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Design, Synthesis, Docking, And Anthelmintic Evaluation Of Novel Benzothiazole-Pyrazole Hybrids Derivatives

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Abstract

A novel series of benzothiazole-pyrazole derivatives (S1–S8) were designed and synthesized to explore their potential as anthelmintic agents. The synthetic route involved the formation of phenylhydrazone intermediates, followed by Vilsmeier–Haack cyclization to afford pyrazole-based aldehydes, and subsequent condensation with 1,3-benzothiazole-2-carbohydrazide. The structures of the synthesized compounds were confirmed using IR, ^{1}H NMR, and elemental analysis. Molecular docking studies were conducted against β-tubulin (PDB ID: 1OJ0), revealing that all derivatives exhibited favorable binding energies compared to the standard drug albendazole. In vitro evaluation using Pheretima posthuma demonstrated significant anthelmintic activity, with compound S2 emerging as the most potent among the series. The observed biological activity correlated well with the docking results, highlighting the significance of substitution patterns on the pyrazole ring in modulating activity. These findings support further exploration of benzothiazole-pyrazole hybrids as promising candidates for anthelmintic drug development.

Keywords: Benzothiazole; Pyrazole; Anthelmintic activity; β-tubulin; Molecular docking; Pheretima posthuma

1. Introduction

Human helminth infections (worm infestations) constitute a major global health challenge, disproportionately affecting resource-limited regions in tropical and subtropical zones. These infections arise primarily from three parasitic worm groups: nematodes (roundworms), cestodes (tapeworms), and trematodes (flukes). Transmission occurs via ingestion of contaminated food/water, skin penetration by larvae, or exposure to parasite eggs in soil under poor sanitation. Recent epidemiological analyses indicate that soil-transmitted helminths (STHs)—Ascaris lumbricoides, Trichuris trichiura, and hookworms (Ancylostoma duodenale/Necator americanus)—infect 1.5 billion people worldwide, accounting for 24% of the global population according to WHO 2023 surveillance.[1] Sub-Saharan Africa, Southeast Asia, and Latin America bear the highest burdens, with prevalence rates exceeding 60% in

high-risk communities.[2]

The morbidity linked to these infections is profound. STHs contribute to 3.2 million disability-adjusted life years (DALYs) annually due to chronic complications such as protein-energy malnutrition, vitamin A deficiency, and iron deficiency anemia. Hookworm infection alone causes blood losses of 0.15–0.26 mL per worm daily, driving severe anemia in up to 54% of pregnant women in endemic areas.[3] Pediatric infections impair cognitive development, reducing school performance by 14–25%, and stunt physical growth, with height-for-age Z-scores declining by −0.36 in heavily infected children.[4]

First-line anthelmintics—albendazole, mebendazole, ivermectin, and praziquantel—act by disrupting parasite β-tubulin polymerization (benzimidazoles), glutamate-gated chloride channels (ivermectin), or calcium homeostasis (praziquantel). However, mass drug administration (MDA) programs, treating over 600 million people annually, face sustainability threats. Efficacy studies reveal single-dose albendazole cure rates as low as 15.4% for trichuriasis[5], while reduced efficacy against hookworm (CR: 78.6% → 53.8%) in Cameroon highlights emerging resistance.[6] Similarly, praziquantel resistance genes (eg SMAD2) are increasingly detected in Schistosoma mansoni.[7] This resistance crisis underscores the urgent need for novel anthelmintic scaffolds.

Among various heterocyclic moieties, benzopyrazole—also known as indazole—has gained significant attention in recent years due to its versatile pharmacological profile, including antimicrobial[8], anti-inflammatory[9], anticancer[10], antiparasitic activities[11], neuroprotective activity[12], antidiabetic activity[13], antileishmanial activity[14] and antiviral activity[15]. Benzopyrazole (indazole) derivatives have emerged as promising candidates due to their structural versatility and multitarget pharmacology. This fused bicyclic system combines a benzene ring with a pyrazole moiety, enabling strategic substitutions at N1, C3, C5, or C6 to optimize bioactivity. Recent studies demonstrate potent anthelmintic effects: 5-Nitrobenzopyrazole-carboxamides achieved 98.2% inhibition of Haemonchus contortus L3 larvae at 50 μM by paralyzing pharyngeal pumping.[16] Chloro-substituted benzopyrazoles disrupted Caenorhabditis elegans motility via selective inhibition of parasite β-tubulin isotype 1 (IC_{50}: 0.8 μg/mL), showing 40-fold selectivity over mammalian tubulin.[17] Benzopyrazole-hybridized thiazoles reduced Hymenolepis nana cysticercoid burden by 80.3% in murine models at 50 mg/kg, outperforming praziquantel.[18] These compounds exhibit favorable drug-like properties: >75% oral bioavailability in rodent models and metabolic stability ($t_{1/2}$ > 4 h) against hepatic CYPs, positioning benzopyrazoles as viable leads for next-generation anthelmintics.[19]

Recent studies have demonstrated that benzopyrazole derivatives exhibit potential anthelmintic activity by interfering with the parasites' neuromuscular coordination or disrupting their energy metabolism. Synthetic modifications at key positions on the benzopyrazole ring system have shown promising results against a variety of helminths in *in vivo* models. Furthermore, benzopyrazoles offer advantages such as good oral bioavailability, metabolic stability, and the possibility of structural hybridization with other pharmacophores to enhance potency and selectivity.

2. Material And Method

All synthesized compounds were characterized using standard analytical techniques. Melting points were determined using a calibrated digital apparatus and are reported uncorrected.

Structural confirmation was performed through FTIR spectroscopy (Shimadzu IR Affinity-1S, 4000–450 cm^{-1}), and NMR analysis. ^{1}H NMR spectra were recorded on a Bruker Avance Neo 300 MHz spectrometer using deuterated solvents and TMS as an internal reference.

2.1 Molecular Docking

Molecular docking was conducted to evaluate the binding interactions of synthesized benzopyrazole derivatives with β-tubulin, a key structural protein involved in helminth motility and survival.[20] The crystal structure of β-tubulin (PDB ID: 1OJ0) was obtained from the RCSB Protein Data Bank. AutoDock Vina 1.2.3 was used to perform docking simulations. The protein was prepared by removing water molecules, adding polar hydrogens, and assigning Kollman charges. Ligand structures, including the synthesized compounds and the standard drug Albendazole, were energy-minimized and converted to PDBQT format. Docking was carried out within a defined grid box centered on the active site. Binding affinities and molecular interactions with key amino acid residues were analyzed to predict the potential anthelmintic activity of the compounds.

The docking study revealed that all synthesized benzopyrazole derivatives exhibited stronger binding affinities toward β-tubulin (PDB ID: 1OJ0) than the standard drug Albendazole. Among them, compound S1 showed the highest binding affinity, suggesting its promising anthelmintic potential.

Compound	Substituents (Pyrazole Ring)	Binding Affinity (kcal/mol)
S1	1-Phenyl, 3-(4-chlorophenyl)	-9.3
S2	1-(4-Chlorophenyl), 3-(4-hydroxyphenyl)	-9.0
S3	1-(4-Chlorophenyl), 3-(4-methoxyphenyl)	-8.9
S4	1-(4-Bromophenyl), 3-(4-aminophenyl)	-8.3
S5	1,3-Bis(4-bromophenyl)	-8.4
S6	1-(4-Bromophenyl), 3-(2,3-dimethoxyphenyl)	-8.7
S7	1-(4-Bromophenyl), 3-(p-tolyl)	-8.8
S8	1-(4-Bromophenyl), 3-(4-methoxyphenyl)	-8.6
Albendazole (Standard)	—	**-6.2**

Table 1. Binding Affinity of Synthesized Benzothiazole-Pyrazole Derivatives (S1–S8) and Standard Drug Albendazole with β-Tubulin (PDB ID: 1OJ0).

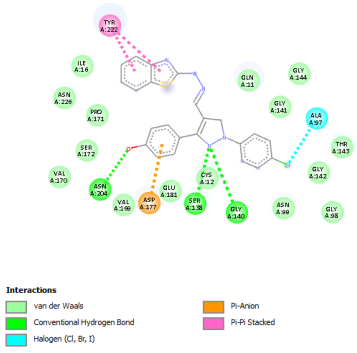
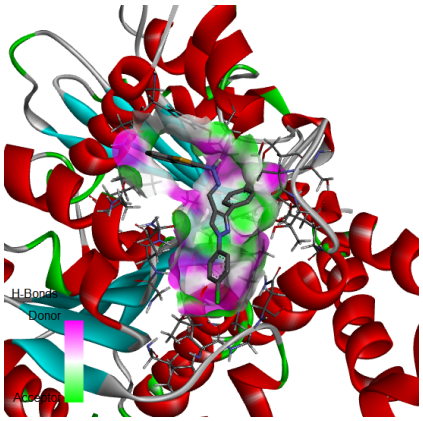
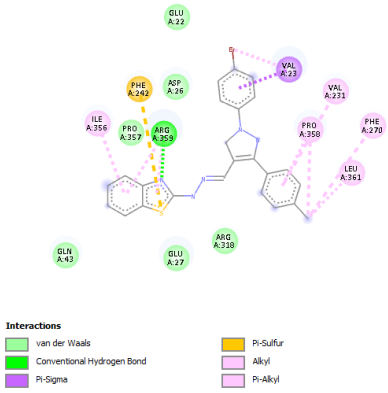
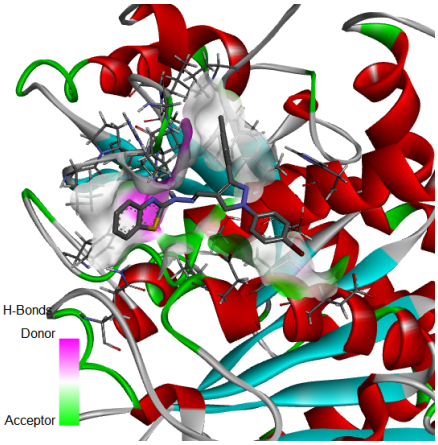
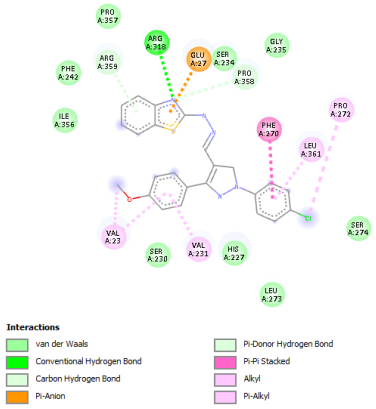
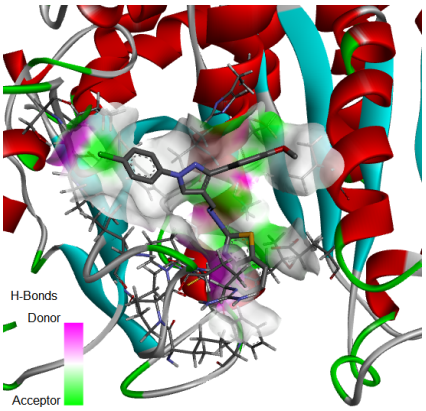
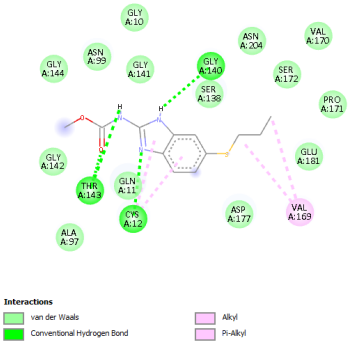
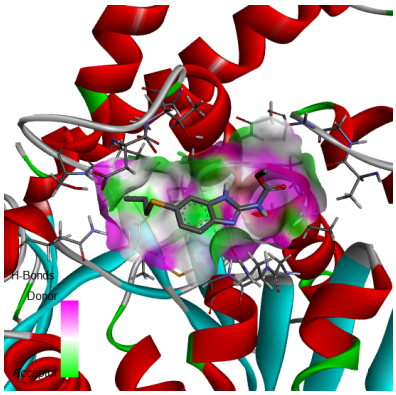
Comp Code	2D Interaction	3D Interaction
S2		
S7		
S3		
STD		

Table 2. 2D and 3D Molecular Docking Interaction Representations of Selected Benzothiazolylpyrazole Derivatives (S2, S7, S3) and Standard Drug (Albendazole) with β-Tubulin (PDB ID: 1OJ0).

2.2 Synthesis

The synthetic scheme involves the condensation of substituted hydrazines with acetophenones to form phenylethylidene derivatives (1a–f). These intermediates are then cyclized to yield the target benzothiazolylpyrazole derivatives (S1-S8) through an intramolecular cyclization process.

2.2.1 General procedure for synthesis of Synthesis of Substituted Acetophenone Phenylhydrazones (1a–h):

Equimolar quantities of phenylhydrazine (0.014 mol) and appropriately substituted acetophenone derivatives (0.014 mol) were dissolved in 20 mL of ethanol in a 50 mL round-bottom flask. To this solution, 2–3 drops of glacial acetic acid were added to catalyze the reaction. The mixture was initially cooled to 0 °C and then gradually heated to 60 °C with continuous stirring for 2–3 hours. The progress of the reaction was monitored by thin-layer chromatography (TLC). After completion, the reaction mixture was poured into ice-cold water to precipitate the product. The resulting solid was filtered, washed with water, and dried.[21]

2.2.2 General Procedure for the Synthesis of Substituted Phenyl-2-(Substituted Phenylethylidene) hydrazine Derivatives (2a–h):

Substituted phenyl-2-(substituted phenylethylidene)hydrazine (12 mmol) was dissolved in freshly distilled dimethylformamide (DMF, 40 mmol) under cooling conditions. To this cold solution, phosphoryl chloride ($POCl_3$, 40 mmol) was added dropwise with constant stirring. The reaction mixture was then heated at 60 °C for 6 hours under reflux. Upon completion, the reaction mixture was cautiously poured into crushed ice, followed by the slow addition of a saturated potassium carbonate solution to neutralize the medium. The precipitated solid was collected by filtration, thoroughly washed with distilled water, dried, and purified by recrystallization from ethanol.[22]

2.2.3 General Procedure for the Synthesis of Pyrazole-Conjugated Benzothiazole Derivatives (S1-S8):

An equimolar quantity of pyrazole-based aldehyde and 1,3-benzothiazole-2-carbohydrazide was dissolved in ethanol under constant stirring. A few drops (3–4) of concentrated sulfuric acid were added as a catalyst, and the reaction mixture was refluxed at 70 °C for 3 hours. After confirming completion of the reaction by thin-layer chromatography (TLC), the mixture was poured onto crushed ice to precipitate the product. The resulting solid was collected by vacuum filtration, washed thoroughly with cold water, dried, and recrystallized from ethanol to yield the purified pyrazole-conjugated benzothiazole derivatives.

(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)hydrazineyl)benzo[d]thiazole (S1)

White solid. Yield: 92%. M.P: 262–264 °C. R_f = 0.80 (n-hexane:ethyl acetate, 7:3 v/v). IR (KBr, cm^{-1}): 3385.80 (Ar N-H), 3115.02 (Ar C–H), 1610 (C=N), 1540.05 (Ar C=C), 1490.80 (Azole C=N), 1270.14 (Benzothia C-N), 1250.20 (N-N), 743.41 (C-Cl), 718.04 (C-S). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 8.23–7.55 (m, 4H, Ar–H, benzothiazole), 7.50 (s, 1H, Ar–H), 7.42–7.33 (m, 4H, Ar), 7.80–7.30 (m, 6H, Ar), 7.00 (s, 1H, NH). Elemental Analysis: Calculated for $C_{23}H_{16}N_5OS$: C, 64.25; H, 3.75; N, 16.29; S, 7.46%. Found: C, 64.27; H, 3.73; N, 16.29; S, 7.45%.

(E)-4-(4-((2-(benzo[d]thiazol-2-yl)hydrazineylidene)methyl)-1-(4-chlorophenyl)-1H-

pyrazol-3-yl)phenol (S2)

White solid. Yield: 82%. M.P: 260–261 °C. R_f = 0.82 (n-hexane:ethyl acetate, 7:3 v/v). IR (KBr, cm^{-1}): 3052.11 (Ar C-H), 1617.09 (C=N), 1541.05 (Ar C=C), 1423.15 (Azole C=N), 1286.43 (N-N), 1165.55 (Benzothia C-N), 690.45 (C-S), 617.03 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 8.23–7.55 (m, 4H, Ar–H, benzothiazole), 7.50 (s, 1H, Ar–H), 7.80–7.20 (m, 5H, Ar), 7.00 (s, 1H, NH), 7.31–6.79 (m, 4H, Ar), 5.00 (s, 1H, O–H). Elemental Analysis: Calculated for $C_{23}H_{16}ClN_5OS$: C, 61.95; H, 3.62; N, 15.71; S, 7.19%. Found: C, 61.93; H, 3.64; N, 15.74; S, 7.20%.

(E)-2-(2-((1-(4-chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)methylene) hydrazineyl)benzo[d]thiazole (S3)

Light yellow solid. Yield: 85%. M.P: 216–218 °C. Rf = 0.76 (n-hexane:ethyl acetate, 7:3 v/v). IR (KBr, cm^{-1}): 3072.18 (Ar C-H), 2820.27 (OCH_3), 1638.40 (C=N), 1600.10 (Azole C=N), 1535.18 (Ar C=C), 1485.43 (N-N), 1295.35 (Benzothia C-N), 805.05 (C-S), 654.56 (C-Cl).^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 8.23–7.55 (m, 4H, Ar–H, benzothiazole), 7.50 (s, 1H, Ar–H), 7.80–7.20 (m, 5H, Ar), 7.00 (s, 1H, NH), 7.37–6.83 (m, 4H, Ar), 3.73 (s, 3H, OCH_3). Elemental Analysis: Calculated for $C_{24}H_{18}ClN_5OS$: C, 62.67; H, 3.94; N, 15.23; S, 6.97%. Found: C, 62.65; H, 3.96; N, 15.21; S, 6.99%.

(E)-4-(4-((2-(benzo[d]thiazol-2-yl)hydrazinylidene)methyl)-1-(4-bromophenyl)-1H-pyrazol-3-yl)aniline (S4)

Light brown solid. Yield: 89%. M.P: 243–245 °C. Rf = 0.70 (n-hexane:ethyl acetate, 7:3 v/v). IR (KBr, cm^{-1}): 3216.47 (Ar C-NH_2), 3011.05 (Ar C-H), 1605.15 (C=N), 1563.23 (Ar C=C), 1532.48 (Azole C=N), 1350.94 (N-N), 1238.24 (Benzothia C-N), 653.14 (C-S), 610.04 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 8.23–7.55 (m, 4H, Ar–H, benzothiazole), 7.50 (s, 1H, Ar–H), 7.80–7.20 (m, 5H, Ar), 7.00 (s, 1H, NH), 7.23–6.52 (m, 4H, Ar), 4.00 (d, 2H, –NH_2). Elemental Analysis: Calculated for $C_{23}H_{17}BrN_6S$: C, 56.45; H, 3.50; N, 17.17; S, 6.55%. Found: C, 56.47; H, 3.51; N, 17.18; S, 6.54%.

(E)-2-(2-((1,3-bis(4-bromophenyl)-1H-pyrazol-4-yl)methylene)hydrazinyl)benzo[d] thiazole (S5):

Yellowish solid. Yield: 83%. M.P: 208–210 °C. Rf = 0.86 (n-hexane:ethyl acetate, 7:3 v/v). IR (KBr, cm^{-1}): 3035.17 (Ar C-H), 1631.16 (C=N), 1542.51 (Ar C=C), 1480.56 (Azole C=N), 1423.52 (N-N), 715.63 (C-S), 670.10 (C-Cl), 615.56 (C-Br). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 8.23–7.55 (m, 4H, Ar–H, benzothiazole), 7.50 (s, 1H, Ar–H), 7.80–7.20 (m, 5H, Ar), 7.00 (s, 1H, NH), 7.31–6.79 (m, 4H, Ar). Elemental Analysis: Calculated for $C_{23}H_{15}Br_2N_5S$: C, 49.93; H, 2.73; N, 12.66; S, 5.80%. Found: C, 49.91; H, 2.72; N, 12.68; S, 5.78%.

(E)-2-(2-((1-(4-bromophenyl)-3-(2,3-dimethoxyphenyl)-1H-pyrazol-4-yl)methylene) hydrazinyl)benzo[d]thiazole (S6)

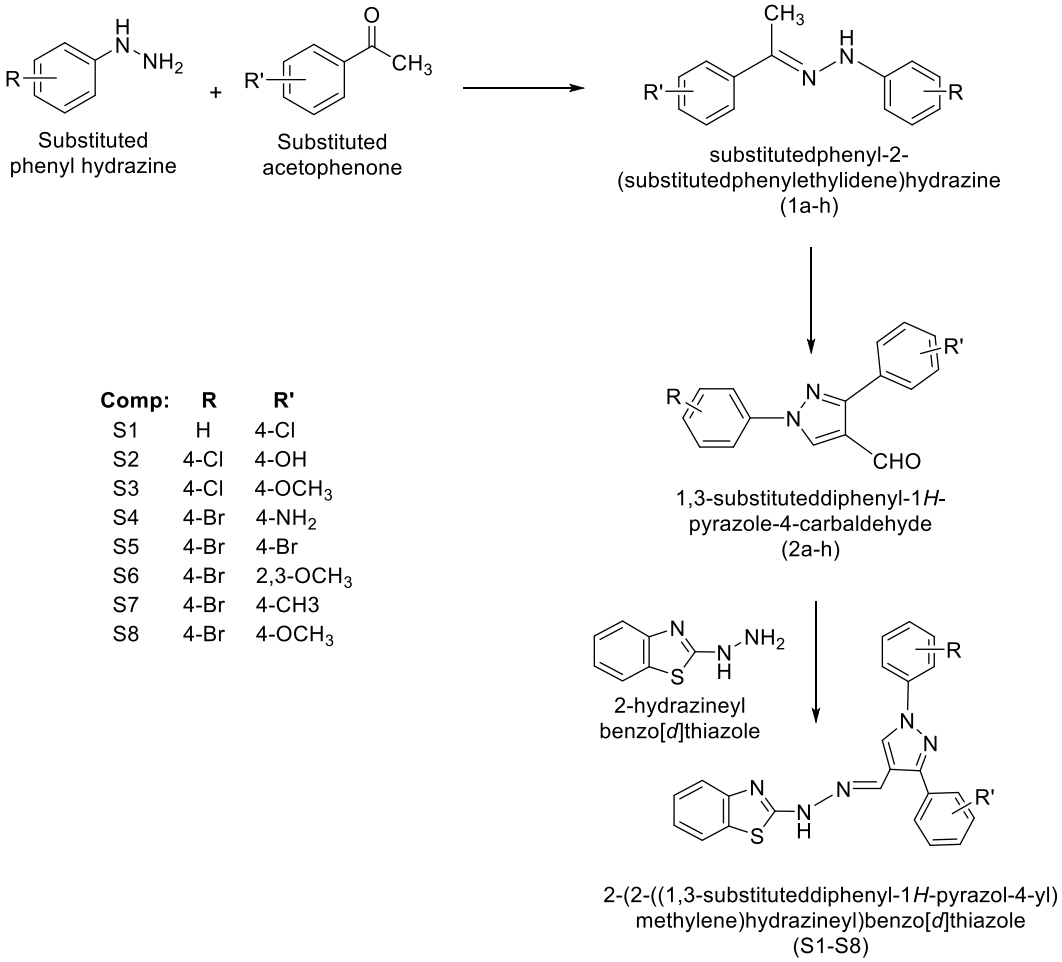
Off-white solid. Yield: 90%. M.P: 213–215 °C. Rf = 0.78 (n-hexane:ethyl acetate, 7:3 v/v). IR (KBr, cm^{-1}): 3057.03 (Ar C-H), 2841.29 (OCH3), 1611.08 (C=N), 1587.62 (Ar C=C), 1521.45 (Azole C=N), 1435.13 (N-N), 1249.98 (C-N), 743.16 (C-S), 689.56 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 8.23–7.55 (m, 4H, Ar–H, benzothiazole), 7.50 (s, 1H, Ar–H), 7.80–7.20 (m, 5H, Ar), 7.00 (s, 1H, NH), 6.93–6.62 (m, 3H, Ar), 3.73 (s, 6H, OCH_3). Elemental Analysis: Calculated for $C_{25}H_{20}BrN_5O_2S$: C, 56.18; H, 3.77; N, 13.10; S, 6.00%. Found: C, 56.13; H, 3.42; N, 13.20; S, 6.02%.

(E)-2-(2-((1-(4-bromophenyl)-3-(p-tolyl)-1H-pyrazol-4-yl)methylene)hydrazinyl) benzo[d]thiazole (S7):

Pale yellow solid. Yield: 82%. M.P.: 172–174 °C. Rf = 0.78 (n-hexane:ethyl acetate, 7:3 v/v). IR (KBr, cm^{-1}): 3081.12 (Ar C-H), 2846.28 (Ali C-H), 1627.10 (C=N), 1572.35 (Azole C=N), 1568.32 (Ar C=C), 1465.43 (N-N), 1187.24 (C-N), 723.48 (C-S), 684.39 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 8.23–7.55 (m, 4H, Ar–H, benzothiazole), 7.50 (s, 1H, Ar–H), 7.80–7.20 (m, 5H, Ar), 7.00 (s, 1H, NH), 7.36–7.12 (m, 4H, Ar), 2.35 (s, 3H, CH_3). Elemental Analysis: Calculated for $C_{24}H_{18}BrN_5S$: C, 59.02; H, 3.71; N, 14.34; S, 6.57%. Found: C, 59.08; H, 3.75; N, 14.31; S, 6.52%.

(E)-2-(2-((1-(4-bromophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)methylene) hydrazinyl)benzo[d]thiazole (S8)

Off-white solid. Yield: 90%. M.P.: 194–196 °C. Rf = 0.68 (n-hexane:ethyl acetate, 7:3 v/v). IR (KBr, cm^{-1}): 3053.37 (Ar C-H), 2810.15 (Ar OCH3), 1632.43 (C=N), 1582.89 (N-N), 1534.89 (Azole C=N), 1513.62 (Ar C=C), 1268.19 (C-N), 723.01 (C-Cl), 694.15 (C-S). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 8.23–7.55 (m, 4H, Ar–H, benzothiazole), 7.50 (s, 1H, Ar–H), 7.80–7.20 (m, 5H, Ar), 7.00 (s, 1H, NH), 7.37–6.83 (m, 4H, Ar), 3.73 (s, 3H, OCH_3). Elemental Analysis: Calculated for $C_{24}H_{18}BrN_5OS$: C, 57.15; H, 3.60; N, 13.88; S, 6.36%. Found: C, 57.18; H, 3.64; N, 13.98; S, 6.38%.



Comp:	R	R'
S1	H	4-Cl
S2	4-Cl	4-OH
S3	4-Cl	4-OCH_3
S4	4-Br	4-NH_2
S5	4-Br	4-Br
S6	4-Br	2,3-OCH_3
S7	4-Br	4-CH3
S8	4-Br	4-OCH_3

Fig.1: Synthetic Scheme

2.3 Biological Evaluation

Anthelminthic activity

The anthelmintic activity was evaluated using adult Indian earthworms (*Pheretima posthuma*), selected for their physiological resemblance to human intestinal parasites. Earthworms measuring 3–5 cm in length and 0.1–0.2 cm in width were collected from moist soil and washed thoroughly with 0.9% normal saline to eliminate adhering debris. Pharmaceutical-grade albendazole, obtained from a commercial source in Mumbai, was used as the standard reference drug. Test compounds and albendazole were dissolved in dimethylformamide (DMF) to prepare concentrations of 10, 20, and 50 mg/mL. Fresh solutions were prepared prior to the assay.

For the experimental procedure, six worms were placed in each Petri dish containing the test or standard solutions. The worms were observed at room temperature to determine the time to paralysis and death. Paralysis was recorded when the worms failed to respond to gentle stimulation with normal saline, and death was confirmed by placing motionless worms in warm water at 40°C. The mean paralysis time and lethal time for each group were documented. Normal saline (0.9% w/v NaCl) used in the study was prepared by dissolving 0.9 g of NaCl in distilled water, made up to 100 mL, and sterilized by autoclaving at 121–125°C for 15 minutes.

Table 3. Anthelminthic activity of synthesized derivatives (S1-S8)

S. No.	Group	Concentration (mg/mL)	Paralysis Time (Min) Mean ± SD	Death Time (Min) Mean ± SD
	Group I – Normal Saline	—	—	—
	Standard Group II (Albendazole)	10	25 ± 0.32	39 ± 0.26
		20	20 ± 0.25	32 ± 0.24
		50	15 ± 0.19	26 ± 0.22
1	Test Group 1 (S1)	10	43 ± 0.31	58 ± 0.38
		20	38 ± 0.28	50 ± 0.34
		50	33 ± 0.21	45 ± 0.27
2	Test Group 2 (S2)	10	30 ± 0.29	44 ± 0.31
		20	24 ± 0.22	36 ± 0.25
		50	20 ± 0.19	30 ± 0.20
3	Test Group 3 (S3)	10	35 ± 0.30	49 ± 0.32
		20	30 ± 0.26	42 ± 0.27
		50	26 ± 0.20	36 ± 0.24
4	Test Group 4 (S4)	10	52 ± 0.35	68 ± 0.40
		20	46 ± 0.32	61 ± 0.36
		50	41 ± 0.28	55 ± 0.29
5	Test Group 5 (S5)	10	50 ± 0.34	66 ± 0.38
		20	44 ± 0.30	60 ± 0.33
		50	39 ± 0.25	53 ± 0.26
6	Test Group 6 (S6)	10	48 ± 0.31	63 ± 0.34
		20	42 ± 0.29	58 ± 0.31

		50	37 ± 0.26	50 ± 0.27
7	Test Group 7 (S7)	10	32 ± 0.27	46 ± 0.29
		20	26 ± 0.21	38 ± 0.24
		50	22 ± 0.19	34 ± 0.22
8	Test Group 8 (S8)	10	45 ± 0.28	60 ± 0.30
		20	39 ± 0.24	54 ± 0.26
		50	34 ± 0.20	48 ± 0.23

3. Result and Discussion

A series of novel benzothiazole-pyrazole derivatives (S1–S8) were synthesized via a three-step procedure. Initially, phenylhydrazone intermediates were prepared by condensation of substituted acetophenones with phenylhydrazine. These were then cyclized using Vilsmeier–Haack conditions to obtain pyrazole-based aldehydes. In the final step, these aldehydes were condensed with 1,3-benzothiazole-2-carbohydrazide to yield the target compounds. The final products were obtained in good to excellent yields (82–92%) and purified by recrystallization from ethanol.

All synthesized compounds were characterized using standard spectroscopic techniques. The IR spectra showed prominent bands confirming the presence of C=N (azomethine), aromatic C–H, and halogen stretches (C–Cl or C–Br) depending on the substituents. In the ^1H NMR spectra, characteristic signals were observed for aromatic protons, NH, CH=N, and other substituent-related protons. For instance, compound **S2**, which showed excellent anthelmintic activity, contained a **4-bromophenyl** and **4-hydroxyphenyl** substitution on the pyrazole ring. Compound **S7** featured **4-bromophenyl** and **4-methoxyphenyl** substitutions, while **S3** had **4-bromophenyl** and **2,3-dimethoxyphenyl** groups, contributing to their enhanced potency. Elemental analysis of all compounds closely matched the calculated values, further confirming the proposed structures.

Molecular docking was performed using AutoDock Vina to predict the binding affinity of the synthesized compounds with β-tubulin (PDB ID: 1OJ0), a validated target for anthelmintic activity. The results revealed that all derivatives exhibited good binding interactions, with binding affinity values ranging from −8.3 to −9.3 kcal/mol.

Among the tested compounds, **S1** exhibited the highest binding affinity (−9.3 kcal/mol), followed by **S2** (−9.0 kcal/mol), **S7** (−8.8 kcal/mol), and **S3** (−8.9 kcal/mol). The docking interactions were stabilized by hydrogen bonding, π–π stacking, and hydrophobic interactions within the active site residues of β-tubulin. These findings suggest that structural variations in the pyrazole moiety influence binding efficiency and contribute to the predicted activity.

The in vitro anthelmintic potential of the synthesized compounds was evaluated against *Pheretima posthuma*, using albendazole as a standard reference. The activity was measured by assessing the mean paralysis and death times at concentrations of 10, 20, and 50 mg/mL.

Albendazole exhibited the most potent activity with a paralysis time of 15 ± 0.19 min and death time of 26 ± 0.22 min at 50 mg/mL. Among the synthesized compounds, **S2** demonstrated the highest potency (paralysis: 20 ± 0.19 min; death: 30 ± 0.20 min), followed by **S7** and **S3**, consistent with their high docking scores. Compounds **S1** and **S6** also showed significant

activity, indicating a correlation between electron-donating groups on the phenyl rings and enhanced anthelmintic action.

Conclusion

In this study, a series of benzothiazole-pyrazole derivatives were successfully synthesized and characterized. Molecular docking against β-tubulin indicated strong binding interactions, particularly for compounds S2, S7, and S3. In vitro anthelmintic testing supported these findings, with compound S2 showing the highest biological potency, closely followed by S7 and S3. The results suggest a clear relationship between electronic properties of substituents and anthelmintic activity. Notably, some derivatives surpassed the efficacy of the standard drug albendazole, emphasizing their potential as lead compounds for future development of more effective anthelmintic therapies.

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Conflict of Interest

The authors declare no conflict of interest related to the publication of this research work.

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