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A Comprehensive Study on in Vivo Assessment of Benzothiazole Derivatives for Antibacterial, Antifungal and Antioxidant Efficacy

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ABSTRACT: This study investigates the in vivo antibacterial, antifungal, and antioxidant activities of novel benzothiazole derivatives. A series of derivatives were evaluated for their biological efficacy using established models. For antibacterial activity, the compounds were tested using a Thigh Infection Model; for antifungal assessments, the benzothiazole derivatives were evaluated against *Candida albicans* in neutropenic mice; and antioxidant potential was assessed using a carbon tetrachloride (CCl_4) induced oxidative toxicity model.

KEYWORDS: Benzothiazole, biological, efficacy, in vivo.

I. INTRODUCTION

The most common types of nitrogen compounds are heterocycles, which are rings with five or six carbon atoms that may include nitrogen, sulphur, and oxygen in different positions. Over eighty-five percent of chemical entities with biological activity include a heterocycle. Accordingly, heterocycles play a significant part in the advancement of contemporary pharmaceuticals. Conversely, medicinal chemists simplified and altered the structures of several heterocyclic compounds that were obtained from natural resources. Medicinal chemistry is "a chemistry-based discipline that also involving aspects of biological, medical, and pharmaceutical sciences" and "concerned with the invention, discovery, design, identification, and preparation of biologically active compounds, their metabolism, the study of their mode of action at the molecular level and the construction of structure-activity relationships." A wide range of natural products and pharmaceutical medications include benzothiazole (BTA) or one of its derivatives, making it one of the most significant heterocyclic compounds. Benzothiazole is a chemical having a benzene ring fused with a thiazole ring; it is heterocyclic. It is a clear, colourless liquid that dissolves in water and has a boiling point of 227° Celsius. The wide range of biological activity shown by benzothiazole derivatives has maintained their attention throughout the years. A wide variety of natural goods and pharmaceutical medications include benzothiazole or one of its derivatives, making it one of the most significant heterocyclic compounds. Benzothiazole derivatives as a class are undeniably interesting due to the wide range of pharmacological actions shown by different molecules. Research and development in medicinal chemistry based on benzothiazoles is a hot and dynamic field right now. Drugs for diabetes, epilepsy, inflammation, ulcers, analgesia, TB, viruses, and bacteria are developed and used in a wide variety of contexts in bioorganic and medicinal chemistry using 2-aminobenzothiazole derivatives. The pharmacological and biological significance of benzothiazoles has made them a leading class of chemical molecules.

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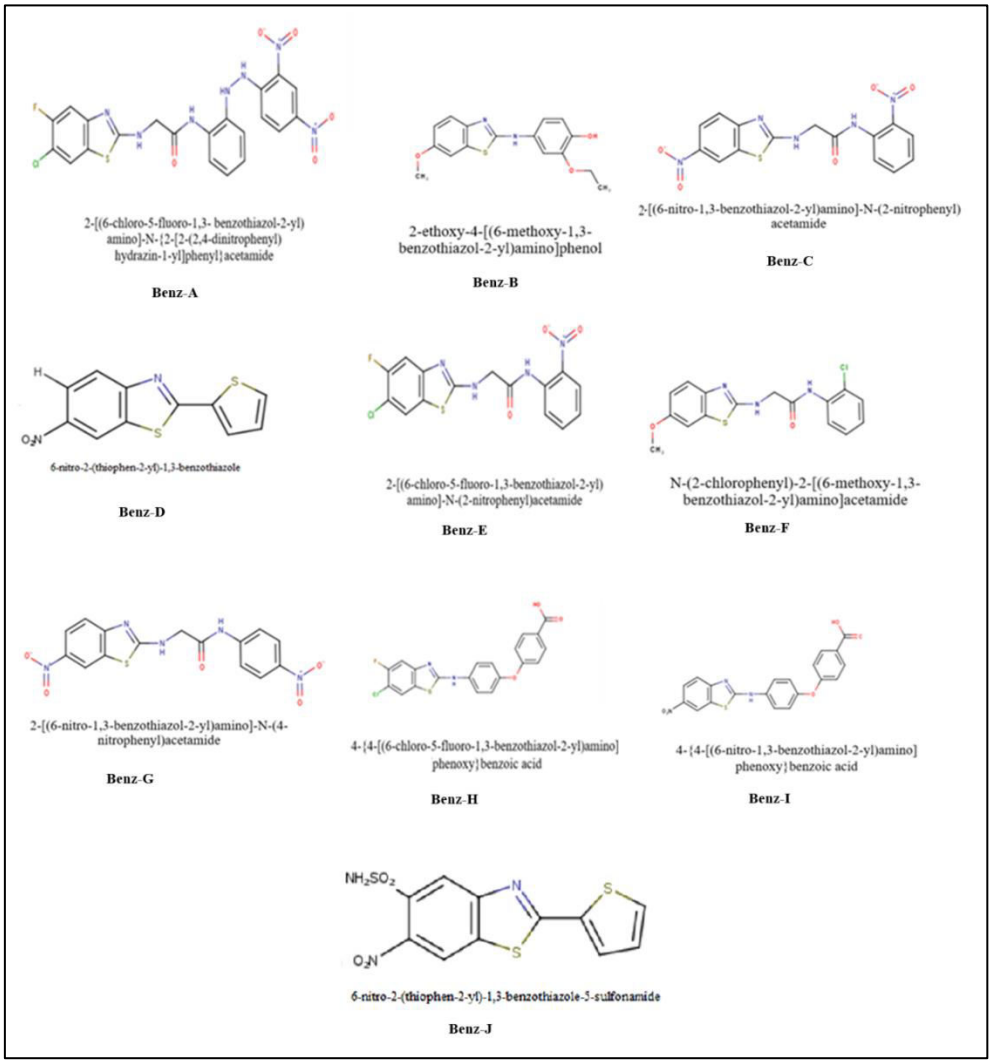
II. RESEARCH METHODOLOGY

Following compounds were studies in vivo for their biological activity:

Fig 1: Novel Benzothiazole Compounds



Methodology for In Vivo Antibacterial Activity Using Thigh Infection Model: The antibacterial efficacy of novel benzothiazole derivatives against *Staphylococcus aureus* were evaluated in a neutropenic mouse thigh infection model. Neutropenic mice (6–8 weeks old, weighing 18–22 g) were selected for the study. Infection was induced in the right thigh muscle of each mouse by injecting 100 µL of the bacterial suspension (approximately 10^5–10^6 CFU). Infection was allowed to establish for 2 hours before treatment begins. Mice were divided into the following groups: Test Compounds (doses of novel benzothiazole derivatives) and Positive Control (Vancomycin at known bactericidal doses). Drug was administered as subcutaneous (s.c.) injection. The test compounds were administered twice daily (bid) at 3- and 6-hours post-infection. Bacterial Load Determination was done 24 hours after infection after euthanizing the mice using humane methods. In order to do this, the infected thighs were removed aseptically, the tissue in 1 mL of sterile saline was homogenized, and serial dilutions were performed. Then, plate the dilutions on agar plates, incubated overnight at 37°C, and the colonies were counted.

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Methodology for In Vivo Antifungal Activity: The in vivo evaluation of antifungal activity for novel benzothiazole derivatives was conducted following a modified version of previously established protocols. Specific-pathogen-free female CD1 mice, six weeks old and weighing between 23-30 grams, were were housed in a controlled environment maintained at a temperature of 22°C and a relative humidity of 30%. A 12-hour light and 12-hour dark cycle was maintained throughout the study. To induce neutropenia, the mice received an intraperitoneal injection of cyclophosphamide, administered at a dose of 150 mg/kg for four consecutive days, followed by a 100 mg/kg dose on the fifth day. On the sixth day, the neutropenic mice were inoculated via the lateral tail vein with 0.1 ml of a *Candida albicans* suspension. Drug treatment commenced two hours post-infection, with the benzothiazole derivatives administered at varying doses via intraperitoneal injection. The antifungal efficacy of the benzothiazole derivatives was assessed in comparison to fluconazole, a known antifungal agent, administered orally at the same doses. After 24 hours of treatment, the mice were euthanized using ether, and both kidneys were harvested as the primary target organs for the study. The kidneys were then homogenized under aseptic conditions and cultured on Sabouraud Dextrose Agar (SDA) media, incubated at 35°C for 24 hours. Following incubation, colony-forming units (CFUs) were enumerated for all groups. The antifungal effects of the tested benzothiazole derivatives were evaluated by comparing the CFU counts from the treated groups against those from the fluconazole control group.

Methodology for In Vivo Antioxidant Activity: For the in vivo evaluation of antioxidant activity of benzothiazole derivatives, Swiss albino mice of either sex, weighing 25-30 g, were used for the experiments. The mice were obtained and kept under standard conditions for one week prior to the experiments to acclimate them. During this time, the mice had free access to a commercial pellet diet and water ad libitum. The in vivo antioxidant potential was assessed using a carbon tetrachloride (CCl_4) induced oxidative toxicity model. Mice were randomly divided into different groups. Group I, the standard group, was treated with silymarin (25 mg/kg body weight) 48 hours after CCl_4 administration. Groups II, III, IV and V received the test benzothiazole derivatives at doses of 100 mg/kg body weight via the IP route. The treatments continued for 7 days. At the end of the treatment period, the mice were anesthetized using ether. The animals were then sacrificed by cervical dislocation. Liver samples were excised, cleaned with ice-cold saline solution (0.9% NaCl), and stored for biochemical analysis. The antioxidant activity was measured through lipid peroxidation (LPO) assay using the thiobarbituric acid reactive substances (TBARS) method. Liver samples were processed, and the malondialdehyde (MDA) content, an indicator of lipid peroxidation, was determined. The assay involved mixing the supernatant with trichloroacetic acid (14%) and thiobarbituric acid (0.6%), followed by heating and centrifugation. The absorbance of the mixture was recorded at 535 nm to determine LPO activity and evaluate the antioxidant capacity of the benzothiazole derivatives.

III. RESULT AND DISCUSSION

In Vivo Antibacterial Activity: The results of the bacterial load analysis, expressed as $\log_{10}$ CFU/lung, for the in vivo antibacterial activity of novel benzothiazole derivatives (Benz-A, Benz-B, Benz-C, Benz-D) and vancomycin reveal important insights into the efficacy of these compounds. Across the tested doses, varying degrees of bacteriostatic activity are observed for the benzothiazole derivatives. Benz-A shows a slight decrease in bacterial burden with increasing doses, from 6.4 $\log_{10}$ CFU at 4 mg/kg to 6.1 $\log_{10}$ CFU at 100 mg/kg, indicating weak antibacterial activity. Benz-B exhibits a similar trend, with a bacterial load reduction from 6.6 $\log_{10}$ CFU at 4 mg/kg to 6.1 $\log_{10}$ CFU at 100 mg/kg. This slight dose-dependent reduction suggests that both Benz-A and Benz-B have limited bacteriostatic effects, with no significant improvement in activity beyond 20 mg/kg. Benz-C shows only a marginal reduction in bacterial load, decreasing from 6.5 $\log_{10}$ CFU at 4 mg/kg to 6.2 $\log_{10}$ CFU at 100 mg/kg, indicating that its antibacterial potency is even lower than Benz-A and Benz-B. On the other hand, Benz-D demonstrates stronger antibacterial activity compared to the other benzothiazole derivatives. It shows a notable decrease in bacterial load from 6.7 $\log_{10}$ CFU at 4 mg/kg to 5.5 $\log_{10}$ CFU at 100 mg/kg, indicating potential bactericidal effects at higher doses. Despite this, Benz-D does not reach the efficacy of vancomycin, the reference. Vancomycin at 4 mg/kg reduces the bacterial load to 6.1 $\log_{10}$ CFU, and at 20 mg/kg, it significantly reduces the load to 5.0 $\log_{10}$ CFU. In comparison, all benzothiazole derivatives primarily exhibit bacteriostatic effects, with only Benz-D showing potential bactericidal activity at the highest dose (100 mg/kg). The dose-response relationship for the benzothiazoles shows gradual reductions in bacterial load with increasing doses.

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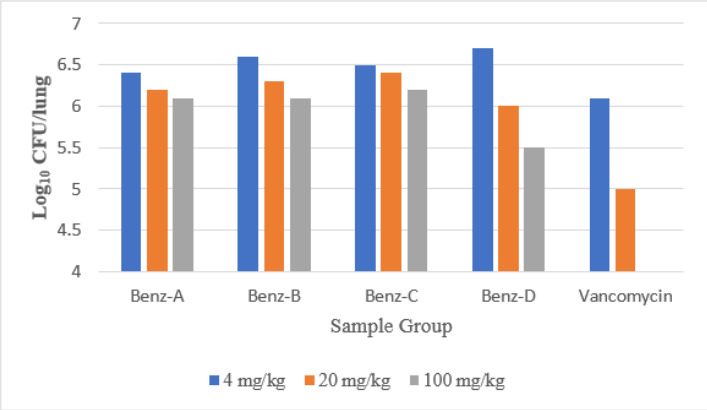
Table 1: Observation for In Vivo Antibacterial Efficacy

Sample Group with Test Compound	Log_{10} CFU/lung		
	4 mg/kg	20 mg/kg	100 mg/kg
Benz-A	6.4	6.2	6.1
Benz-B	6.6	6.3	6.1
Benz-C	6.5	6.4	6.2
Benz-D	6.7	6	5.5
Vancomycin	6.1	5	-

Fig 2: Observation for In Vivo Antibacterial Efficacy



In Vivo Antifungal Activity: The analysis of the antifungal efficacy of the benzothiazole derivatives, as indicated by the colony-forming unit (CFU) counts, reveals notable insights into the effectiveness of the different compounds at varying doses. The CFU counts for each sample group at different doses indicate that all tested benzothiazole derivatives exhibit some degree of antifungal activity, but the efficacy varies significantly across different compounds and dosages. Benz-E, Benz-F, and Benz-G demonstrate relatively high CFU counts at the lowest dose of 10 mg/kg, with values ranging from approximately 442.80 to 466.24. As the dosage increases, the CFU counts for these compounds show a slight decrease but remain relatively high, suggesting that these benzothiazole derivatives may have a moderate antifungal effect but are less effective at reducing fungal growth compared to the control group. Benz-D stands out with significantly lower CFU counts, particularly at the higher doses. At 10 mg/kg, the CFU count is 220.46, which is considerably higher than the counts seen with fluconazole. However, at 20 mg/kg and particularly at 50 mg/kg, the CFU count drops dramatically to 129.90 and 69.24, respectively. This trend indicates that Benz-D may exhibit stronger antifungal activity at higher concentrations, achieving a more substantial reduction in fungal viability, especially at 50 mg/kg. Overall, while some benzothiazole derivatives, particularly Benz-D, show promise at higher dosages. The results indicate that further investigation is warranted to optimize the dosing and formulation of the benzothiazole derivatives to enhance their antifungal activity and establish their potential as alternative treatments.

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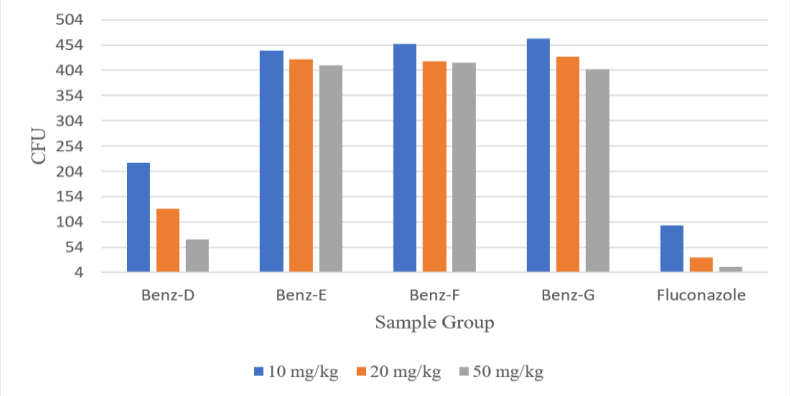
Table 2: Observation for In Vivo Antifungal Efficacy

Sample Group with Test Compound	CFU		
	10 mg/kg	20 mg/kg	50 mg/kg
Benz-D	220.46	129.90	69.24
Benz-E	442.80	425.89	413.65
Benz-F	456.32	421.37	419.16
Benz-G	466.24	431.35	405.67
Fluconazole	97.12	34.19	15.33

Fig 3: Observation for In Vivo Antifungal Efficacy



In Vivo Antioxidant Activity: The analysis of the lipid peroxidation (LPO) inhibition activity shows that the benzothiazole derivatives possess varying levels of antioxidant activity, as indicated by their ability to inhibit LPO. The percentage inhibition values for the test compounds Benz-H, Benz-I, Benz-D, and Benz-J, compared to the reference antioxidant silymarin, provide insight into their relative antioxidant potential. Benz-H and Benz-I demonstrated modest antioxidant activity, with % inhibition values of 34% and 31%, respectively. These results suggest that these two compounds offer some protection against oxidative damage, but their efficacy is limited compared to the other tested compounds. Benz-D showed a significant improvement in LPO inhibition, with a value of 62%, indicating a stronger antioxidant potential. This suggests that Benz-D is more effective in reducing oxidative stress and protecting against lipid peroxidation in biological tissues. Benz-J exhibited the highest LPO inhibition among the test compounds, with 68% inhibition. This result highlights its potent antioxidant activity, approaching the efficacy of silymarin, which achieved 85% inhibition of LPO activity.

Table 3: Observation for In Vivo Antioxidant Efficacy

Sample Group with Test Compound	% Inhibition of LPO activity
Benz-D	62
Benz-H	34
Benz-I	31
Benz-J	68
Silymarin	85

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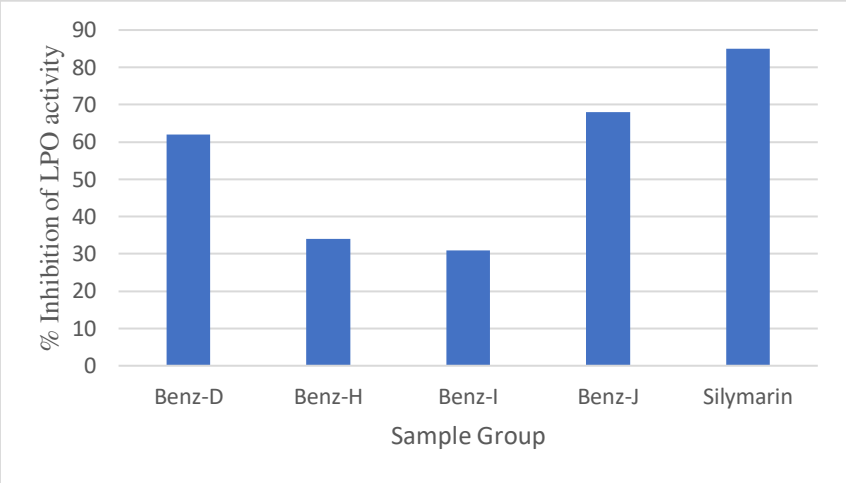
Fig 4: Observation for In Vivo Antioxidant Efficacy



IV. CONCLUSION

This study assessed the in vivo antibacterial activity of novel benzothiazole derivatives using a thigh infection model. While these derivatives exhibited varying levels of antibacterial effects, none matched the efficacy of vancomycin. Benz-D showed the most promise, indicating potential bactericidal properties at higher doses. However, overall, the compounds primarily displayed antibacterial activity with limited reductions in bacterial burden. Similarly, the in vivo evaluation of the antifungal activity of benzothiazole derivatives revealed varying degrees of effectiveness across different compounds and doses. Among the tested compounds, Benz-D exhibited the most promising antifungal activity, particularly at higher doses, where it significantly reduced CFU counts compared to other derivatives. While Benz-D shows potential as a candidate for further development, additional studies are necessary to optimize its antifungal efficacy and compare its long-term therapeutic benefits with established antifungal agents like fluconazole. Additionally, the evaluation of benzothiazole derivatives for antioxidant activity showed that Benz-J and Benz-D demonstrated significant lipid peroxidation (LPO) inhibition rates of 68% and 62%, respectively, indicating strong antioxidant potential. In comparison, Benz-H and Benz-I exhibited lower inhibition rates of 34% and 31%, suggesting limited antioxidant capacity. These results highlight the potential of specific benzothiazole derivatives as effective antioxidants, warranting further investigation into their therapeutic applications.

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