: N-hexane
6	f	C10H9N4	205.17	182-186	55-60	78-84	0.74	Ethyl acetate: N-hexane
7	g	C8H7N40	240.24	200-204	58-64	81-86	0.76	Ethyl acetate: N-hexane
8	h	C8H8N4O3S	176.18	228-232	62-66	85-90	0.80	Ethyl acetate: N-hexane

FTIR spectral analysis :- FTIR spectral analysis confirmed the successful synthesis of substituted 1,2,4- triazole derivatives spectral of compound shown in figure 1 and 2 and their band value are shown in table 2 and 3. Characteristic absorption bands corresponding to N-H stretching vibration were observed in the region of 3300-3100 cm^{-1}. Aromatic C-H stretching vibration appeared near 3090 cm^{-1}, while C=N stretching associated with the triazole ring was observed around 1498 cm^{-1}. Nitro substituted compound exhibited characteristic asymmetric and symmetric NO_2 stretching vibration near 1542cm^{-1} and 1371 cm^{-1}, respectively. The presence of C-N and N-N stretching vibration further confirmed formation of the triazole ring system.

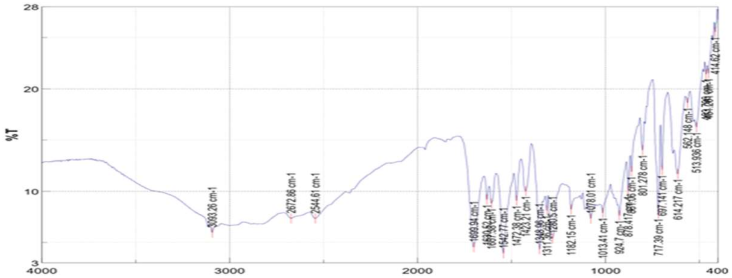


Figure 1 IR spectrum of 3-(3,5-dinitrophenyl)-1*H*-1,2,4-triazol-5-amine

Table 2 IR spectrum value of 3-(3,5-dinitrophenyl)-1*H*-1,2,4-triazol-5-amine

Sr no.	Wave number (cm^{-})	Vibration type	Functional group assignment
1	3300-3100(cm^{-})	N-H stretching	Primary amine
2	3093(cm^{-})	Aromatic C-H stretching	Benzene ring
3	2672(cm^{-})	N-H stretching weak	Triazole ring
4	2544(cm^{-})	Hydrogen bonded n-h	Amine ring
5	1600-1500(cm^{-})	C=C stretching	Aromatic ring
6	1542(cm^{-})	Asymmetric stretching	-NO2 group
7	1498(cm^{-})	C=N Stretching	Triazole ring
8	1423(cm^{-})	C-N stretching	Aromatic amine
9	1371(cm^{-})	Asymmetric stretching	-NO2 group
10	1317(cm^{-})	C-N stretching	Aromatic amine
11	1182(cm^{-})	C-N stretching	triazole
12	1078(cm^{-})	N-N stretching	Triazole ring
13	1013(cm^{-})	C-N stretching	amine
14	924(cm^{-})	C-H bending	Aromatic ring
15	717(cm^{-})	C-H out of plane bending	Substituted benzene
16	654(cm^{-})	N-O stretching	Nitro group
17	513(cm^{-})	Ring deformation	Aromatic ring

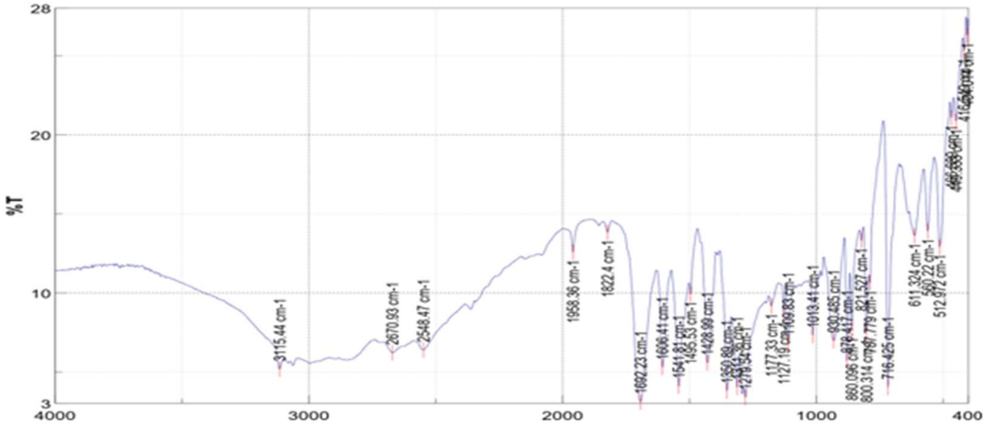


Figure 2. IR spectrum of 4-(5-amino-1*H*-1,2,4-triazol-3-yl)benzoic acid

Table 3. IR spectrum value of 4-(5-amino-1*H*-1,2,4-triazol-3-yl)benzoic acid

Sr no.	**Wave number (cm^{-})**	**Vibration type**	**Functional group assignment**
1	3400-3200	O-H/N-H Stretching	Amine/hydrogen bonding
2	3090-3000	Aromatic C-H stretching	Benzene rig
3	2920-2850	C-H stretching	Alkane ring
4	1650-1600	C=C stretching	Aromatic ring
5	1590-1500	C=N stretching	Heterocyclic ring
6	1540-1520	Asymmetric stretching	Nitro group
7	1470-1450	C-H bending	Aromatic ring
8	1375-1350	Symmetric stretching	Nitro group

9	1310-1250	C-N stretching	Aromatic amine
10	1200-1100	C-N stretching	Heterocyclic amine
11	1050-1000	N-N stretching	Triazole ring
12	900-700	C-H out of plane bending	Substituted benzene
13	650-500	N-O bending	Nitro group

1H NMR spectral analysis:-The 1H NMR spectra showed figure 3 and 4 and their value in table 4 and 5 characteristic chemical shift corresponding to triazole N-H, aromatic protons, and amino groups the triazole N-H proton appeared in the downfield region around 13.5-14.0 ppm due to hydrogen bonding. Aromatic protons were observed between 7.6-9.2 ppm depending on substituent effect, whereas amino protons appeared around 5.5-6.2 ppm

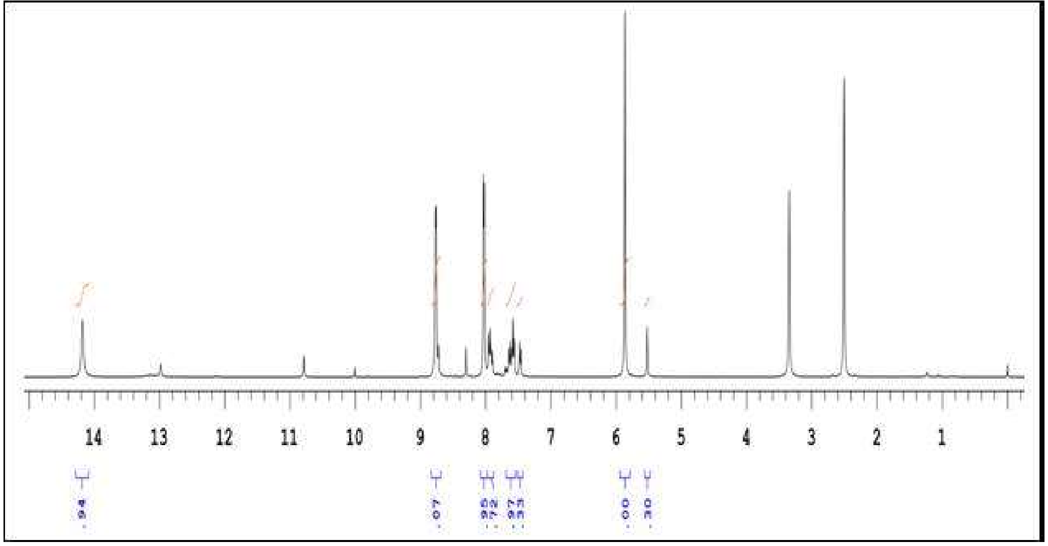


Figure 3. NMR spectrum of 3-(3,5-dinitrophenyl)-1*H*-1,2,4-triazol-5-amine

Table 4. NMR spectrum value for compound b

Sr no.	Chemical shift	Proton assignments
1	13.8-14.0	N-H (triazole)
2	10.5-11.0	N-H (secondary amine)
3	8.9-9.2	Aromatic proton (AR-H) near no2
4	8.4-8.7	Aromatic proton (AR-H)
5	8.0-8.3	Aromatic proton (AR-H)
6	7.6-7.9	Aromatic proton (AR-H)
7	5.8-6.2	-NH2 (primary amine)
8	2.5-2.6	DMSO solvent
9	3.3-3.5	Water impurity peak

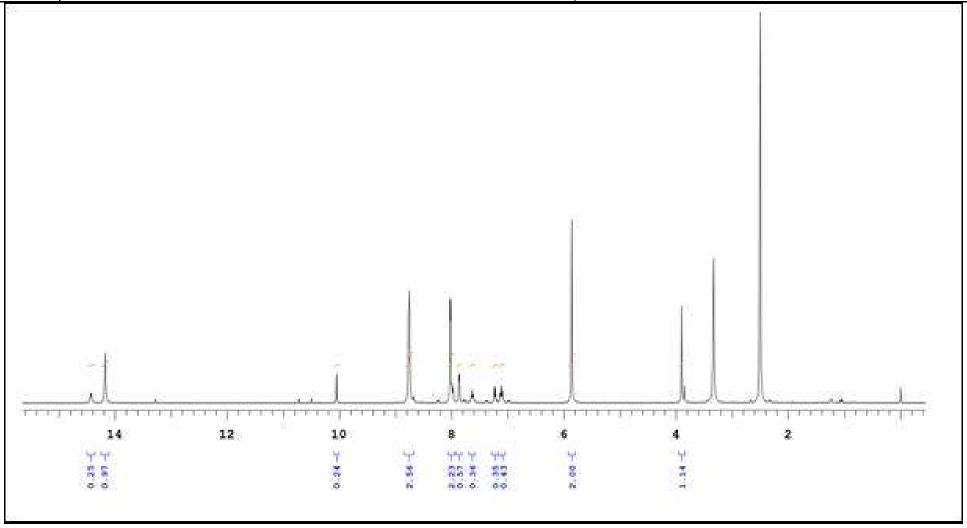


Figure 4. NMR spectrum of 4-(5-amino-1*H*-1,2,4-triazol-3-yl) benzoic acid

Table 5. NMR spectrum value for 4-(5-amino-1*H*-1,2,4-triazol-3-yl) benzoic acid

Sr no.	Chemical shift	Proton assignments
1	13.5- 14.0	COOH (carboxylic acid proton)
2	10.0-10.5	N-H (triazole ring)
3	8.0-8.3	Aromatic protons (H2,H6) Ortho to COOH
4	7.6-7.9	Aromatic protons (H3,H5)
5	5.5-6.0	NH2(primary amine)
6	2.5-2.6	DMSO- d6 solvent peak
7	3.3-3.5	Water impurity peak

Mass spectral analysis:-Mass spectral studies confirmed the molecular masses of synthesized derivatives protonated molecular ion peak corresponding to the expected molecular weight were observed in all compounds as shown in figure 5 and 6 and their value in table 6 and 7. Characteristic fragmentation patterns involving loss of NH2, NO2, and aromatic fragments supported the proposed structures.

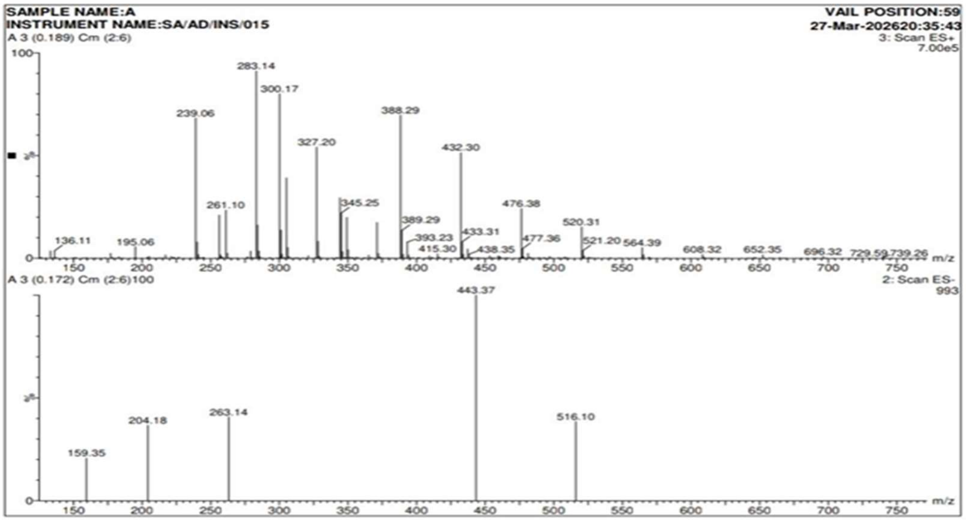


Figure 5. mass spectrum of 3-(3,5-dinitrophenyl)-1*H*-1,2,4-triazol-5-amine

Table 6 mass spectrum of 3-(3,5-dinitrophenyl)-1*H*-1,2,4-triazol-5-amine

Sr no.	Structural change	Structural formula of fragment	Actual m/z	Observed m/z
1	Molecular ion	$C_8H_6N_6O_4^+$	250	-
2	Protonated molecular ion	$C_8H_7N_6O_4^+$	261	261.10
3	Loss of NH2	$C_8H_4N_5O_4^+$	234	239.06
4	Loss of NO2	$C_8H_6N_5O_2^+$	204	204.18
5	Phenyl triazole fragment	$C_7H_5N_4^+$	195	195.06
6	Nitrobenzene fragment	$C_6H_4NO_2^+$	136	136.11
7	Ring cleavage fragment	$C_7H_7N_3O_2^+$	263	263.14

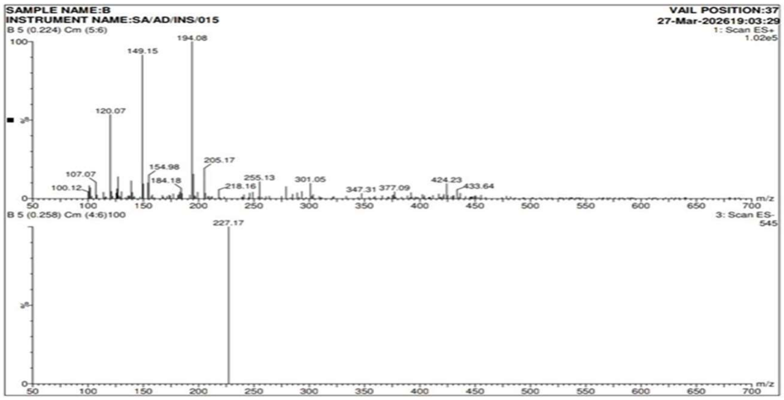


Figure 6. mass spectrum of 4-(5-amino-1*H*-1,2,4-triazol-3-yl)benzoic acid

Table 7. mass spectrum of 4-(5-amino-1*H*-1,2,4-triazol-3-yl)benzoic acid

Sr no.	**Structural change**	**Structural formula of fragment**	**Actual m/z**	**Observed m/z**
1	Molecular ion	$C_9H_8N_4O_2^+$	204	-
2	Protonated molecular ion	$C_9H_9N_4O_2^+$	205	205.17
3	Loss of COOH	$C_8H_8N_4^+$	160	154.98
4	Loss of NH_2	$C_9H_6N_3O_2^+$	188	184.18
5	Benzene ring fragment	$C_7H_5O_2^+$	121	120.07
6	Aromatic fragment	$C_6H_5^+$	77	100.12
7	Triazole fragment	$C_2H_3N_3^+$	69	107.07
8	Rearrangement fragment	$C_8H_7N_3O^+$	149	149.15
9	Base peak fragment	Stable rearranged ion	227	227.17

Molecular Docking Studies:-Docking studies against 6 COX protein demonstrated promising anti inflammatory potential of the synthesized compound. Compound b exhibited the highest binding affinity (-8.0 kcal/mol) which was comparable to the standard drug celecoxib (-8.2kcal/mol) as shown in table 8. The compound formed stable interaction within the active binding pocket through hydrogen bonding and hydrophobic interaction

Similarly, antimicrobial docking studies against 1TH4 protein revealed that compound e showed the strong binding affinity (-8.6kcal/mol), surpassing the standard drug amoxicillin (-8.2 kcal/mol) as shown in table 9. The docking result indicated strong ligand protein interaction, suggesting significant antimicrobial potential.

Table 8 :- Anti-inflammatory activity results of molecular docking studies by binding affinity (6COX)

Sr No.	**Compound Code**	**Molecular Structure**	**Binding affinity**
1.	a		-7.8
2.	b		**-8**

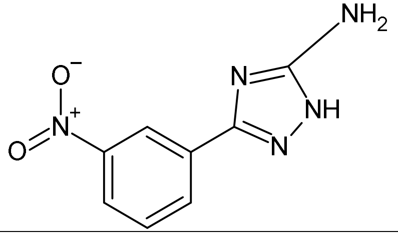
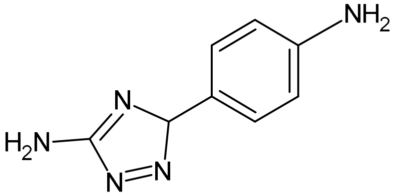
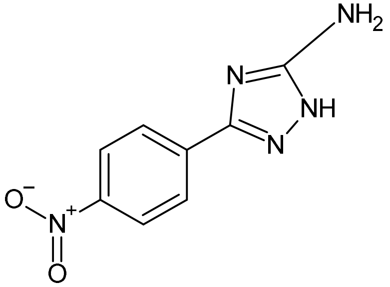
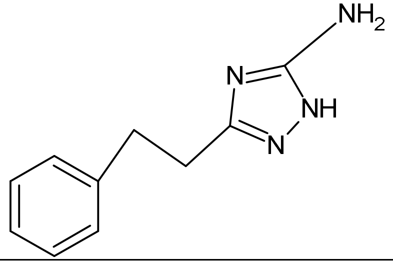
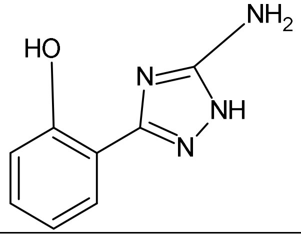
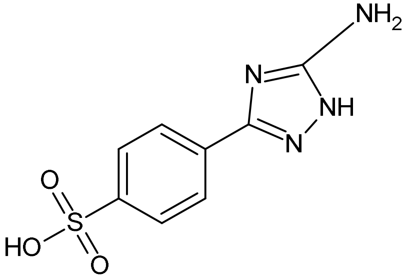
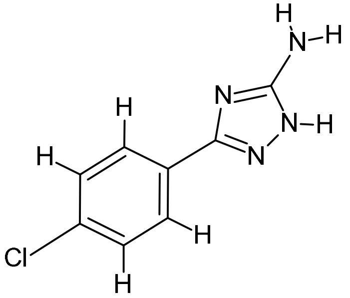
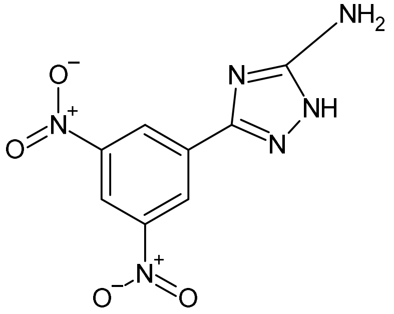
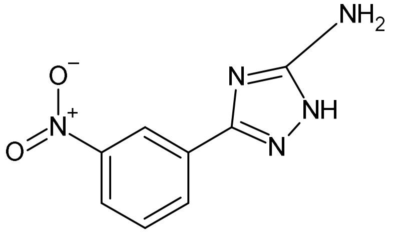
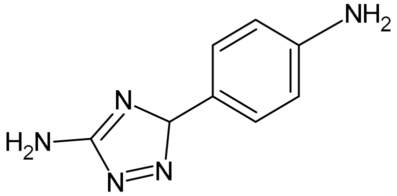
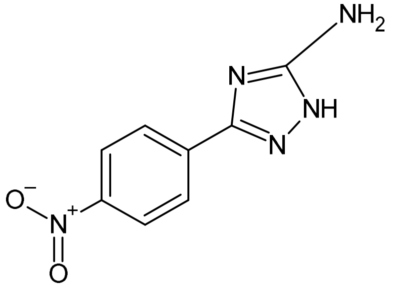
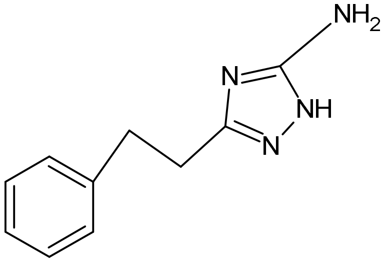
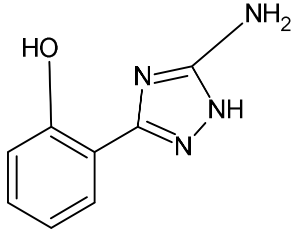
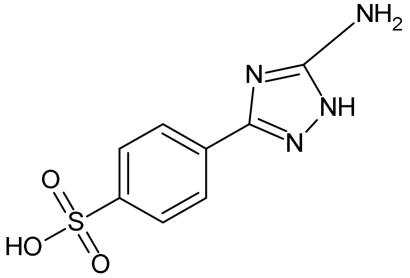
3.	c		-7.9
4.	d		-7.1
5.	e		-7.4
6.	f		-6.4
7.	g		-6.8
8.	h		-7.4

Table 9 :- Antimicrobial activity results of molecular docking studies by binding affinity (1TH4)

Sr No.	Compound Code	Molecular Structure	Binding affinity

1.	a		-7.4
2.	b		-7.6
3.	c		-8.5
4.	d		-6.6
5.	e		**-8.6**
6.	f		-7.1

7.	g		-8.4
8.	h		-8.3

Toxicity prediction:-In silico toxicity studies revealed that most compounds exhibited acceptable toxicity profiles. Compound b and e showed inactive cardiotoxicity and nephotoxicity profile with moderate LD50 value, as shown in table 10 suggested relatively safer pharmacological behaviour.

Table 10 Toxicity study by synthesized compound

Sr No.	Compound code	Smile id	Target	Prediction	LD50 Mg/kg
1.	a	NC1=NC(=NN1)C1=CC=C(Cl)C=C1	Hepatotoxicity	Active	680
			Neurotoxicity	Active	
			Nephrotoxicity	Inactive	
			Respiratory toxicity	Active	
			Cardiotoxicity	Inactive	
2.	b	NC1=NC(=NN1)C1=CC(=CC(=C1)[N+]([O-])=O)[N+]([O-])=O	Hepatotoxicity	Active	680
			Neurotoxicity	Inactive	
			Nephrotoxicity	Inactive	
			Respiratory toxicity	Inactive	
			Cardiotoxicity	Inactive	
3.	c	NC1=NC(=NN1)C1=CC=C(N)C=C1	Hepatotoxicity	Active	680
			Neurotoxicity	Active	
			Nephrotoxicity	Inactive	
			Respiratory toxicity	Active	
			Cardiotoxicity	Inactive	
4.	d	NC1=NC(CCC2=CC=CC=C2)=NN1	Hepatotoxicity	Inactive	800
			Neurotoxicity	Active	
			Nephrotoxicity	Inactive	
			Respiratory toxicity	Active	
			Cardiotoxicity	Inactive	
5.	e	NC1=NC(=NN1)C1=CC=CC(=C1)[N+]([O-])=O	Hepatotoxicity	Active	680
			Neurotoxicity	Inactive	
			Nephrotoxicity	Inactive	
			Respiratory toxicity	Inactive	
			Cardiotoxicity	Inactive	
6.	f	NC1=NC(=NN1)C1=CC=C(C=C1)[N+]([O-	Hepatotoxicity	Active	680

		])=O			
			Neurotoxicity	Inactive	
			Nephrotoxicity	Inactive	
			Respiratory toxicity	Inactive	
			Cardiotoxicity	Inactive	
7.	g	NC1=NC(=NN1)C1=CC=C(C=C1)S(O)(=O)=O	Hepatotoxicity	Inactive	500
			Neurotoxicity	Inactive	
			Nephrotoxicity	Active	
			Respiratory toxicity	Inactive	
			Cardiotoxicity	Inactive	
8.	h	NC1=NC(=NN1)C1=CC=CC=C1O	Organ toxicity	Active	680
			Organ toxicity	Active	
			Organ toxicity	Active	
			Organ toxicity	Active	
			Organ toxicity	Inactive	

Anti-inflammatory activity:-Compound code e demonstrated concentration-dependant anti-inflammatory activity in the protein denaturation assay. The compound exhibited 46.90% inhibition at 200 µg/ml, which increased progressively to 80.62% at 1000µg/ml diclofenace sodium showed higher inhibition values from 72..87% to 85.27% across the tested concentration

The result indicate that compound e possesses significant protein stabilization potential and effectively inhibits heat-induced protein denaturation, which is a key mechanism associated with inflammatory processes. At higher concentration, the activity of the synthesized compound approached that of the standard drug, suggesting promising anti-inflammatory efficacy. These findings support the potential of compound e as a candidate for further development as an anti-inflammatory agent.

Antimicrobial activity:-The synthesized compound exhibited weak antibacterial activity against *staphylococcus aureus*. Streptomycin produced a pronounced zone of inhibition of 25mm at 1mg/ml, confirming the sensitivity of the test organism and validity of the assay. In comparison, compound code e showed 1mm and 4 mm zone of inhibition at concentration of 50 and 100 mg/ml respectively. The observed result suggested that compound e possesses minimal antibacterial potential against the tested gram positive bacterial strain. Although a slight increase in inhibition was observed with increasing concentration the activity remained substantially lower that the standard antibiotic the limited antibacterial efficacy may be attributed to poor diffusion of the compound in agar medium of insufficient interaction with bacterial cellular targets. Further structural optimization may improve the antimicrobial profile of the synthesized compound.

Conclusion

A series of novel substituted 1,2,4-triazole derivative were successfully synthesized using both conventional and microwave assisted method. The microwave assisted synthesis proved to be superior approach by providing higher yield, shorter reaction time, and improved reaction efficiency confirmed the successful synthesis of the desired compounds.

Molecular docking studies demonstrated favourable interaction of the synthesized derivative with anti-inflammatory and antimicrobial target protein, including their therapeutic potential. Among the synthesized compounds, compound b showed excellent binding affinity toward the 6 COX protein, while compound e exhibited the strongest interaction with the antibacterial target protein 1TH4. Toxicity prediction studies further suggested relatively safe pharmacological profile for selected compounds.

The in vitro anti-inflammatory studies revealed that compound e possesses significant protein denaturation inhibitory activity comparable to the standard drug diclofenac sodium, indicating promising anti-inflammatory potential. Although the antimicrobial activity against Staphylococcus aureus was comparatively weak, the obtained result provide valuable insight into the structure activity relationship of the synthesized triazole derivatives.

Overall, the present investigation highlight the importance of substituted 1,2,4-triazole derivatives as promising bioactive scaffolds for the development of new anti-inflammatory agents. Further structural modification, detailed mechanistic studies and, in vivo pharmacological evaluation are recommended to enhance their biological efficacy and therapeutic applicability

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