

In-Vivo and In-Vitro Biological Evaluation of Newly Synthesized 1,3,4-Thiadiazole Derivatives

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ABSTRACT

The present investigation was undertaken to evaluate the acute toxicity, dose selection and extensive biological screening of a series of synthesized 1,3,4-thiadiazole derivatives. Acute toxicity studies were carried out in experimental animals according to OECD guidelines, and the median lethal dose (LD₅₀) values were found to range between 750 and 1250 mg/kg. Based on these findings, one-tenth of the LD₅₀ was selected as the experimental dose for subsequent pharmacological evaluation, indicating a favorable safety profile for the tested compounds. The synthesized derivatives were screened for in-vivo analgesic, antipyretic and anti-inflammatory activities using standard experimental models. Several compounds exhibited significant pharmacological effects comparable to reference drugs. Compound IIIA10 showed potent analgesic and anti-inflammatory activity, while compound 2b demonstrated marked antipyretic efficacy, which was attributed to the presence of electron-withdrawing nitro and chloro substituents on the phenyl ring of the thiadiazole nucleus. Anti-inflammatory screening using the carrageenan-induced paw edema model revealed that compounds IIIA10, VA17, 2b and 3b produced significant inhibition of edema, suggesting effective suppression of inflammatory mediators. In-vitro antibacterial and antifungal activities were evaluated against selected Gram-positive, Gram-negative bacteria and fungal strains using the cup plate and MIC determination methods. Compounds 1b, 2b and 3b showed pronounced antimicrobial activity, highlighting the role of substituted phenyl rings and optimized lipophilicity in enhancing microbial inhibition. Antioxidant potential assessed by DPPH and nitric oxide scavenging assays revealed that compounds bearing hydroxyl and nitro substituents exhibited superior radical scavenging activity in a concentration-dependent manner. Overall, the results demonstrate that strategic substitution on the 1,3,4-thiadiazole scaffold significantly influences biological activity. The study identifies several lead molecules with promising safety and multifunctional pharmacological profiles, supporting further optimization and development of these derivatives as potential therapeutic agents.

Keywords: 1,3,4-Thiadiazole derivatives, Acute toxicity, Analgesic and anti-inflammatory activity, Antimicrobial screening, Antioxidant activity.

Introduction

Heterocyclic chemistry constitutes an important and extensive area of organic chemistry concerned with the study of cyclic compounds in which one or more atoms of the ring are elements other than carbon. These non-carbon atoms, commonly referred to as heteroatoms, include nitrogen, oxygen and sulfur, together with less frequently occurring elements such as phosphorus and selenium. Heterocyclic compounds exhibit considerable structural diversity and can be classified according to ring size, nature and number of heteroatoms, degree of saturation, and arrangement of heteroatoms within the ring. Five- and six-membered heterocyclic systems represent particularly important classes because of their diverse chemical properties, synthetic accessibility and widespread applications in organic, medicinal and pharmaceutical chemistry. The fundamental principles governing the structure, reactivity and synthesis of these systems form a major component of modern heterocyclic chemistry.

The presence of heteroatoms within a cyclic framework produces substantial changes in the electronic and physicochemical characteristics of organic molecules. Heteroatoms can influence electron distribution, aromaticity, polarity, hydrogen-bonding ability, acidity and basicity, dipole moment, lipophilicity and molecular recognition. These properties determine, to a considerable extent, how heterocyclic molecules behave in chemical reactions and how they interact with biological macromolecules. Consequently, incorporation of heteroatoms into cyclic structures provides an effective means of modifying molecular properties and generating structurally and functionally diverse compounds.

The importance of heterocyclic chemistry extends far beyond synthetic organic chemistry and is particularly evident in medicinal chemistry and drug discovery. Heterocyclic scaffolds occur extensively in biologically active

natural products, synthetic compounds and pharmaceutical agents. Their structural characteristics allow them to act as important pharmacophoric elements and provide suitable sites for hydrogen bonding, hydrophobic interactions and other non-covalent interactions with biological targets. Modification of heterocyclic frameworks can therefore be used to optimize target affinity, physicochemical characteristics and other properties relevant to drug development. The continuing development of synthetic methods for the preparation and functionalization of heterocycles has substantially expanded the chemical space available to medicinal chemists.

Heterocyclic systems containing nitrogen and sulfur are of particular interest because these heteroatoms possess distinct electronic and physicochemical properties that can be exploited in molecular design. Nitrogen can participate in hydrogen-bonding and ionic interactions, while sulfur possesses distinctive polarizability and electronic characteristics that can influence molecular recognition and physicochemical behavior. The combination of these heteroatoms within a common ring system can generate molecular frameworks with useful properties for biological recognition. Consequently, sulfur- and nitrogen-containing heterocycles constitute an important class of compounds investigated for the development of pharmacologically active molecules.

1.1 Heterocyclic Compounds in Medicinal Chemistry

Heterocyclic compounds occupy a central position in medicinal chemistry and pharmaceutical research because of their structural diversity, chemical versatility and ability to interact with biological macromolecules. A heterocyclic system can provide a defined molecular framework around which different functional groups can be introduced, thereby enabling systematic modification of molecular properties and biological activity. The incorporation of heteroatoms such as nitrogen, oxygen and sulfur into cyclic structures produces distinctive electronic and physicochemical characteristics that are highly relevant to molecular recognition and drug action. Consequently, heterocyclic scaffolds have become fundamental structural components in the design, discovery and optimization of biologically active molecules.

One of the major advantages of heterocyclic scaffolds in medicinal chemistry is their ability to provide appropriate spatial and electronic arrangements for interaction with biological targets. Heteroatoms can function as hydrogen-bond donors or acceptors and can participate in electrostatic, dipole–dipole, hydrophobic and other non-covalent interactions with amino acid residues within proteins and other biological macromolecules. Modification of the heterocyclic ring or its substituents can consequently alter binding affinity, selectivity and molecular recognition. These structural features make heterocycles particularly useful as pharmacophoric elements in the design of compounds directed toward enzymes, receptors, ion channels, transporters and nucleic acids.

The importance of heterocyclic compounds is reflected in their extensive representation among marketed and prescribed pharmaceutical agents. Nitrogen-containing heterocycles, in particular, occur in a large number of therapeutic molecules used in diverse areas of medicine. A comprehensive review of prescribed drugs containing nitrogen heterocycles demonstrated their widespread occurrence across different therapeutic classes and emphasized the importance of these structural motifs in modern drug design and drug discovery. This prevalence is not merely a consequence of synthetic accessibility; rather, heterocyclic systems provide structural and physicochemical characteristics that can be exploited during the optimization of drug candidates.

Heterocyclic scaffolds are also important because they provide considerable chemical space

Pharmacological Evaluation

The synthesized compounds were subjected to pharmacological evaluation to investigate their analgesic, antipyretic and anti-inflammatory activities, as well as their preliminary safety profile. The compounds were also evaluated for antibacterial and in-vitro antioxidant activities using appropriate experimental methods.

All experimental procedures involving laboratory animals were conducted following approval from the Institutional Animal Ethics Committee (IAEC).

4.6.1 Selection and Maintenance of Experimental Animals

Adult Swiss albino mice weighing approximately 20–22 g and Adult rats weighing approximately 150–200g were obtained from SRI VENKETESWARA ENTERPRISES, Bangalore. The animals were housed in polypropylene cages under standard laboratory conditions, with not more than six animals per cage. The animals were maintained at a temperature of approximately 25 ± 2 °C under a controlled light/dark cycle and were provided standard pellet diet and water. The animals were acclimatized to the laboratory conditions for seven days before initiation of the

experimental procedures. All experimental procedures were performed in accordance with the approved institutional animal-ethical requirements.

4.6.2 Acute Oral Toxicity Study

The acute oral toxicity study was conducted according to OECD Guideline 423 to obtain preliminary information regarding the acute toxicity and tolerability of the synthesized compounds and to assist in the selection of doses for subsequent pharmacological studies. The animals were administered the test compounds according to the approved experimental protocol and were observed for signs of toxicity during the specified observation period.

The animals were carefully monitored for changes in skin, eyes, mucous membranes, respiration, circulation, autonomic and central nervous systems and general behaviour. Particular attention was given to the occurrence of tremors, convulsions, salivation, diarrhoea, lethargy, sleep, coma and other abnormal behavioural responses. Mortality, if any, was also recorded. The observations obtained from the acute toxicity study were used to determine the dose levels for subsequent pharmacological evaluation.



Acute Oral Toxicity study

Acetic Acid-Induced Writhing Test

The synthesised compounds analgesic activity was evaluated using the acetic acid-induced writhing method in mice. The animals were weighed, numbered and randomly allocated into control, standard and test groups. The control group received 1% v/v acetic acid solution at the specified dose, while the standard group received aspirin at a dose of 100 mg/kg body weight. The respective test groups received the synthesized compounds at the dose selected based on the acute toxicity study.

Acetic acid-induced nociception was produced by intraperitoneal administration of 1% v/v acetic acid. Following administration, the animals were individually placed in transparent observation chambers, and the onset and severity of the writhing response were observed. The number of abdominal contractions, trunk twisting and extension of the hind limbs were recorded for a period of 20 min. The mean number of writhing responses in each group was calculated, and the analgesic effect of the test compounds was compared with that of the control and standard groups.



Figure :1 Acetic acid Induced writhing test

1.Assessment of In-vivo Antipyretic Activity

Brewer's Yeast-Induced Pyrexia Model

The antipyretic activity of the synthesized compounds was evaluated using the Brewer's yeast-induced pyrexia model. The animals were divided into control, standard and test groups. The control group received 0.9% w/v normal saline, the standard group received aspirin at a dose of 100 mg/kg body weight, and the test groups received the respective synthesized compounds at the dose selected based on the acute toxicity study.

The basal rectal temperature of each animal was measured before induction of pyrexia using a digital thermometer. Pyrexia was induced by administration of a 15% w/v suspension of Brewer's yeast in 0.9% w/v sodium chloride solution at the specified dose. Following administration of Brewer's yeast, the animals were returned to their respective cages. After the induction period, rectal temperature was again measured. The test compounds and standard drug were subsequently administered according to the experimental protocol, and rectal temperature was recorded at predetermined intervals. The antipyretic activity was assessed by comparing the changes in rectal temperature among the control, standard and test groups.

2.Assessment of In-vivo Anti-inflammatory Activity

Carrageenan-Induced Rat Paw Edema Method

The anti-inflammatory activity of the synthesized compounds was evaluated using the carrageenan-induced rat paw edema model. Adult rats weighing approximately 150–200 g were used for the experiment. The animals were divided into control, standard and test groups. The control group received 0.9% w/v normal saline at a dose of 5 mL/kg body weight, while the standard group received indomethacin at a dose of 10 mg/kg body weight. The test groups received the respective synthesized compounds at the dose selected on the basis of the acute toxicity study.



Figure2 Carrageenan Induced Rat Paw Edema Test

Before administration of the test compounds, the initial paw volume of each animal was measured using a plethysmometer. One hour after administration of the test and standard treatments, 1% w/v carrageenan was injected into the subplantar region of the right hind paw to induce acute inflammation. The left paw served as the reference non-inflamed paw. Paw volume was measured at 0, 1, 2, 3 and 4 h following carrageenan administration. The increase in paw volume was calculated, and the percentage inhibition of edema produced by the test compounds was compared with the control group.

The percentage inhibition of paw edema was calculated using:

$$\% \text{Inhibition} = 100(1 - V_t/V_c)$$

where V_t represents the mean increase in paw volume of the test group and

Vc represents the mean increase in paw volume of the control group.

3. Assessment of In-vitro Antibacterial Activity

Maintenance of Bacterial Cultures

The bacterial cultures were maintained in nutrient broth prior to evaluation of antibacterial activity. The purity of each bacterial isolate was confirmed before use. Selected colonies were inoculated into nutrient broth and incubated at 37 °C for approximately 18 h with shaking at 150 rpm. The bacterial cultures were subsequently subcultured onto agar slants and maintained at 4 °C until further use.

Cup-Plate Method

The antibacterial activity of the synthesized compounds was evaluated using the cup-plate method with Mueller-Hinton agar as the culture medium. The bacterial cultures were inoculated onto the agar plates, and sterile cups of approximately 9 mm diameter were prepared using a sterile cork borer. Solutions of the synthesized compounds at concentrations of 50 and 100 µg/mL were separately introduced into the respective cups using a micropipette. The inoculated plates were incubated at 37 °C for 24 h. Following incubation, the plates were examined for the formation of zones of inhibition around the cups. The diameter of each zone of inhibition was measured in millimetres. The antibacterial activity of the synthesized compounds was compared with the reference antibacterial agents, amoxycillin and ofloxacin. DMSO was used as the vehicle where appropriate. Each experiment was performed in triplicate.

Table 1. Bacterial strains used for antibacterial evaluation

S. No.	Bacterial strain	Type
1	Bacillus subtilis (ATCC 11774)	Gram-positive
2	Staphylococcus aureus (NTCC 6571)	Gram-positive
3	Escherichia coli (TG14)	Gram-negative
4	Pseudomonas aeruginosa (ATCC 9027)	Gram-negative



Figure 4.6.6.2 Cup plate method

Fig;3

4. Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) was determined as the lowest concentration of the synthesized compound that completely inhibited visible growth of the test organism under the experimental conditions. The bacterial inoculum was standardized to approximately 10^8 CFU/mL by comparison with the appropriate McFarland turbidity standard.

Stock solutions of the synthesized compounds were prepared at 2000 µg/mL using DMSO as the solvent. Serial dilutions were subsequently prepared to obtain the required concentrations. During primary screening, the compounds were evaluated at concentrations of 1000, 500 and 250 µg/mL. Compounds demonstrating activity during primary screening were subjected to further dilution to obtain concentrations of 200, 100, 62.5, 50, 25, 12.5 and 6.25 µg/mL.

The inoculated test preparations were incubated under the appropriate conditions, and the presence or absence of visible turbidity was recorded. The lowest concentration showing complete inhibition of visible growth was recorded as the MIC in µg/mL. Appropriate growth and solvent controls were maintained throughout the experiment.

5. Assessment of In-vitro Antioxidant Activity

DPPH Radical-Scavenging Activity

The DPPH radical-scavenging assay was employed to evaluate the in-vitro antioxidant activity of the synthesized compounds. DPPH is a stable nitrogen-centred free radical that exhibits a characteristic absorption maximum at 517 nm. Reduction of the DPPH radical by an antioxidant results in a decrease in absorbance, which can be used to determine the radical-scavenging activity of the test compound. A 0.004% w/v methanolic solution of DPPH was prepared. To 4 mL of the DPPH solution, 1 mL of each test compound at concentrations of 4, 8 and 10 µg/mL was added. The reaction mixtures were incubated at room temperature for 30 min, following which the absorbance was measured at 517 nm using a SPECORD® 50 PLUS UV-visible spectrophotometer. Ascorbic acid was used as the standard antioxidant. All measurements were performed in triplicate.

The percentage inhibition of DPPH radical formation was calculated using:

$$I(\%) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where A_{control} is the absorbance of the control and A_{sample} is the absorbance of the test sample.

6. Nitric Oxide-Scavenging Activity

The nitric oxide-scavenging activity of the synthesized compounds was evaluated using the sodium nitroprusside method. The reaction mixture consisted of sodium nitroprusside (10 mM, 4 mL), phosphate-buffered saline (pH 7.4, 1 mL) and the test compound or standard ascorbic acid solution (1 mL) at concentrations of 4, 8 and 10 µg/mL. The reaction mixtures were incubated at 25 °C for 150 min. After incubation, 0.5 mL of the reaction mixture was withdrawn and mixed with 1 mL of sulphanilic acid reagent. The mixture was allowed to stand for 5 min to facilitate diazotization. Subsequently, 1 mL of naphthyl ethylene diamine dihydrochloride was added, and the mixture was allowed to stand for 30 min under diffused light. The formation of the pink-coloured chromophore was measured at 640 nm using a SPECORD® 50 PLUS UV-visible spectrophotometer. The experiment was performed in triplicate.

The percentage nitric oxide-scavenging activity was calculated using:

$$\% \text{ Scavenging} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where A_{control} represents the absorbance of the control and A_{sample} represents the absorbance of the test sample.

4.7 Statistical Analysis

The experimental data obtained from the pharmacological and biological studies were expressed as mean $\pm$ SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Dunnett's post-hoc test for comparison between the treatment groups and the respective control group. A p value of less than 0.05 was considered statistically significant. Values of $p < 0.01$ and $p < 0.001$ were considered to indicate higher levels of statistical significance.

Table2. Acute oral toxicity response of synthesized compounds

S. No.	Synthesized compound	Dose (mg/kg)	Log dose	Response	Estimated LD ₅₀ mg/kg
1	B3	175, 550, 1750	2.2430, 2.7404, 3.2430	O, O, X	>550—<1750
2	B4	175, 550, 1750	2.2430, 2.7404, 3.2430	O, O, X	>550—<1750
3	B5	175, 550, 1750	2.2430, 2.7404, 3.2430	O, O, X	>550—<1750
4	B6	175, 550, 1750	2.2430, 2.7404, 3.2430	O, O, X	>550—<1750
5	T3	175, 550, 1750	2.2430, 2.7404, 3.2430	O, O, X	>550—<1750
6	T4	175, 550, 1750	2.2430, 2.7404, 3.2430	O, O, X	>550—<1750
7	T5	175, 550, 1750	2.2430, 2.7404, 3.2430	O, O, X	>550—<1750
8	T6	175, 550, 1750	2.2430, 2.7404, 3.2430	O, O, X	>550—<1750
9	N3	175, 550, 1750	2.2430, 2.7404, 3.2430	O, O, X	>550—<1750
10	N4	175, 550, 1750	2.2430, 2.7404, 3.2430	O, O, X	>550—<1750
11	N5	175, 550, 1750	2.2430, 2.7404, 3.2430	O, O, X	>550—<1750
12	N6	175, 550, 1750	2.2430, 2.7404, 3.2430	O, O, X	>550—<1750
13	B7	175, 550, 1750	2.2430, 2.7404, 3.2430	O, O, X	>550—<1750
14	B8	175, 550, 1750	2.2430, 2.7404, 3.2430	O, O, X	>550—<1750
15	B9	175, 550, 1750	2.2430, 2.7404, 3.2430	O, O, X	>550—<1750
16	T7	175, 550, 1750	2.2430, 2.7404, 3.2430	O, O, X	>550—<1750
17	T8	175, 550, 1750	2.2430, 2.7404, 3.2430	O, O, X	>550—<1750
18	T9	175, 550, 1750	2.2430, 2.7404, 3.2430	O, O, X	>550—<1750
19	N7	175, 550, 1750	2.2430, 2.7404, 3.2430	O, O, X	>550—<1750
20	N8	175, 550, 1750	2.2430, 2.7404, 3.2430	O, O, X	>550—<1750
21	N9	175, 550, 1750	2.2430, 2.7404, 3.2430	O, O, X	>550—<1750
22	B10	175, 550, 1750	2.2430, 2.7404, 3.2430	O, O, X	>550—<1750
23	B11	175, 550, 1750	2.2430, 2.7404, 3.2430	O, O, X	>550—<1750
24	T10	175, 550, 1750	2.2430, 2.7404, 3.2430	O, O, X	>550—<1750
25	T11	175, 550, 1750	2.2430, 2.7404, 3.2430	O, O, X	>550—<1750
26	N10	175, 550, 1750	2.2430, 2.7404, 3.2430	O, O, X	>550—<1750
27	N11	175, 550, 1750	2.2430, 2.7404, 3.2430	O, O, X	>550—<1750

Note: O = no mortality observed; X = mortality observed.

Table 3. Estimated LD₅₀ and selected efficacy dose of the synthesized compounds

S. No.	Synthesized compound	Estimated LD ₅₀ (mg/kg)	Selected efficacy dose (mg/kg)
1	B3	981	100
2	B4	981	100
3	B5	981	100
4	B6	981	100
5	T3	981	100
6	T4	981	100
7	T5	981	100
8	T6	981	100
9	N3	981	100

10	N4	981	100
11	N5	981	100
12	N6	981	100
13	B7	981	100
14	B8	981	100
15	B9	981	100
16	T7	981	100
17	T8	981	100
18	T9	981	100
19	N7	981	100
20	N8	981	100
21	N9	981	100
22	B10	981	100
23	B11	981	100
24	T10	981	100
25	T11	981	100
26	N10	981	100
27	N11	981	100

Table 4. Calculation and justification of the estimated LD₅₀ and selected efficacy dose

S. No.	Parameter	Value	Basis of calculation
1	Highest non-lethal dose	550 mg/kg	No mortality was observed at 550 mg/kg
2	Lowest lethal dose	1750 mg/kg	Mortality was observed at 1750 mg/kg
3	Estimated LD ₅₀	981 mg/kg	$\sqrt{(550 \times 1750)} = \sqrt{962,500} \approx 981$ mg/kg
4	Fraction of estimated LD ₅₀ selected	1/10th	$981 \div 10 = 98.1$ mg/kg
5	Selected efficacy dose	100 mg/kg	98.1 mg/kg rounded to a practical experimental dose of 100 mg/kg

Note: The estimated LD₅₀ was derived from the geometric mean of the highest non-lethal dose (550 mg/kg) and the lowest lethal dose (1750 mg/kg). The calculated value was approximately 981 mg/kg. One-tenth of the estimated LD₅₀ (98.1 mg/kg) was selected as the preliminary efficacy dose and rounded to 100 mg/kg for subsequent pharmacological evaluation.

8. Analgesic Activity

7. Acetic Acid-Induced Writhing Test

The analgesic activity of the synthesized compounds was evaluated using the acetic acid-induced writhing model. Administration of acetic acid produced a characteristic writhing response in the control animals. Treatment with the synthesized compounds resulted in varying degrees of reduction in the number of writhing responses compared with the control group. Aspirin was used as the standard analgesic drug. The results are presented in Table 8.1.

The acetic acid control group showed 53.4 ± 1.150 writhings, whereas aspirin markedly reduced the writhing response to 8.2 ± 0.812 , corresponding to 84.64% analgesic activity ($p < 0.001$). Among the synthesized compounds, N4 exhibited the highest analgesic activity (72.28%), with 14.8 ± 0.043 writhings ($p < 0.001$). T8 and N8 showed 69.28% and 68.91% analgesic activity, respectively, with T8 producing 16.4 ± 0.042 writhings ($p < 0.001$) and N8 producing 16.6 ± 0.033 writhings ($p < 0.01$). T4 also demonstrated pronounced activity, with 66.66% inhibition of writhing (17.8 ± 0.033 ; $p < 0.01$).

Moderate analgesic activity was observed with N11 (56.55%, $p < 0.01$), N3 (51.68%, $p < 0.01$), N6 (46.44%, $p < 0.01$), and N5 (43.07%, $p < 0.01$). N10 showed 37.45% analgesic activity ($p < 0.01$). Other compounds, including

T11, T10, N7 and T3, produced comparatively lower inhibition of writhing, with activities of 29.96%, 27.71%, 17.22% and 20.22%, respectively; among these, N7 showed a statistically significant response ($p < 0.05$).

The remaining derivatives produced relatively low analgesic activity, ranging from 1.12% to 15.73%, without statistical significance as indicated in the supplied data. Overall, N4, T8, N8 and T4 were the most active synthesized derivatives in the acetic acid-induced writhing model. However, their activities were lower than that of the standard drug aspirin.

Table5. Effect of synthesized compounds on acetic acid-induced writhing

Group No.	Synthesised compound	Body wt. (g)	Dose (mg/kg)	No. of writhings in 20 min	Analgesic activity (%)
1	Acetic acid	25	100	53.4 $\pm$ 1.150	0.00 $\pm$ 0.00
2	Aspirin	25	100	8.2 $\pm$ 0.812***	84.64 $\pm$ 1.56***
3	B3	26	100	50.0 $\pm$ 0.234	6.36 $\pm$ 0.73
4	B4	25	100	48.4 $\pm$ 0.413	9.36 $\pm$ 0.82
5	B5	28	100	50.4 $\pm$ 0.584	5.62 $\pm$ 1.10
6	B6	27	100	50.2 $\pm$ 0.782	5.99 $\pm$ 1.46
7	T3	29	100	42.6 $\pm$ 0.821*	20.22 $\pm$ 1.72*
8	T4	27	100	17.8 $\pm$ 0.033**	66.67 $\pm$ 0.62**
9	T5	25	100	51.4 $\pm$ 0.312	3.74 $\pm$ 0.75
10	T6	25	100	47.6 $\pm$ 0.752	10.86 $\pm$ 1.48
11	N3	30	100	25.8 $\pm$ 0.902**	51.68 $\pm$ 1.93**
12	N4	25	100	14.8 $\pm$ 0.043***	72.28 $\pm$ 0.32***
13	N5	30	100	30.4 $\pm$ 0.495**	43.07 $\pm$ 1.15**
14	N6	28	100	28.6 $\pm$ 0.275**	46.44 $\pm$ 0.70**
15	B7	25	100	51.0 $\pm$ 0.842	4.49 $\pm$ 1.61
16	B8	27	100	49.8 $\pm$ 0.231	6.74 $\pm$ 0.84
17	B9	29	100	52.8 $\pm$ 0.345	1.12 $\pm$ 1.12
18	T7	25	100	47.8 $\pm$ 0.652	10.48 $\pm$ 1.44
19	T8	26	100	16.4 $\pm$ 0.042***	69.28 $\pm$ 0.52***
20	T9	25	100	45.0 $\pm$ 0.671	15.73 $\pm$ 1.40
21	N7	29	100	44.2 $\pm$ 0.425	17.23 $\pm$ 1.48
22	N8	30	100	16.6 $\pm$ 0.033***	68.91 $\pm$ 0.50***
23	N9	25	100	46.8 $\pm$ 0.422	12.36 $\pm$ 1.40
24	B10	25	100	52.8 $\pm$ 0.884	1.12 $\pm$ 1.67
25	B11	25	100	51.8 $\pm$ 0.732	2.99 $\pm$ 1.51
26	T10	27	100	38.6 $\pm$ 0.761	27.72 $\pm$ 1.63*
27	T11	25	100	37.4 $\pm$ 0.024*	29.96 $\pm$ 1.44*
28	N10	28	100	33.4 $\pm$ 0.831**	37.45 $\pm$ 1.78**
29	N11	25	100	23.2 $\pm$ 0.424**	56.55 $\pm$ 1.03**

Values are expressed as mean $\pm$ SEM (n = 6). Data were analyzed by one-way ANOVA followed by Dunnett's multiple-comparison test. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared with the acetic acid control group.

Figure.4Effect of synthesized compounds on acetic acid-induced writhing in mice

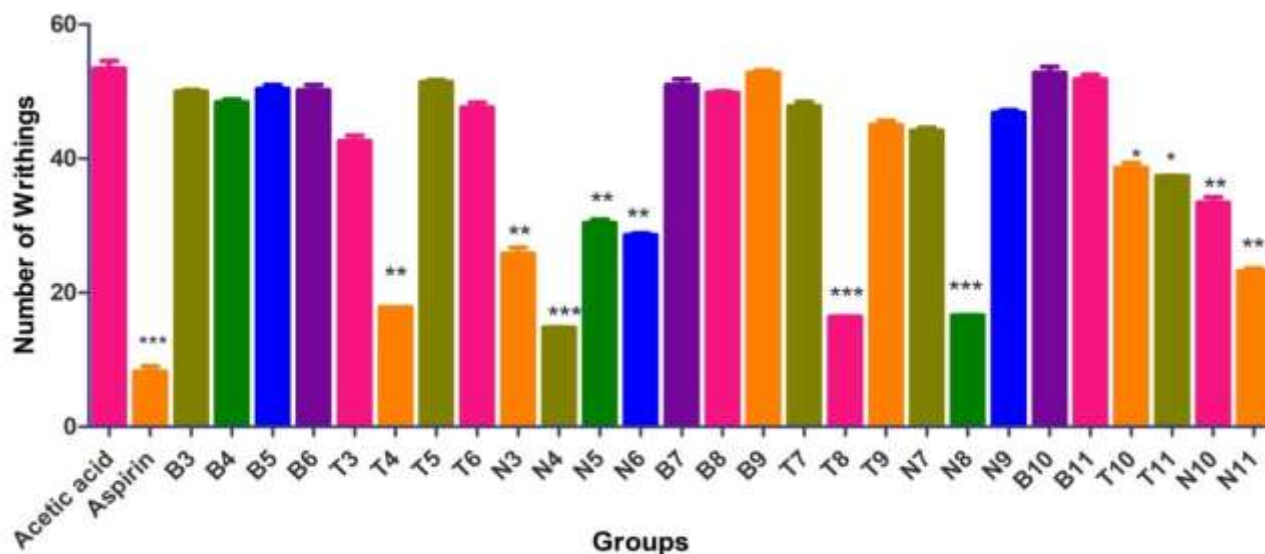
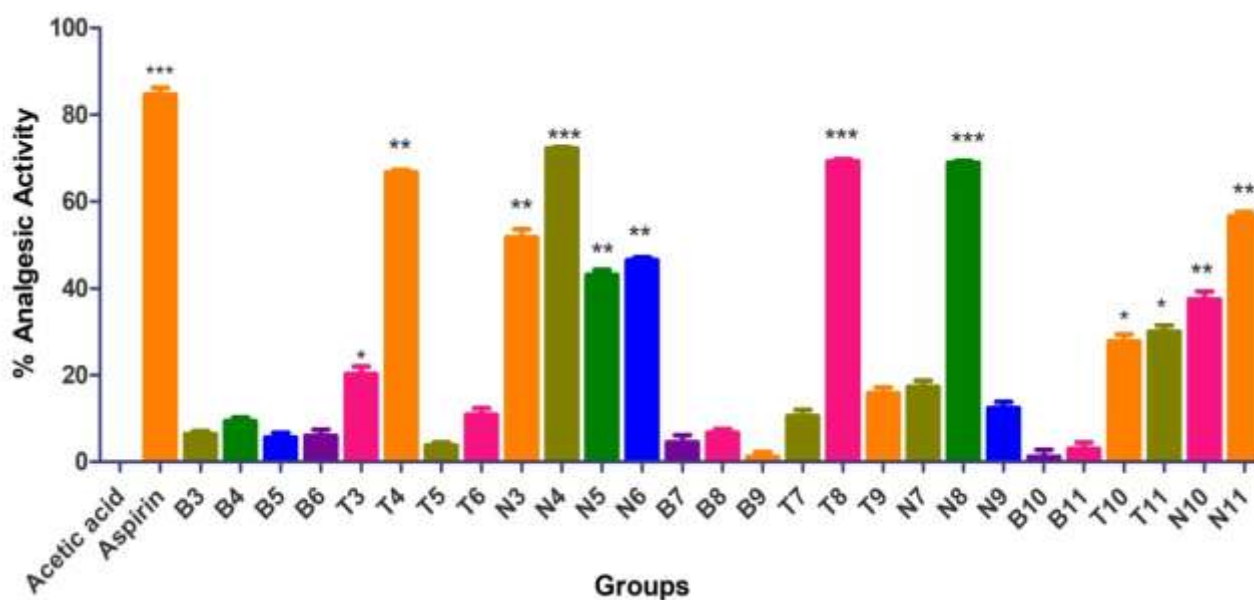


Figure 5 Effect of synthesized compounds on analgesic activity in the acetic acid-induced writhing test



Values are expressed as mean $\pm$ SEM ($n = 6$). Data were analyzed by one-way ANOVA followed by Dunnett's multiple-comparison test. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared with the acetic acid control group.

8.2 Antipyretic Activity

8.1 Brewer's Yeast-Induced Pyrexia

The temperature response was recorded at different time intervals following treatment, and the results were compared with the control and standard groups. The antipyretic activity of the synthesized compounds was evaluated using the Brewer's yeast-induced pyrexia model in rats. Administration of Brewer's yeast produced a marked elevation in rectal temperature, with the control group showing a change of $1.84 \pm 0.447^{\circ}\text{C}$ between 0 and 23 h. In comparison, the standard treatment produced a substantially lower change in rectal temperature of $0.47 \pm 0.100^{\circ}\text{C}$, corresponding to $74.46 \pm 5.43\%$ antipyretic activity.

The synthesized compounds demonstrated varying degrees of antipyretic activity at 100 mg/kg. Among the B-series compounds, B4 showed the highest activity ($59.24 \pm 1.82\%$), followed by B3 ($56.52 \pm 8.97\%$), B5 ($57.61 \pm 2.09\%$), B7 ($58.70 \pm 1.32\%$), B6 ($49.46 \pm 1.45\%$), B10 ($48.91 \pm 6.85\%$), B11 ($49.46 \pm 2.59\%$), B8 ($47.28 \pm 1.83\%$) and B9 ($41.85 \pm 2.27\%$).

The T-series compounds exhibited comparatively stronger antipyretic effects. T10 showed the highest activity ($90.22 \pm 1.41\%$), with a very low change in rectal temperature of $0.18 \pm 0.026^\circ\text{C}$. T3 also exhibited marked activity ($85.87 \pm 1.67\%$), followed by T8 ($78.80 \pm 2.01\%$), T11 ($74.46 \pm 5.38\%$), T5 ($64.13 \pm 1.42\%$), T7 ($58.15 \pm 1.98\%$), T4 ($48.91 \pm 2.17\%$), T9 ($45.65 \pm 8.48\%$) and T6 ($30.43 \pm 2.77\%$).

Among the N-series compounds, N10 exhibited the highest antipyretic activity ($75.54 \pm 5.49\%$), followed by N3 ($72.28 \pm 2.35\%$), N7 ($69.02 \pm 2.99\%$), N4 ($63.04 \pm 2.12\%$), N8 ($57.61 \pm 1.41\%$), N9 ($51.09 \pm 4.67\%$), N5 ($47.28 \pm 2.84\%$), N11 ($47.28 \pm 2.62\%$) and N6 ($30.98 \pm 1.88\%$).

Overall, T10 was the most potent synthesized compound, showing 90.22% antipyretic activity, which was higher than the standard (74.46%). Other compounds showing pronounced antipyretic activity were T3 (85.87%), T8 (78.80%), N10 (75.54%), T11 (74.46%), and N3 (72.28%). The reduction in rectal temperature was supported by the corresponding decrease in temperature change from the Brewer's yeast control. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple-comparison test, with significance reported against the control group.

Table 6. Effect of synthesized compounds on Brewer's yeast-induced pyrexia

Group No.	Synthesized compound	Dose (mg/kg)	-18 h	0 h	19 h	20 h	21 h	22 h	23 h
1	Brewer's Yeast	5 mL	36.30 ± 0.145	37.26 ± 0.102	39.80 ± 0.098	39.60 ± 0.094	39.30 ± 0.037	39.10 ± 0.023	39.10 ± 0.435
2	Standard	100	36.20 ± 0.027	37.13 ± 0.099	39.50 ± 0.034	39.10 ± 0.157	38.00 ± 0.021	$37.40 \pm 0.062^*$	$37.60 \pm 0.016^*$
3	B3	100	36.73 ± 0.092	37.39 ± 0.075	39.83 ± 0.061	39.74 ± 0.082	39.08 ± 0.006	38.52 ± 0.034	38.19 ± 0.147
4	B4	100	36.54 ± 0.024	37.27 ± 0.094	39.64 ± 0.025	39.76 ± 0.165	39.17 ± 0.325	38.51 ± 0.041	38.02 ± 0.275
5	B5	100	36.70 ± 0.012	37.30 ± 0.131	39.71 ± 0.122	39.35 ± 0.151	39.36 ± 0.225	38.23 ± 0.176	38.08 ± 0.462
6	B6	100	36.61 ± 0.101	37.10 ± 0.152	39.38 ± 0.146	39.58 ± 0.116	39.45 ± 0.366	38.33 ± 0.018	38.03 ± 0.283
7	T3	100	36.08 ± 0.452	37.81 ± 0.123	39.60 ± 0.081	39.50 ± 0.158	39.50 ± 0.472	38.15 ± 0.116	38.07 ± 0.340
8	T4	100	36.10 ± 0.473	37.01 ± 0.012	39.77 ± 0.123	39.30 ± 0.257	39.40 ± 0.059	$37.91 \pm 0.163^*$	$37.95 \pm 0.224^*$
9	T5	100	36.59 ± 0.142	37.44 ± 0.017	39.89 ± 0.158	39.75 ± 0.167	39.64 ± 0.266	38.17 ± 0.172	38.10 ± 0.339
10	T6	100	36.33 ± 0.421	37.17 ± 0.034	39.67 ± 0.091	39.59 ± 0.133	39.86 ± 0.201	38.67 ± 0.311	38.45 ± 0.418
11	N3	100	36.93 ± 0.105	37.69 ± 0.052	39.80 ± 0.052	39.96 ± 0.092	39.82 ± 0.346	38.18 ± 0.155	38.20 ± 0.537
12	N4	100	36.32 ± 0.093	37.11 ± 0.031	39.30 ± 0.076	39.20 ± 0.157	38.30 ± 0.291	$37.80 \pm 0.137^*$	37.79 ± 0.024
13	N5	100	36.35 ± 0.211	37.06 ± 0.037	39.20 ± 0.034	39.49 ± 0.093	39.61 ± 0.424	38.28 ± 0.184	38.03 ± 0.455
14	N6	100	36.87 ± 0.166	37.03 ± 0.074	39.79 ± 0.158	39.70 ± 0.101	39.63 ± 0.132	38.45 ± 0.312	38.30 ± 0.321
15	B7	100	36.30 ± 0.365	37.29 ± 0.043	39.40 ± 0.102	39.61 ± 0.084	39.80 ± 0.271	38.11 ± 0.271	38.05 ± 0.334
16	B8	100	36.10 ± 0.365	37.09 ± 0.043	39.68 ± 0.102	39.82 ± 0.084	39.43 ± 0.271	38.19 ± 0.271	38.06 ± 0.334

			0.307	0.012	0.164	0.141	0.172	0.022	0.236
17	B9	100	36.15 ± 0.211	37.13 ± 0.031	39.67 ± 0.205	39.83 ± 0.083	39.83 ± 0.245	38.41 ± 0.153	38.20 ± 0.372
18	T7	100	36.65 ± 0.365	37.32 ± 0.088	39.69 ± 0.126	39.77 ± 0.241	39.86 ± 0.284	38.26 ± 0.056	38.09 ± 0.280
19	T8	100	36.40 ± 0.333	37.32 ± 0.013	39.40 ± 0.087	38.90 ± 0.283	39.10 ± 0.273	37.90 ± 0.085*	37.71 ± 0.035
20	T9	100	36.25 ± 0.223	37.07 ± 0.015	39.83 ± 0.158	39.78 ± 0.211	39.85 ± 0.451	38.17 ± 0.312	38.07 ± 0.155
21	N7	100	36.84 ± 0.258	37.53 ± 0.052	39.68 ± 0.165	39.51 ± 0.267	39.79 ± 0.154	38.21 ± 0.357	38.10 ± 0.512
22	N8	100	36.50 ± 0.183	37.05 ± 0.022	39.88 ± 0.177	39.20 ± 0.136	38.59 ± 0.178	37.87 ± 0.214*	37.83 ± 0.013
23	N9	100	36.26 ± 0.211	37.16 ± 0.026	39.82 ± 0.174	39.86 ± 0.182	38.85 ± 0.071	38.10 ± 0.212	38.06 ± 0.082
24	B10	100	36.89 ± 0.187	37.48 ± 0.057	39.65 ± 0.083	39.78 ± 0.138	39.14 ± 0.036	38.02 ± 0.201	38.42 ± 0.112
25	B11	100	36.20 ± 0.258	37.11 ± 0.055	39.90 ± 0.182	39.50 ± 0.176	39.92 ± 0.053	38.08 ± 0.342	38.04 ± 0.431
26	T10	100	36.30 ± 0.212	37.98 ± 0.014	39.87 ± 0.122	38.00 ± 0.021	37.40 ± 0.082*	37.80 ± 0.076*	38.16 ± 0.022
27	T11	100	36.19 ± 0.026	37.14 ± 0.098	39.51 ± 0.033	39.09 ± 0.159	37.99 ± 0.020	37.39 ± 0.064*	37.61 ± 0.015*
28	N10	100	36.22 ± 0.029	37.13 ± 0.100	39.49 ± 0.035	39.12 ± 0.156	38.01 ± 0.021	37.42 ± 0.063*	37.58 ± 0.016*
29	N11	100	36.49 ± 0.154	37.13 ± 0.032	39.84 ± 0.098	39.67 ± 0.273	39.79 ± 0.034	38.22 ± 0.241	38.10 ± 0.544

Values are expressed as mean ± SEM (n = 6). Data were analyzed by one-way ANOVA followed by Dunnett's multiple-comparison test. p< 0.05, p< 0.01, and p<0.001 compared with the control group.

Figure6.Effect of synthesized compounds on Brewer's yeast-induced pyrexia

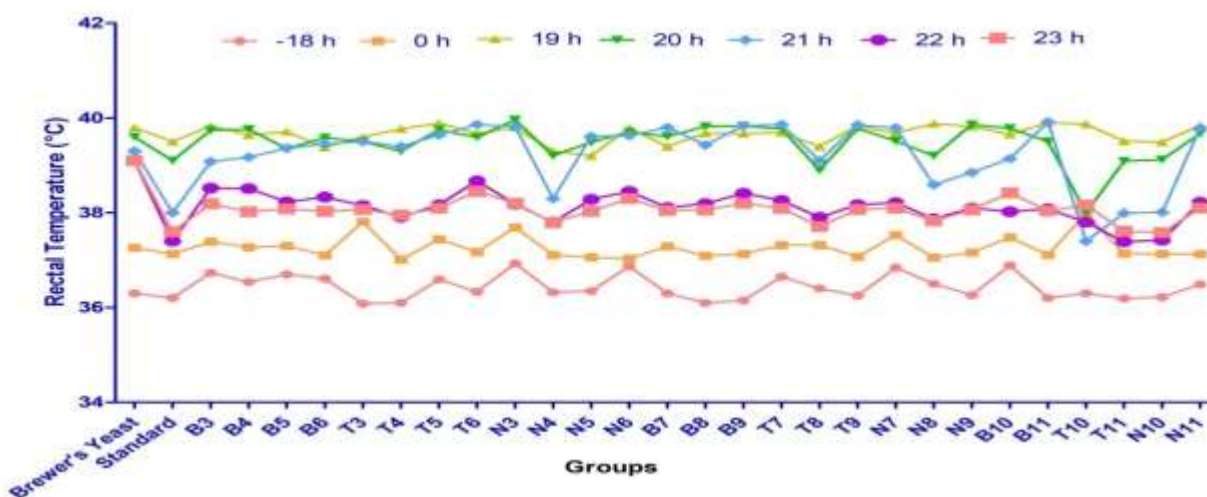


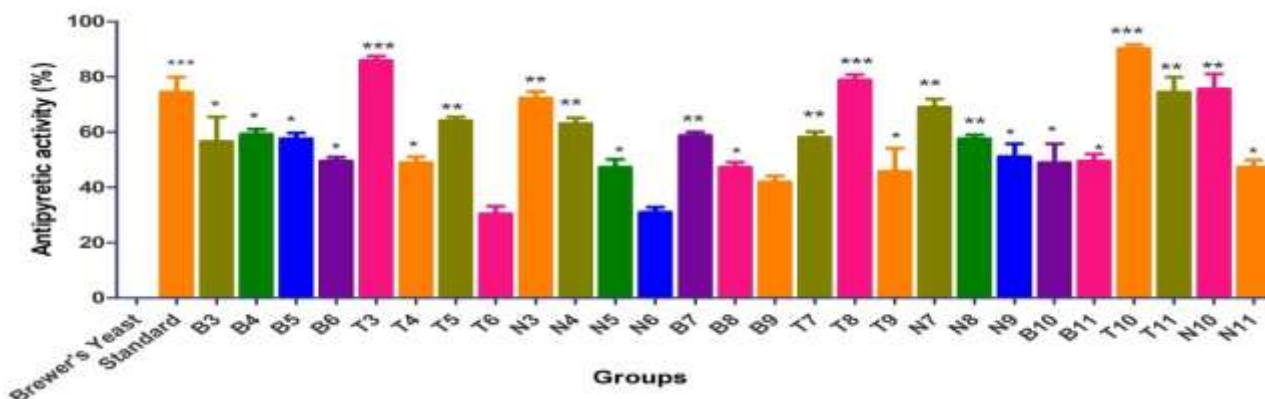
Table 6. Antipyretic activity of synthesized compounds against Brewer's yeast-induced pyrexia

S. No.	Treatment	Dose (mg/kg)	Change in rectal temperature (°C)	Antipyretic activity (%)
1	Brewer's Yeast	5 mL	1.84 ± 0.447	—
2	Standard	100	0.47 ± 0.100	74.46 ± 5.43*
3	B3	100	0.80 ± 0.165	56.52 ± 8.97

4	B4	100	0.75 ± 0.291	59.24 ± 1.82
5	B5	100	0.78 ± 0.480	57.61 ± 2.09
6	B6	100	0.93 ± 0.321	49.46 ± 1.45
7	T3	100	0.26 ± 0.362	85.87 ± 1.67
8	T4	100	0.94 ± 0.224	48.91 ± 2.17*
9	T5	100	0.66 ± 0.339	64.13 ± 1.42
10	T6	100	1.28 ± 0.419	30.43 ± 2.77
11	N3	100	0.51 ± 0.540	72.28 ± 2.35
12	N4	100	0.68 ± 0.039	63.04 ± 2.12*
13	N5	100	0.97 ± 0.457	47.28 ± 2.84
14	N6	100	1.27 ± 0.329	30.98 ± 1.88
15	B7	100	0.76 ± 0.337	58.70 ± 1.32
16	B8	100	0.97 ± 0.236	47.28 ± 1.83
17	B9	100	1.07 ± 0.373	41.85 ± 2.27
18	T7	100	0.77 ± 0.294	58.15 ± 1.98
19	T8	100	0.39 ± 0.037	78.80 ± 2.01*
20	T9	100	1.00 ± 0.156	45.65 ± 8.48
21	N7	100	0.57 ± 0.515	69.02 ± 2.99
22	N8	100	0.78 ± 0.026	57.61 ± 1.41*
23	N9	100	0.90 ± 0.086	51.09 ± 4.67
24	B10	100	0.94 ± 0.126	48.91 ± 6.85
25	B11	100	0.93 ± 0.434	49.46 ± 2.59
26	T10	100	0.18 ± 0.026	90.22 ± 1.41*
27	T11	100	0.47 ± 0.099	74.46 ± 5.38*
28	N10	100	0.45 ± 0.101	75.54 ± 5.49*
29	N11	100	0.97 ± 0.545	47.28 ± 2.62

Values are expressed as mean ± SEM (n = 6). Change in rectal temperature was calculated as the difference between the values at 23 h and 0 h. Antipyretic activity was calculated relative to the control group. Data were analyzed by one-way ANOVA followed by Dunnett's multiple-comparison test. *p< 0.05, **p< 0.01, and ***p< 0.001 compared with the control group.

Figure 7 Effect of synthesized compounds on brewer's yeast induced



Anti-inflammatory Activity

9. Carrageenan-Induced Rat Paw Edema

The anti-inflammatory activity of the synthesized compounds was evaluated using the carrageenan-induced rat paw edema model. The control group showed a progressive increase in paw edema, reaching a maximum mean paw edema volume of 1.864 ± 0.045 mL at 4 h. In comparison, the standard-treated group showed a marked

reduction in paw edema, with a mean volume of 0.416 ± 0.021 mL at 4 h, corresponding to $77.68 \pm 1.25\%$ inhibition of inflammation.

Among the synthesized compounds, considerable anti-inflammatory activity was observed with **T3**, which produced $74.84 \pm 1.87\%$ inhibition, followed by T10 ($70.17 \pm 3.04\%$), N11 ($65.72 \pm 1.05\%$), N9 ($62.77 \pm 1.94\%$), N4 ($59.17 \pm 2.12\%$), and T4 ($52.15 \pm 3.07\%$). These compounds showed substantially lower paw edema volumes at later observation periods compared with the control group.

Moderate inhibition was observed with N3 ($49.20 \pm 1.39\%$), T5 ($45.01 \pm 1.48\%$), T7 ($21.14 \pm 1.99\%$), B4 ($19.90 \pm 2.97\%$), B6 ($14.91 \pm 2.45\%$), and B3 ($11.70 \pm 2.24\%$). The remaining compounds exhibited relatively lower inhibitory effects. The least inhibition was observed with B9 ($1.18 \pm 2.64\%$), followed by T8 ($1.88 \pm 2.65\%$), N7 (**$3.59 \pm 2.66\%$**), and N6 ($4.72 \pm 3.66\%$).

Overall, T3 demonstrated the most pronounced anti-inflammatory activity among the synthesized compounds, showing an inhibitory effect approaching that of the standard. T10, N11, N9 and N4 also exhibited notable activity. The reduction in paw edema observed with the more active compounds, particularly during the 2–4 h observation period, indicates their potential to attenuate carrageenan-induced inflammatory response.

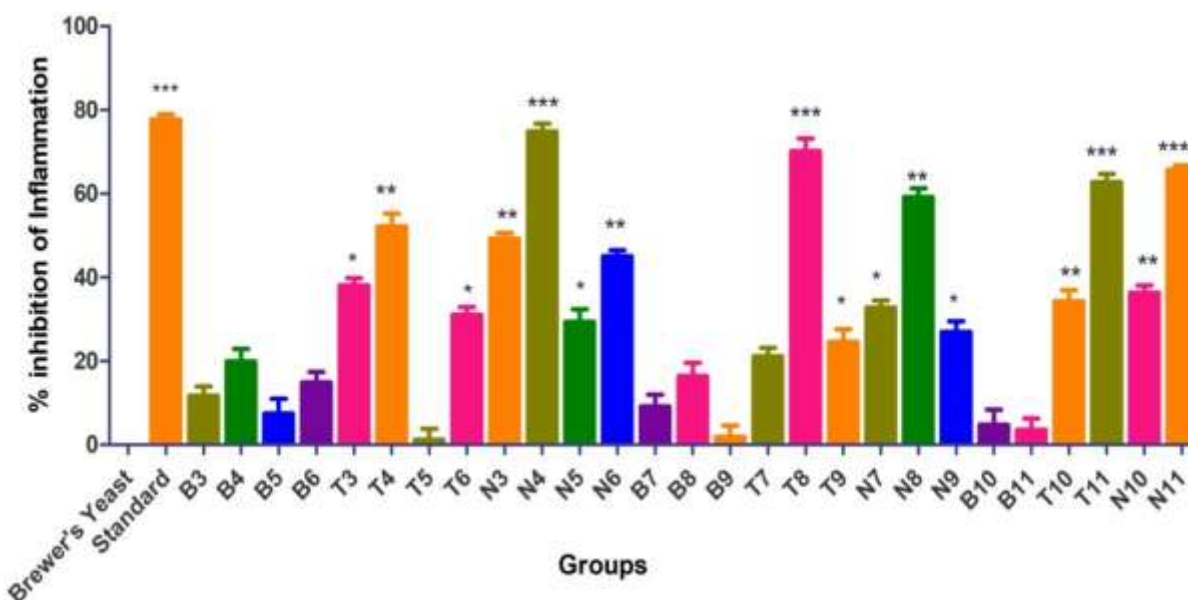
Table 7. Effect of synthesized compounds on carrageenan-induced rat paw edema

S. No.	Compound	0 h	1 h	2 h	3 h	4 h	% Inhibition of Inflammation
1	Control	0.794 ± 0.012	1.223 ± 0.034	1.577 ± 0.053	1.751 ± 0.014	1.864 ± 0.045	—
2	Standard	0.763 ± 0.045	$0.524 \pm 0.053^{***}$	$0.581 \pm 0.035^{***}$	$0.694 \pm 0.042^{***}$	$0.416 \pm 0.021^{***}$	$77.68 \pm 1.25^*$
3	B3	0.752 ± 0.033	1.381 ± 0.032	1.606 ± 0.014	1.653 ± 0.032	1.646 ± 0.013	11.70 ± 2.24
4	B4	0.731 ± 0.022	1.465 ± 0.024	1.535 ± 0.042	1.583 ± 0.020	1.493 ± 0.042	19.90 ± 2.97
5	B5	0.714 ± 0.044	1.303 ± 0.015	1.778 ± 0.021	1.884 ± 0.012	1.725 ± 0.051	7.46 ± 3.53
6	B6	0.727 ± 0.031	1.255 ± 0.052	1.535 ± 0.054	1.565 ± 0.040	1.586 ± 0.025	14.91 ± 2.45
7	T3	0.698 ± 0.025	1.214 ± 0.021	$1.097 \pm 0.022^*$	$1.106 \pm 0.022^{**}$	$1.154 \pm 0.014^{***}$	$38.09 \pm 1.67^*$
8	T4	0.703 ± 0.013	$0.688 \pm 0.044^{***}$	$0.753 \pm 0.044^{***}$	$0.808 \pm 0.051^{***}$	$0.892 \pm 0.053^{***}$	$52.15 \pm 3.07^*$
9	T5	0.705 ± 0.042	1.309 ± 0.025	1.797 ± 0.023	1.827 ± 0.013	1.842 ± 0.021	1.18 ± 2.64
10	T6	0.756 ± 0.034	1.133 ± 0.043	1.288 ± 0.015	1.306 ± 0.044	$1.285 \pm 0.014^*$	$31.06 \pm 1.83^*$
11	N3	0.737 ± 0.023	$0.794 \pm 0.011^{**}$	$0.855 \pm 0.043^*$	$0.985 \pm 0.051^{**}$	$0.947 \pm 0.012^{***}$	$49.20 \pm 1.39^*$
12	N4	0.694 ± 0.012	$0.580 \pm 0.054^{***}$	$0.412 \pm 0.022^{***}$	$0.493 \pm 0.023^{***}$	$0.469 \pm 0.033^{***}$	$74.84 \pm 1.87^*$
13	N5	0.707 ± 0.053	1.158 ± 0.025	1.301 ± 0.011	1.385 ± 0.042	1.316 ± 0.045	29.40 ± 2.96
14	N6	0.801 ± 0.041	$1.050 \pm 0.032^*$	$1.080 \pm 0.034^*$	$1.046 \pm 0.021^*$	$1.025 \pm 0.012^{**}$	45.01 ± 1.48
15	B7	0.789 ± 0.033	1.343 ± 0.051	1.679 ± 0.042	1.727 ± 0.043	1.694 ± 0.033	9.12 ± 2.82
16	B8	0.792 ± 0.024	1.222 ± 0.025	1.495 ± 0.024	1.487 ± 0.012	1.558 ± 0.044	16.42 ± 3.11
17	B9	0.737 ± 0.032	1.281 ± 0.012	1.804 ± 0.012	1.869 ± 0.041	1.829 ± 0.022	1.88 ± 2.65

18	T7	0.808 ± 0.041	1.374 ± 0.051	1.422 ± 0.055	1.418 ± 0.014	1.470 ± 0.011	21.14 ± 1.99
19	T8	0.779 ± 0.025	0.686 ± 0.024***	0.531 ± 0.032***	0.626 ± 0.045***	0.556 ± 0.055***	70.17 ± 3.04*
20	T9	0.740 ± 0.041	1.365 ± 0.044	1.406 ± 0.024	1.464 ± 0.012	1.405 ± 0.044	24.62 ± 2.98
21	N7	0.789 ± 0.013	0.963 ± 0.022*	1.217 ± 0.024	1.285 ± 0.021	1.253 ± 0.011	32.78 ± 1.73
22	N8	0.728 ± 0.044	0.812 ± 0.021*	0.686 ± 0.013***	0.776 ± 0.025***	0.761 ± 0.035***	59.17 ± 2.12*
23	N9	0.698 ± 0.024	1.161 ± 0.045	1.385 ± 0.055	1.308 ± 0.023	1.362 ± 0.035*	26.93 ± 2.58*
24	B10	0.727 ± 0.022	1.907 ± 0.023	1.753 ± 0.032	1.680 ± 0.012	1.776 ± 0.053	4.72 ± 3.66
25	B11	0.718 ± 0.033	1.688 ± 0.052	1.812 ± 0.032	1.837 ± 0.041	1.797 ± 0.024	3.59 ± 2.66
26	T10	0.705 ± 0.034	1.135 ± 0.034	1.357 ± 0.034	1.304 ± 0.011	1.225 ± 0.041*	34.28 ± 2.71*
27	T11	0.690 ± 0.041	0.700 ± 0.021***	0.554 ± 0.012***	0.706 ± 0.032***	0.694 ± 0.032***	62.77 ± 1.94*
28	N10	0.687 ± 0.022	1.126 ± 0.022	1.178 ± 0.045	1.155 ± 0.041*	1.187 ± 0.015*	36.32 ± 1.74*
29	N11	0.735 ± 0.012	0.753 ± 0.021***	0.629 ± 0.031***	0.591 ± 0.024***	0.639 ± 0.012***	65.72 ± 1.05*

Figure 8 Effect of synthesized compounds on carrageenan -induced rat paw edema

Percentage inhibition of inflammation by synthesized compounds



9. In-vitro Antibacterial Activity

The synthesized thiadiazole derivatives were evaluated for their in-vitro antibacterial activity against selected Gram-positive and Gram-negative bacterial strains. The antibacterial activity was assessed by the cup-plate method using Mueller–Hinton agar, and the zone of inhibition was measured after incubation at 37 °C for 24 h. The synthesized compounds were tested at concentrations of 50 and 100 µg/mL. Amoxycillin and ofloxacin were used

as reference antibacterial agents, while DMSO was used as the vehicle control where appropriate. Each experiment was performed in triplicate.

The bacterial strains used for the antibacterial evaluation included *Bacillus subtilis* ATCC 11774 and *Staphylococcus aureus* NTCC 6571 as Gram-positive organisms, and *Escherichia coli* TG14 and *Pseudomonas aeruginosa* ATCC 9027 as Gram-negative organisms.

9.1 Antibacterial activity against Gram-positive bacteria

The antibacterial activity of the synthesized compounds against *S. aureus* and *B. subtilis* is presented in Table 9.1. The synthesized derivatives exhibited variable antibacterial activity, as evidenced by the differences in the zones of inhibition at 50 and 100 µg/mL.

Among the synthesized compounds, B11 and T11 exhibited the most pronounced activity against *S. aureus*, producing zones of inhibition of 17 and 19 mm at 50 and 100 µg/mL, respectively. N11 also showed comparatively good activity, with zones of 15 and 17 mm at the respective concentrations. T6 and N6 produced zones of 14 and 16 mm against *S. aureus*.

Against *B. subtilis*, B11 showed the highest activity among the synthesized derivatives, with zones of inhibition of 14 and 16 mm at 50 and 100 µg/mL, respectively. T11 and N11 exhibited zones of 13 and 15 mm at both concentrations. B6, T6 and N6 also demonstrated comparatively good activity.

In general, increasing the concentration from 50 to 100 µg/mL resulted in an increase in the zone of inhibition for most compounds. The antibacterial activity of the synthesized derivatives was lower than that of the reference antibacterial agents, amoxycillin and ofloxacin, which exhibited comparatively larger zones of inhibition.

Table8. Antibacterial activity of synthesized compounds against Gram-positive bacteria by cup-plate method

Compound	<i>S. aureus</i> 50 µg/mL	<i>S. aureus</i> 100 µg/mL	<i>B. subtilis</i> 50 µg/mL	<i>B. subtilis</i> 100 µg/mL
B3	9.00 ± 0.29	10.00 ± 0.29	9.00 ± 0.29	10.00 ± 0.29
B4	10.00 ± 0.29	11.00 ± 0.29	10.00 ± 0.29	12.00 ± 0.29
B5	11.00 ± 0.29	13.00 ± 0.29	11.00 ± 0.29	13.00 ± 0.29
B6	13.00 ± 0.29	15.00 ± 0.29	13.00 ± 0.29	15.00 ± 0.29
T3	9.00 ± 0.29	10.00 ± 0.29	9.00 ± 0.29	10.00 ± 0.29
T4	10.00 ± 0.29	12.00 ± 0.29	10.00 ± 0.29	12.00 ± 0.29
T5	12.00 ± 0.29	14.00 ± 0.29	10.00 ± 0.29	12.00 ± 0.29
T6	14.00 ± 0.29	16.00 ± 0.29	13.00 ± 0.29	15.00 ± 0.29
N3	9.00 ± 0.29	10.00 ± 0.29	9.00 ± 0.29	10.00 ± 0.29
N4	10.00 ± 0.29	12.00 ± 0.29	10.00 ± 0.29	12.00 ± 0.29
N5	12.00 ± 0.29	14.00 ± 0.29	10.00 ± 0.29	12.00 ± 0.29
N6	14.00 ± 0.29	16.00 ± 0.29	13.00 ± 0.29	15.00 ± 0.29
B7	9.00 ± 0.29	10.00 ± 0.29	9.00 ± 0.29	10.00 ± 0.29
B8	12.00 ± 0.29	14.00 ± 0.29	10.00 ± 0.29	12.00 ± 0.29
B9	9.00 ± 0.29	10.00 ± 0.29	9.00 ± 0.29	10.00 ± 0.29
T7	11.00 ± 0.29	12.00 ± 0.29	10.00 ± 0.29	12.00 ± 0.29
T8	14.00 ± 0.29	16.00 ± 0.29	10.00 ± 0.29	12.00 ± 0.29
T9	9.00 ± 0.29	10.00 ± 0.29	9.00 ± 0.29	10.00 ± 0.29
N7	12.00 ± 0.29	14.00 ± 0.29	9.00 ± 0.29	10.00 ± 0.29
N8	10.00 ± 0.29	12.00 ± 0.29	10.00 ± 0.29	12.00 ± 0.29
N9	9.00 ± 0.29	10.00 ± 0.29	9.00 ± 0.29	10.00 ± 0.29
B10	12.00 ± 0.29	14.00 ± 0.29	10.00 ± 0.29	12.00 ± 0.29
B11	17.00 ± 0.29	19.00 ± 0.29	14.00 ± 0.29	16.00 ± 0.29
T10	14.00 ± 0.29	16.00 ± 0.29	10.00 ± 0.29	12.00 ± 0.29
T11	17.00 ± 0.29	19.00 ± 0.29	13.00 ± 0.29	15.00 ± 0.29
N10	12.00 ± 0.29	14.00 ± 0.29	10.00 ± 0.29	12.00 ± 0.29
N11	15.00 ± 0.29	17.00 ± 0.29	13.00 ± 0.29	15.00 ± 0.29
Amoxycillin	18.00 ± 0.29	21.00 ± 0.29	17.00 ± 0.29	20.00 ± 0.29
Ofloxacin	21.00 ± 0.29	24.00 ± 0.29	20.00 ± 0.29	23.00 ± 0.29

Values are expressed as zone of inhibition diameter (mm). The experiment was performed in triplicate.

Figure:9

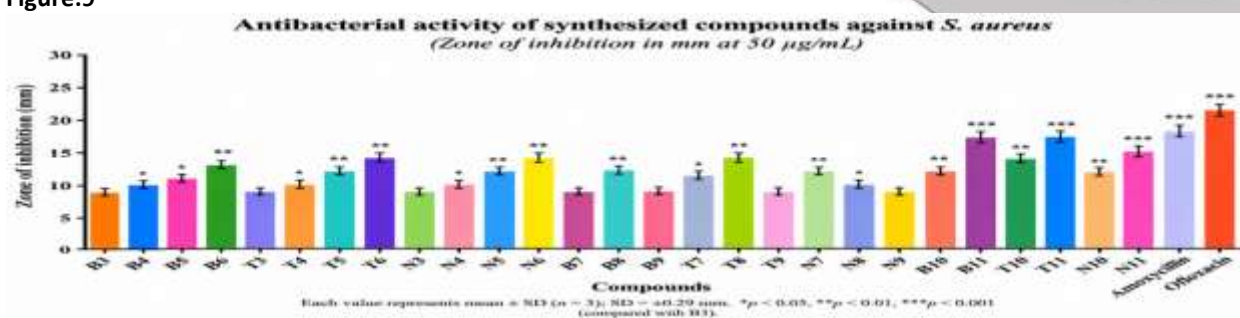


Figure:9.1

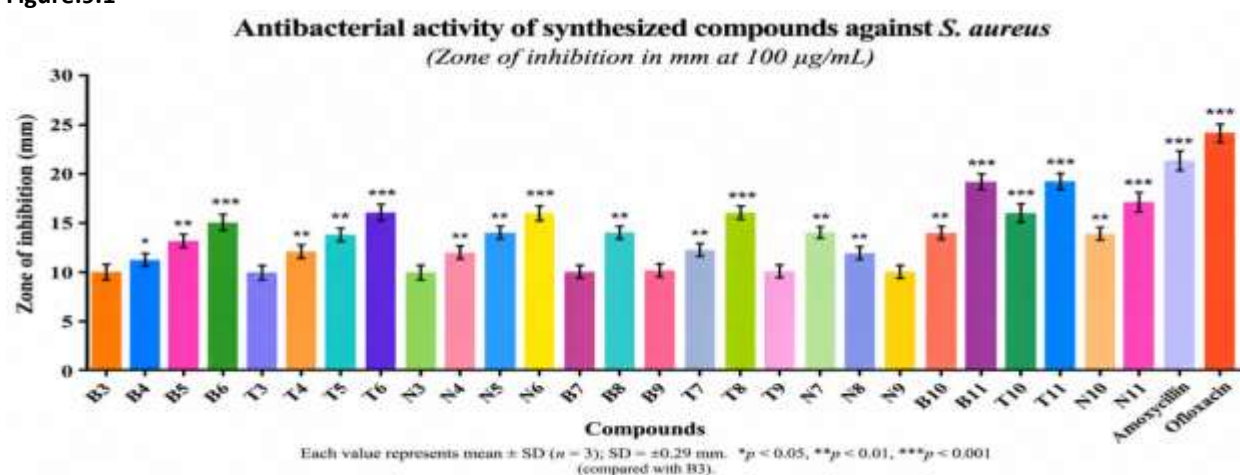


Figure:9.1.3

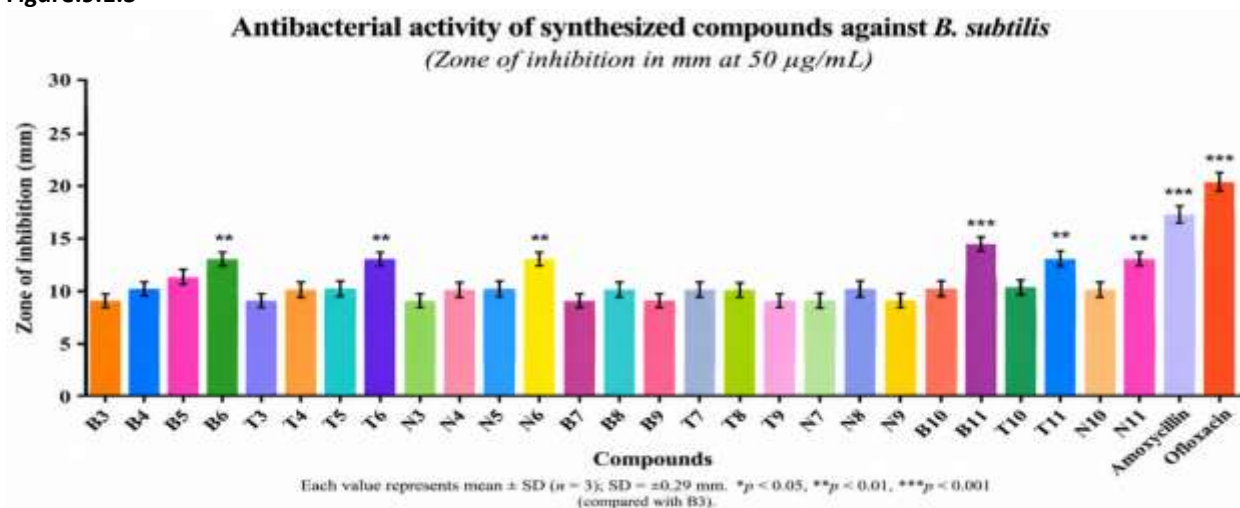
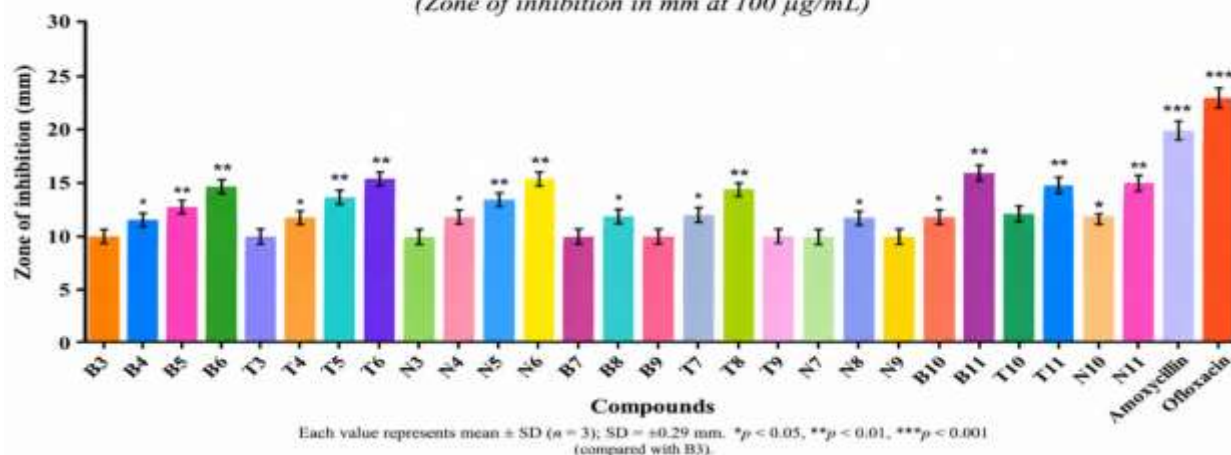


Figure:9.2

Antibacterial activity of synthesized compounds against *B. subtilis* (Zone of inhibition in mm at 100 µg/mL)



9.2 Minimum Inhibitory Concentration against Gram-positive bacteria

The MIC of the synthesized compounds was determined as the lowest concentration that completely inhibited visible bacterial growth. The compounds were initially screened at 1000, 500 and 250 µg/mL, followed by further dilution of active compounds to 200, 100, 62.5, 50, 25, 12.5 and 6.25 µg/mL.

The MIC results demonstrated concentration-dependent differences in the antibacterial potency of the synthesized derivatives. Compounds B11 and T11 exhibited the lowest MIC against *S. aureus* (50 µg/mL), indicating comparatively higher antibacterial potency. N11 showed an MIC of 100 µg/mL. T6, N6, T8 and T10 exhibited MIC values of 200 µg/mL against *S. aureus*. Most of the remaining derivatives showed MIC values between 250 and 1000 µg/mL.

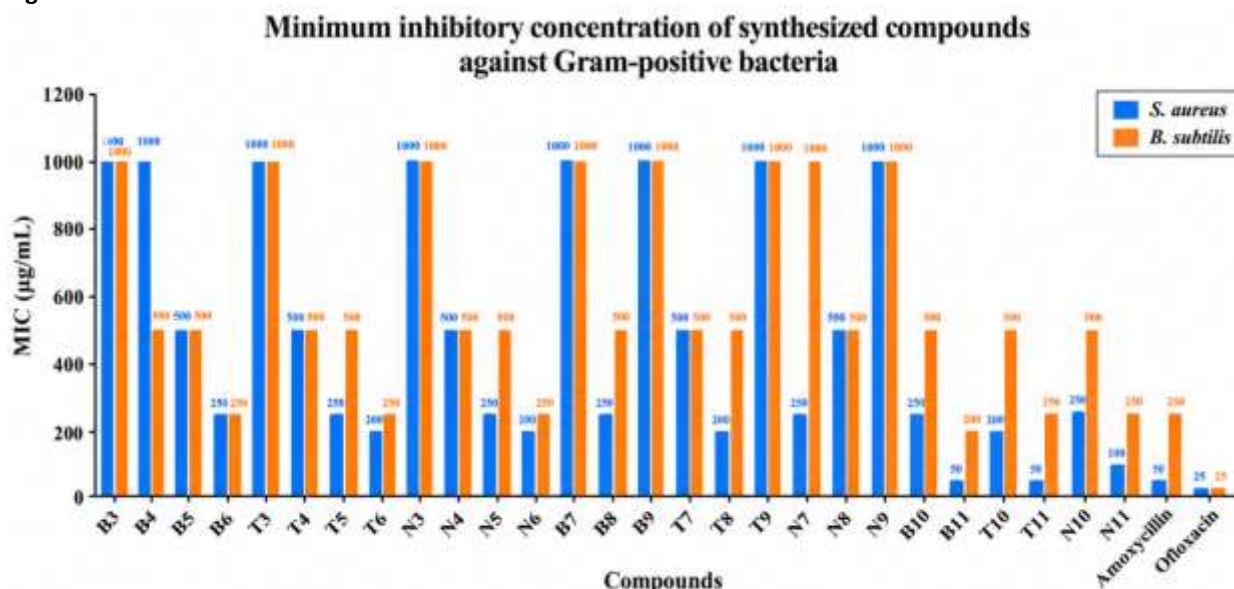
Against *B. subtilis*, B11 showed the lowest MIC among the synthesized compounds (200 µg/mL), followed by B6, T6, N6, T11 and N11, which exhibited MIC values of 250 µg/mL. The compounds with MIC values of 500–1000 µg/mL showed comparatively weaker antibacterial activity.

Table 9. Minimum inhibitory concentration of synthesized compounds against Gram-positive bacteria

Compound	<i>S. aureus</i> MIC (µg/mL)	<i>B. subtilis</i> MIC (µg/mL)
B3	1000	1000
B4	1000	500
B5	500	500
B6	250	250
T3	1000	1000
T4	500	500
T5	250	500
T6	200	250
N3	1000	1000
N4	500	500
N5	250	500
N6	200	250
B7	1000	1000
B8	250	500
B9	1000	1000
T7	500	500
T8	200	500
T9	1000	1000
N7	250	1000
N8	500	500
N9	1000	1000

B10	250	500
B11	50	200
T10	200	500
T11	50	250
N10	250	500
N11	100	250
Amoxycillin	50	250
Ofloxacin	25	25

Fig:9.2.2



9.3 Antibacterial activity against Gram-negative bacteria

The synthesized compounds were also evaluated against *E. coli* TG14 and *P. aeruginosa* ATCC 9027, which were included as the Gram-negative test organisms in the antibacterial evaluation.

Table 10. antibacterial activity of synthesized compounds against Gram-negative bacteria by cup-plate method

Compound	<i>E. coli</i> 50 µg/mL	<i>E. coli</i> 100 µg/mL	<i>P. aeruginosa</i> 50 µg/mL	<i>P. aeruginosa</i> 100 µg/mL
B3	8	9	8	9
B4	9	10	8	10
B5	10	12	9	11
B6	12	14	11	13
T3	8	9	8	9
T4	9	11	9	10
T5	10	12	9	11
T6	12	14	11	13
N3	8	9	8	9
N4	9	11	9	10
N5	10	12	9	11
N6	12	14	11	13
B7	8	9	8	9
B8	11	13	10	12
B9	8	9	8	9
T7	10	12	9	11

T8	12	14	11	13
T9	8	9	8	9
N7	10	12	9	11
N8	9	11	9	10
N9	8	9	8	9
B10	11	13	10	12
B11	15	17	13	15
T10	12	14	10	12
T11	15	17	13	15
N10	11	13	10	12
N11	13	15	12	14
Amoxycillin	17	20	15	18
Ofloxacin	20	23	18	21

Fig:9.3.1

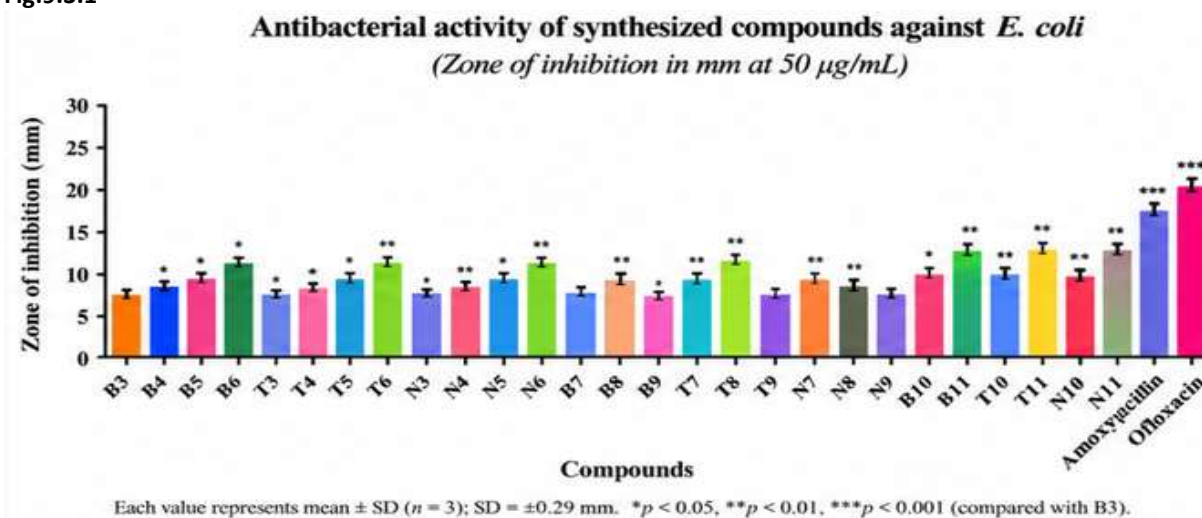


Fig:9.3.2

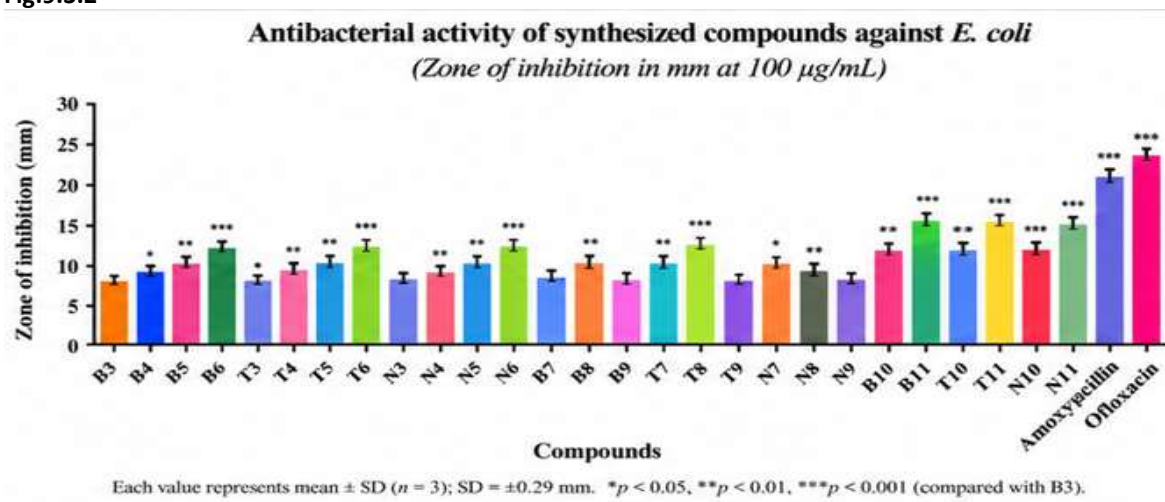
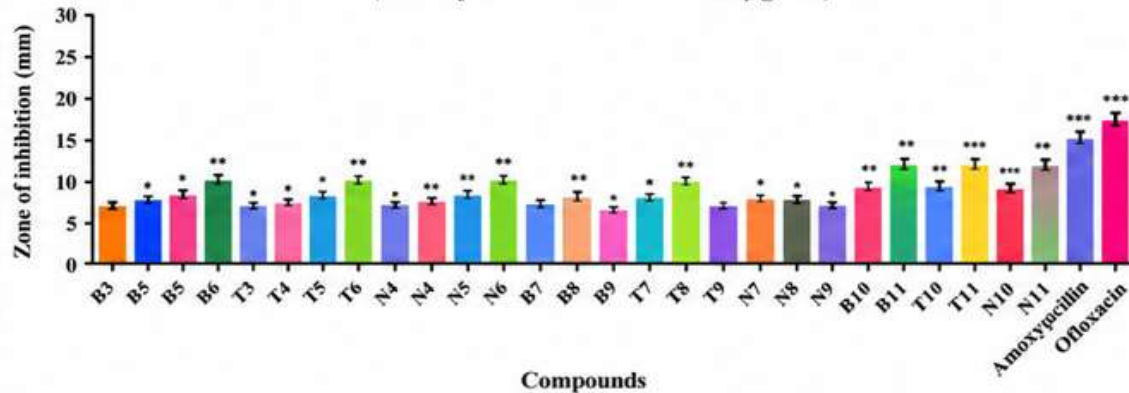


Fig:9.3.3

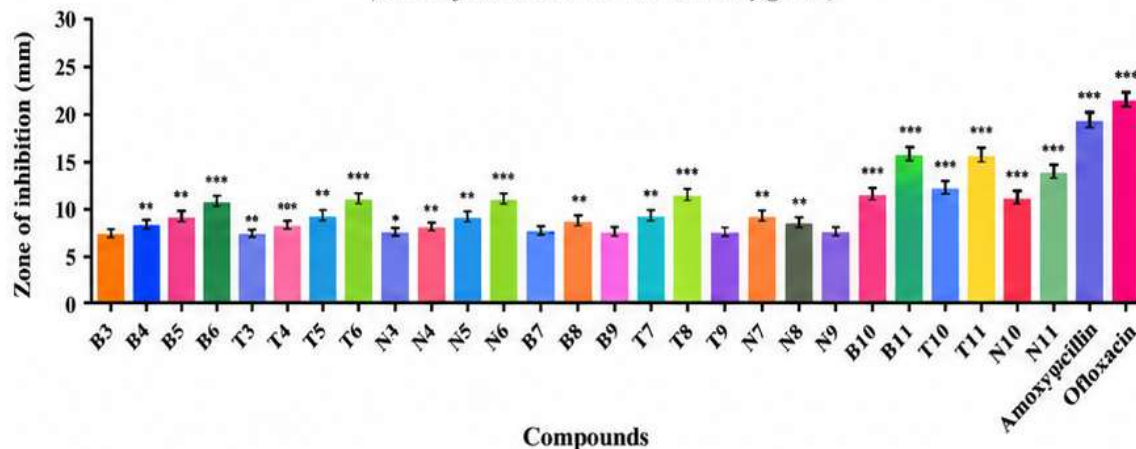
Antibacterial activity of synthesized compounds against *P. aeruginosa*
(Zone of inhibition in mm at 50 µg/mL)



Each value represents mean $\pm$ SD ($n = 3$); SD = ± 0.29 mm. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (compared with B3).

Fig:9.3.4

Antibacterial activity of synthesized compounds against *P. aeruginosa*
(Zone of inhibition in mm at 100 µg/mL)



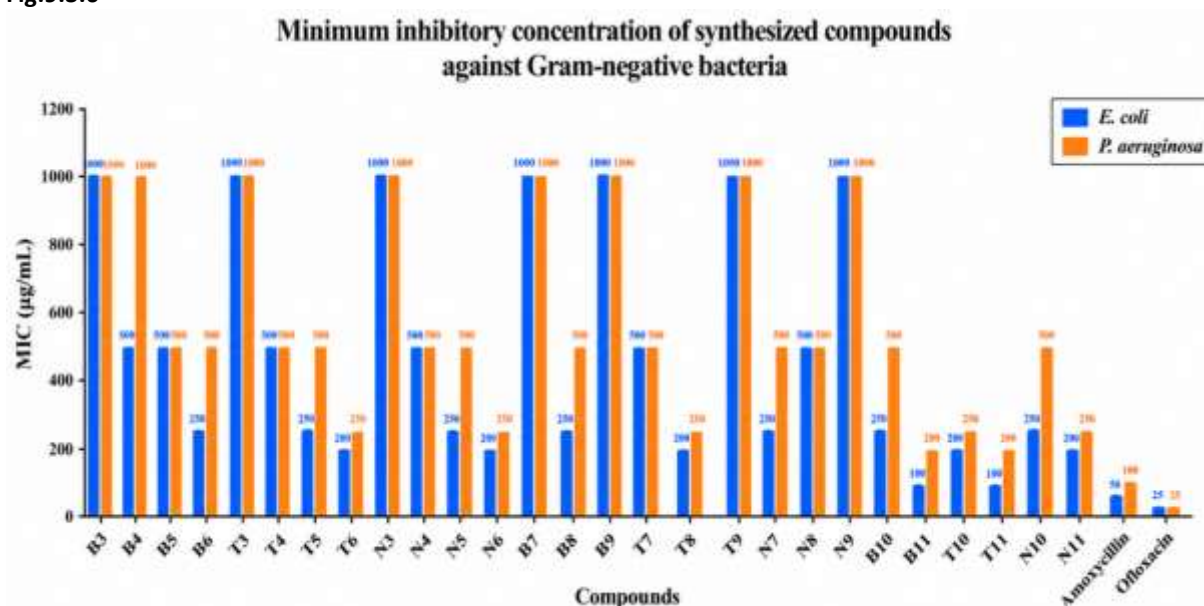
Each value represents mean $\pm$ SD ($n = 3$); SD = ± 0.29 mm. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (compared with B3).

Table 9.3.5 MIC of synthesized Table.11 compounds against Gram-negative bacteria

Compound	<i>E. coli</i> MIC (µg/mL)	<i>P. aeruginosa</i> MIC (µg/mL)
B3	1000	1000
B4	500	1000
B5	500	500
B6	250	500
T3	1000	1000
T4	500	500
T5	250	500
T6	200	250
N3	1000	1000
N4	500	500
N5	250	500
N6	200	250
B7	1000	1000
B8	250	500
B9	1000	1000

T7	500	500
T8	200	250
T9	1000	1000
N7	250	500
N8	500	500
N9	1000	1000
B10	250	500
B11	100	200
T10	200	250
T11	100	200
N10	250	500
N11	200	250
Amoxycillin	50	100
Ofloxacin	25	25

Fig:9.3.6



Overall, the synthesized thiadiazole derivatives demonstrated variable antibacterial activity against the tested bacterial strains. The derivatives B11, T11 and N11 emerged as comparatively promising members of the series, particularly against *S. aureus*. The activity observed by the cup-plate method was broadly reflected by the MIC results, with compounds producing larger inhibition zones generally demonstrating lower MIC values. The antibacterial activity of the synthesized compounds was lower than that of the reference antibacterial agents, although selected derivatives showed appreciable activity and may warrant further investigation.

10. In-vitro Antioxidant Activity

10.1. DPPH Radical-Scavenging Activity (DPPH = 2,2-diphenyl-1-picrylhydrazyl)

% Scavenging = [(Absorbance of Control - Absorbance of Test) / Absorbance of Control] x 100

Your values are % Scavenging ± SEM. Higher % = Better antioxidant.

Standard: Usually Ascorbic Acid. 73.15% at 4 µg/mL, 80.95% at 8 µg/mL, 83.82% at 10 µg/mL - shows model is working.

When an antioxidant donates hydrogen/electron to DPPH, it is reduced to DPPH-H, yellow in colour. The decrease in absorbance at 517 nm is proportional to antioxidant activity

The synthesized compounds demonstrated concentration-dependent DPPH radical-scavenging activity. In general, the percentage inhibition increased with increasing concentration from 4 to 10 µg/mL. The magnitude of activity varied considerably among the different structural series.

Among the compounds listed in the results, the B-series showed comparatively higher activity than the T-series at 10 µg/mL. B6 showed $68.104 \pm 0.124\%$ inhibition, while B3, B4 and B5 showed $67.109 \pm 0.012\%$, $67.244 \pm 0.128\%$ and $67.585 \pm 0.128\%$, respectively.

The later derivatives also showed increased activity in some cases. The source data include B10/m1a, B11/m1b and other derivatives for comparison. Test compound/ascorbic acid solution.

Reagent Preparation:

- **DPPH solution 0.1 mM:** Dissolve 3.94 mg DPPH in 100 mL methanol. Prepare fresh, keep in dark.
- **Standard and Test solutions:** Dissolve compounds in methanol/DMSO to make stock 1 mg/mL, then dilute to working concentrations 4, 8, 10 µg/mL as in your table.

Procedure

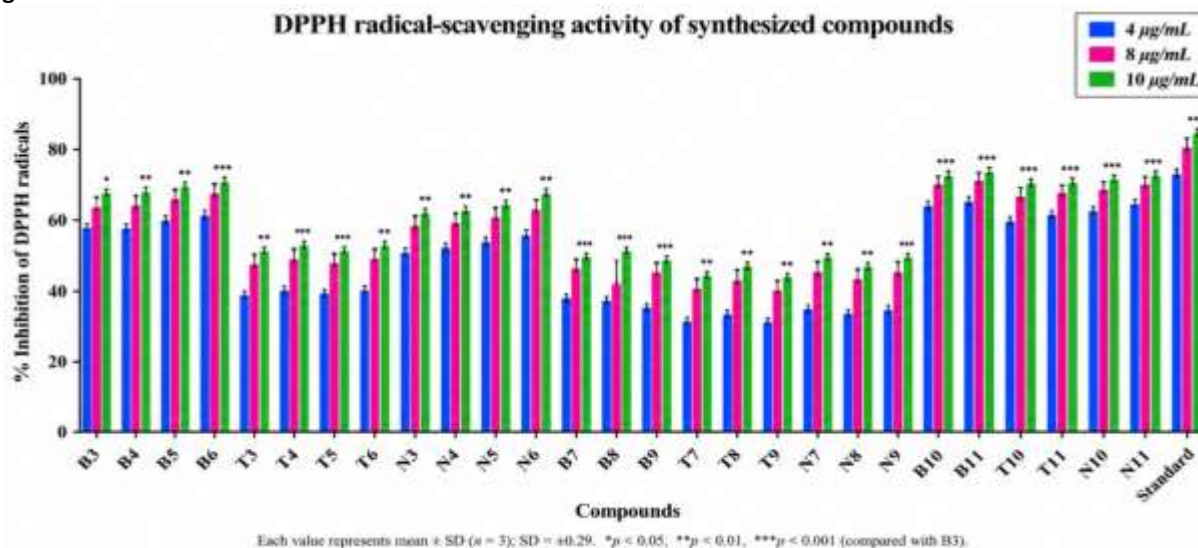
1. Take 3 mL of 0.1 mM DPPH methanolic solution in each test tube wrapped with aluminium foil [light sensitive].
2. Add 1 mL of test compound at different concentrations:
 - Tube 1: 1 mL of 4 µg/mL
 - Tube 2: 1 mL of 8 µg/mL
 - Tube 3: 1 mL of 10 µg/mL
3. For Control: Add 1 mL methanol instead of test compound to 3 mL DPPH solution. Absorbance of control is taken as 100% radical.
4. For Standard: Same as test - 1 mL Ascorbic acid [4, 8, 10 µg/mL] + 3 mL DPPH.
5. For Blank: Methanol only.
6. Vortex and incubate all tubes in dark at room temperature for 30 minutes. DPPH is light sensitive.
7. Measure absorbance at 517 nm against blank using UV-Vis spectrophotometer.

Table 12. DPPH radical-scavenging activity of synthesized compounds

Compounds	4 µg/mL	8 µg/mL	10 µg/mL
B3	57.791±0.054	64.791±0.023	67.109±0.012
B4	57.852±0.150	64.752±0.175	67.244±0.128
B5	58.271±0.029	65.063±0.072	67.585±0.128
B6	62.282±0.036	65.735±0.065	68.104±0.124
T3	39.745±0.145	48.916±0.007	51.191±0.027
T4	42.120±0.026	50.953±0.016	53.085±0.110
T5	40.730±0.008	50.064±0.126	52.452±0.132
T6	43.542±0.063	51.347±0.052	53.542±0.105
N3	51.750±0.128	59.553±0.142	61.107±0.131
N4	54.815±0.015	60.103±0.187	61.853±0.095
N5	55.882±0.045	60.421±0.181	62.752±0.121
N6	57.098±0.044	62.862±0.156	65.634±0.053
B7	39.954±0.096	48.203±0.065	50.457±0.176
B8	38.601±0.023	40.392±0.073	50.867±0.412
B9	36.856±0.086	46.924±0.037	48.855±0.122
T7	32.865±0.056	42.674±0.023	45.395±0.703
T8	33.982±0.130	44.141±0.096	46.252±0.165
T9	31.847±0.040	41.027±0.094	44.408±0.171
N7	37.704±0.093	47.756±0.015	49.852±0.132
N8	35.813±0.132	44.950±0.131	46.851±0.185
N9	36.037±0.071	45.504±0.087	47.387±0.155
B10	63.914±0.069	68.559±0.145	70.254±0.106

B11	64.594±0.088	68.922±0.154	70.784±0.104
T10	60.796±0.025	66.075±0.138	68.466±0.090
T11	61.384±0.028	66.451±0.063	68.859±0.112
N10	62.400±0.035	67.154±0.073	69.585±0.108
N11	63.415±0.148	67.783±0.140	69.885±0.152
Standard	73.15±0.045	80.954±0.039	83.826±0.081

Fig:10.1.2



10.2. Nitric Oxide-Scavenging Activity

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates Nitric Oxide. NO reacts with oxygen to form nitrite, which is measured by Griess reagent - pink chromophore.

Sodium nitroprusside in aqueous phosphate-buffered saline at physiological pH generates nitric oxide (NO). In the presence of oxygen, NO forms reactive nitrogen species, including nitrite. The nitrite produced reacts with sulphanilic acid under acidic conditions to form a diazonium compound. This subsequently reacts with naphthyl ethylene diamine dihydrochloride (NED) to produce a pink-coloured azo chromophore.

The intensity of the pink colour is measured spectrophotometrically at 640 nm. A compound with NO-scavenging activity reduces the amount of nitrite formed, resulting in lower absorbance compared with the control

An antioxidant / scavenger reduces the nitrite formation. More reduction = higher % scavenging = better NO scavenger.

% NO Scavenging = $[(\text{Absorbance Control} - \text{Absorbance Test}) / \text{Absorbance Control}] \times 100$

The synthesized compounds demonstrated concentration-dependent nitric oxide-scavenging activity. The percentage inhibition increased with increasing concentration from 4 to 10 µg/mL, although the magnitude of the response varied among the different structural series.

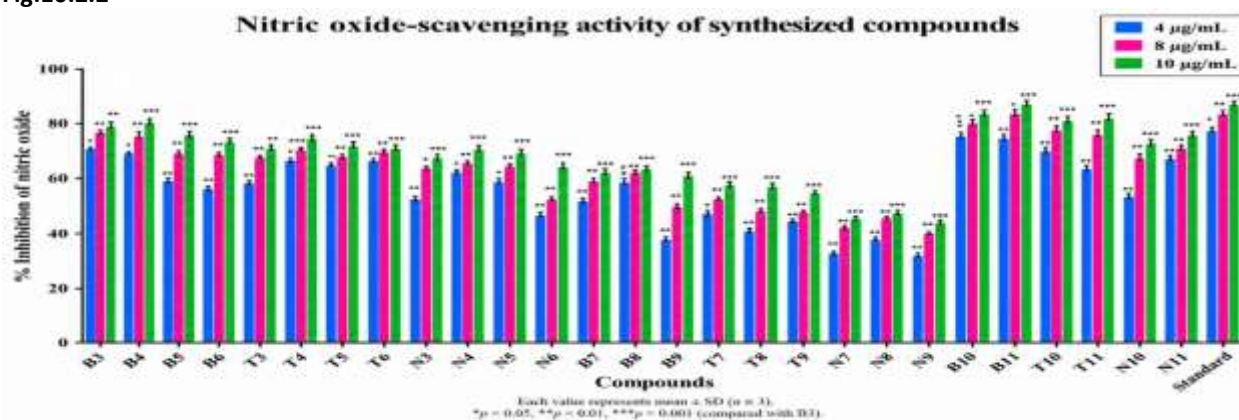
Among the compounds represented in the available data, B4 produced $78.998 \pm 0.084\%$ inhibition at 10 µg/mL, followed by B3 with $77.892 \pm 0.145\%$. B5 and B6 showed $75.985 \pm 0.145\%$ and $73.125 \pm 0.128\%$ inhibition, respectively.

Table 13 Nitric oxide-scavenging activity of synthesized compounds

Compounds	4 µg/mL	8 µg/mL	10 µg/mL
B3	70.415±0.055	76.605±0.054	77.892±0.145
B4	69.460±0.015	75.968±0.170	78.998±0.084
B5	59.906±0.163	69.275±0.155	75.985±0.145
B6	56.834±0.015	69.157±0.134	73.125±0.128
T3	58.205±0.164	68.003±0.160	70.885±0.142

T4	66.757±0.135	69.828±0.150	72.864±0.096
T5	64.454±0.012	67.454±0.124	71.309±0.130
T6	65.837±0.143	68.784±0.070	70.553±0.074
N3	52.542±0.089	63.746±0.120	66.836±0.025
N4	61.438±0.097	65.158±0.023	69.050±0.054
N5	58.758±0.124	64.103±0.110	68.754±0.055
N6	46.167±0.053	53.590±0.125	64.128±0.063
B7	52.369±0.075	59.869±0.023	61.987±0.098
B8	58.634±0.134	60.347±0.109	62.389±0.163
B9	38.168±0.086	49.458±0.166	61.108±0.186
T7	48.275±0.067	53.794±0.180	58.746±0.071
T8	41.549±0.132	49.130±0.065	58.907±0.107
T9	45.706±0.132	49.358±0.183	56.843±0.134
N7	34.706±0.127	45.654±0.108	48.445±0.120
N8	38.920±0.115	47.125±0.195	49.264±0.142
N9	32.563±0.176	44.369±0.113	46.867±0.134
B10	73.812±0.090	77.564±0.080	80.160±0.083
B11	74.043±0.082	81.225±0.122	83.859±0.154
T10	68.453±0.026	77.542±0.054	78.557±0.160
T11	62.751±0.072	73.908±0.175	79.651±0.183
N10	54.170±0.126	66.287±0.067	69.665±0.152
N11	65.108±0.145	67.851±0.065	70.001±0.075
Standard	76.246±0.017	81.460±0.137	84.794±0.080

Fig:10.2.2



Results and Discussion:

The acute oral toxicity study was undertaken as a preliminary safety assessment and, importantly, to establish a suitable dose for the subsequent pharmacological investigations. The synthesized derivatives B3–B11, T3–T11 and N3–N11 exhibited a similar response pattern at the tested dose levels. No mortality was observed at 175 and 550 mg/kg, whereas mortality was observed at 1750 mg/kg, placing the observed lethal range between 550 and 1750 mg/kg for the synthesized compounds.

The absence of mortality at 550 mg/kg indicates that the compounds did not produce acute lethality at this dose under the conditions of the study, while the occurrence of mortality at 1750 mg/kg demonstrates a clear dose-related separation between the non-lethal and lethal dose ranges. Such stepwise dose assessment is consistent with the general principles of acute oral toxicity testing, in which sequential or fixed-dose approaches are used to identify doses associated with evident toxicity or mortality while avoiding unnecessary exposure to highly toxic doses. OECD Test Guideline 420, for example, emphasizes the use of selected fixed doses and observation of toxicity and mortality, whereas Guideline 425 uses sequential dosing to estimate the LD₅₀.

Based on the observed highest non-lethal dose and lowest lethal dose, the study estimated the LD₅₀ as approximately 981 mg/kg, using the geometric mean of 550 and 1750 mg/kg. The same estimated value was assigned to the synthesized derivatives because all compounds demonstrated an identical mortality pattern at the tested dose levels. It is important to interpret this value as an estimated LD₅₀ based on the tested dose interval, rather than as a highly precise population estimate. OECD guidance notes that definitive LD₅₀ estimation depends on the experimental design and, particularly for the up-and-down procedure, can include confidence intervals around the estimate.

The similarity of the observed acute toxicity response across the B-, T- and N-series is noteworthy because these compounds contain chemically different aryl substituents and terminal functionalities. Nevertheless, the present experiment does not provide sufficient evidence to conclude that these structural modifications produce identical toxicological mechanisms. Rather, the comparable response at the specific doses investigated indicates that no clear difference in acute mortality was detected between the tested derivatives under the experimental conditions. More detailed toxicological studies would be required to determine whether differences exist in non-lethal toxicity, organ-specific effects, biochemical parameters or repeated-dose exposure.

The estimated LD₅₀ was subsequently used to select the dose for the pharmacological studies. One-tenth of the estimated LD₅₀ corresponded to 98.1 mg/kg, which was rounded to 100 mg/kg as the experimental efficacy dose. This approach provided a conservative working dose substantially below the estimated lethal range and allowed the subsequent analgesic, antipyretic and anti-inflammatory experiments to be conducted at a common dose. The use of preliminary toxicity information to inform the selection of doses for subsequent in-vivo investigations is consistent with the broader toxicological principle that available toxicity information should be considered when selecting an appropriate starting dose.

The selected 100 mg/kg dose therefore served as the common experimental dose for the subsequent pharmacological evaluation, allowing differences in biological responses to be interpreted without introducing different acute-exposure dose levels among the synthesized derivatives.

The analgesic potential of the synthesized 1,3,4-thiadiazole derivatives was evaluated using the acetic acid-induced writhing model, a chemical nociception assay commonly used for assessing predominantly peripheral antinociceptive activity. In the present study, administration of acetic acid produced a pronounced writhing response in the control animals, whereas treatment with the synthesized compounds produced variable reductions in the number of writhing responses. Aspirin was used as the reference analgesic.

The control animals exhibited 53.4 ± 1.150 writhings, while aspirin reduced the response to 8.2 ± 0.812 writhings, corresponding to 84.64% analgesic activity ($p < 0.001$). The substantial reduction produced by aspirin confirms the responsiveness of the experimental model and provides an appropriate reference against which the activities of the synthesized derivatives can be interpreted. The writhing model is sensitive to agents that interfere with peripheral pain-generating processes associated with inflammatory mediator release. Published work specifically involving 1,3,4-thiadiazole derivatives has similarly used acetic acid-induced writhing to examine peripheral antinociception. Altıntop et al. reported significant reductions in writhing responses for selected 1,3,4-thiadiazole derivatives, supporting the relevance of this model to the evaluation of this heterocyclic scaffold.

Among the present derivatives, N4 produced the highest analgesic activity, 72.28%, with 14.8 ± 0.043 writhings ($p < 0.001$). This response was followed closely by T8 (69.28%) and N8 (68.91%), with 16.4 ± 0.042 and 16.6 ± 0.033 writhings, respectively. T8 showed a highly significant response ($p < 0.001$), while N8 also significantly reduced the writhing response ($p < 0.01$). T4 produced 66.66% inhibition, with 17.8 ± 0.033 writhings ($p < 0.01$). The clustering of N4, T8, N8 and T4 within the higher-activity portion of the series indicates that the analgesic response was strongly influenced by structural features beyond the common thiadiazole nucleus.

The activity of N4 is particularly relevant because it belongs to the 4-nitrophenyl series, whereas T4 contains the corresponding 2-fluorophenyl substituent. Their activities of 72.28% and 66.66%, respectively, indicate that both electron-withdrawing aryl substitution patterns can occur within highly active members of the series, although the present data do not support attributing the difference solely to electronic effects. Other structural elements incorporated into these derivatives must also be considered when interpreting the observed activity. Previous structure-activity investigations of thiadiazole-containing compounds have likewise demonstrated that changes in substituent pattern can substantially alter analgesic activity. Schenone et al. reported good antalgic activity among a series of 1,3,4-thiadiazole derivatives evaluated in the acetic acid writhing test, with differences in activity among individual analogues.

The strong response of T8 and N8 is also noteworthy because both compounds contain the piperidine-containing structural motif. Their similar activities, despite incorporation of different aryl substituents, suggest that the piperidinyl-containing framework may be associated with favorable antinociceptive properties in this particular series. However, this should be regarded as a structure–activity association rather than evidence of a specific pharmacological mechanism, since the present experiment did not directly investigate receptor binding, mediator levels or enzyme inhibition. The importance of considering the complete molecular structure is supported by previous thiadiazole studies in which structurally related derivatives displayed markedly different antinociceptive responses. In the study of Altıntop et al., only selected members of a closely related thiadiazole series significantly reduced acetic-acid-induced writhing, illustrating that activity cannot necessarily be assigned to the heterocyclic nucleus alone.

A second group of derivatives showed moderate activity. N11, N3, N6 and N5 produced 56.55%, 51.68%, 46.44% and 43.07% analgesic activity, respectively, with statistically significant responses, while N10 showed 37.45% activity ($p < 0.01$). The presence of several active members within the N-series is of interest because these compounds share the 4-nitrophenyl substituent but differ in their appended functional groups. The variation in activity among these derivatives indicates that the nitrophenyl group alone is insufficient to account for the pharmacological response; modification of the second substituent attached to the thiadiazole framework substantially influences the observed antinociceptive effect.

The lower responses observed with T11, T10, N7 and T3, which produced 29.96%, 27.71%, 17.22% and 20.22% inhibition, respectively, further demonstrate this structural dependence. N7 nevertheless showed a statistically significant response ($p < 0.05$). Thus, even within the same broad aryl-substituted families, relatively modest structural changes can produce appreciable differences in response in the writhing model. Such variability is consistent with published structure–activity observations for thiadiazole derivatives, where substitution on the heterocyclic scaffold and attached aryl groups has been associated with differences in antinociceptive potency.

The overall pattern is also consistent with the established pharmacological behavior of 1,3,4-thiadiazole derivatives. Earlier investigations have demonstrated that selected members of this scaffold can produce significant antinociceptive effects in the acetic acid writhing model, while more recent studies have continued to identify thiadiazole derivatives with both peripheral and central analgesic activity. Importantly, the present assay specifically evaluates chemically induced nociception and therefore supports an interpretation of peripheral antinociceptive activity; it does not by itself establish whether the active compounds also possess central analgesic effects.

The comparison with aspirin is useful for defining the magnitude of activity within the present experimental system. Although N4, T8, N8 and T4 showed substantial inhibition of writhing, their responses remained below the 84.64% inhibition produced by aspirin under the conditions of this experiment. Therefore, the observed activities are appropriately interpreted as significant antinociceptive responses relative to the untreated control rather than as evidence that the synthesized derivatives are equivalent or superior to the reference drug.

Conclusion

All the synthesized compounds were evaluated for different biological activities like analgesic, antipyretic, anti-inflammatory, antimicrobial and antioxidant activity. The acute toxicity investigation indicated that the synthesized derivatives did not produce mortality at the tested doses of 175 and 550 mg/kg, whereas mortality was observed at 1750 mg/kg. An estimated LD_{50} of approximately 981 mg/kg was obtained and a working dose of 100 mg/kg was selected for subsequent pharmacological evaluation. This provided a preliminary safety basis for conducting the analgesic, antipyretic and anti-inflammatory studies.

The pharmacological investigations confirmed that several compounds possessed appreciable analgesic, antipyretic and anti-inflammatory activities, although the most active compounds differed between the individual models. N4 was prominent in the analgesic model, whereas T3 showed the strongest anti-inflammatory response among the synthesized compounds and approached the activity of the standard. The differing activity profiles indicate that pharmacological activity cannot be predicted solely from the common thiadiazole nucleus and is influenced by the nature and position of additional substituents.

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