RESEARCH PAPER

Integrated Synthesis, Molecular Modeling, and Biological Assessment of Novel Albendazole Derivatives as Potential Anthelmintic Agents

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ABSTRACT

The emergence of resistance to conventional anthelmintic drugs has necessitated the development of novel therapeutic agents with enhanced efficacy and safety profiles. In the present study, a series of novel albendazole derivatives were designed, synthesized, and evaluated as potential anthelmintic agents through an integrated approach involving chemical synthesis, molecular modelling, and biological assessment. Structural modifications were introduced into the albendazole scaffold to improve its pharmacological properties and target-binding affinity. The synthesized compounds were characterized using standard spectroscopic techniques, including FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry, confirming their chemical structures and purity. Molecular docking studies were performed to investigate the interaction of the synthesized derivatives with key parasitic target proteins. Several compounds exhibited favorable binding energies and strong interactions with critical amino acid residues, suggesting enhanced target specificity and inhibitory potential. In silico pharmacokinetic and drug-likeness analyses further indicated acceptable ADME properties and compliance with Lipinski's rule of five. The biological efficacy of the synthesized derivatives was evaluated using established in vitro anthelmintic assays. Most of the compounds demonstrated significant anthelmintic activity, exhibiting reduced paralysis and death times compared with the parent drug albendazole. Structure–activity relationship analysis revealed that specific substituents on the benzimidazole nucleus contributed positively to biological activity.

Keywords: Albendazole derivatives, Anthelmintic activity, Lipophilicity, Mannich Base, Pheretima posthuma.

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INTRODUCTION

Helminth infections continue to pose a major public health burden worldwide, particularly in tropical and subtropical regions where poor sanitation and limited access to healthcare facilitate the transmission of parasitic worms.[1] These infections affect billions of humans and livestock, leading to significant morbidity, malnutrition, impaired growth, reduced productivity, and substantial economic losses.[2] Despite the availability of several anthelmintic drugs, the widespread and prolonged use of existing therapies has resulted in the emergence of drug-resistant helminth strains, thereby diminishing treatment efficacy and highlighting the urgent need for novel therapeutic alternatives.[3]

Albendazole, a benzimidazole derivative, is one of the most extensively used broad-spectrum anthelmintic agents due to its effectiveness against a wide range of nematodes, cestodes, and trematodes. Its mechanism of action primarily involves the inhibition of microtubule polymerization through selective binding to β-tubulin, leading to impaired glucose uptake, disruption of cellular metabolism, and eventual parasite death. However, limitations such as poor aqueous solubility, variable bioavailability, and the increasing prevalence of resistance have encouraged researchers to explore structural modifications of the albendazole scaffold to improve its pharmacological profile and therapeutic efficacy. [4,5,6]

Recent advances in medicinal chemistry have demonstrated that strategic modifications of

benzimidazole-based compounds can significantly influence their biological activity, selectivity, and pharmacokinetic properties. The integration of computational approaches, particularly molecular modeling and molecular docking studies, has become a valuable tool in modern drug discovery by enabling the prediction of ligand–target interactions, binding affinities, and structure–activity relationships prior to biological evaluation. These in silico techniques facilitate the rational design of novel derivatives with enhanced therapeutic potential while reducing the time and cost associated with experimental screening.

In the present study, a series of novel albendazole derivatives were designed and synthesized through appropriate synthetic methodologies. The synthesized compounds were characterized using standard spectroscopic techniques to confirm their chemical structures and purity. Molecular modeling studies were performed to investigate the binding interactions of the derivatives with selected parasitic target proteins and to predict their potential mechanism of action. Furthermore, the biological activities of the synthesized compounds were evaluated using established in vitro anthelmintic assays.

The integrated approach combining chemical synthesis, molecular modeling, and biological assessment aims to identify promising albendazole-based derivatives with improved anthelmintic activity. The findings of this study may contribute to the development of next-generation antiparasitic agents capable of overcoming the limitations associated with currently available therapies and addressing the growing challenge of anthelmintic resistance.

In-silico study

An in-silico evaluation of the physicochemical properties of the synthesized compounds was carried out and their drug-likeness profiles were analyzed using the SwissADME platform.

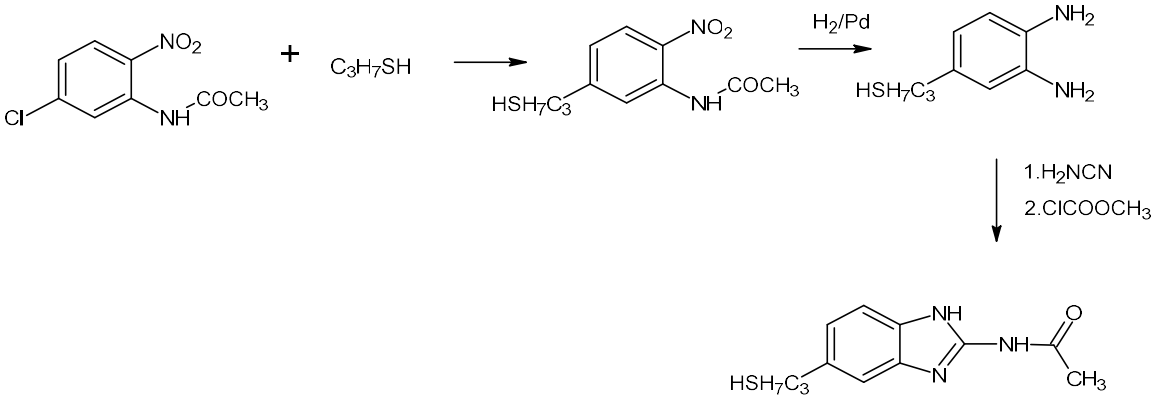
Docking refers to predicting a ligand's structure and orientation at a certain binding site. This study identified tubulin alpha-1B and tubulin beta chains as the primary targets for anthelmintic drugs, based on research on anthelmintic receptor proteins. [7]

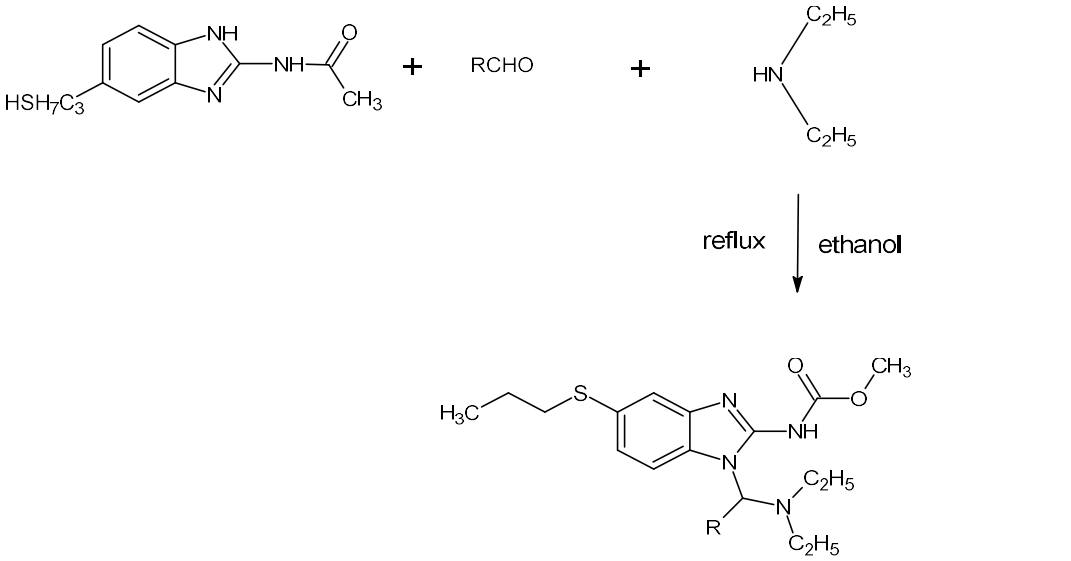
MATERIALS AND MATHODS:

Molecular Docking Studies

- Molecular docking studies were carried out to evaluate the anthelmintic potential of the synthesized Mannich base derivatives of albendazole.
- The docking study was performed using AutoDock Vina software.
- The three-dimensional crystal structure of the target protein (PDB ID: 5D73) was retrieved from the Protein Data Bank (PDB).
- The protein structure was prepared by removing water molecules and other unwanted molecules, followed by the addition of hydrogen atoms and charges required for docking.
- The chemical structures of the synthesized Mannich base derivatives were drawn using ChemSketch software.
- The ligand structures were converted into three-dimensional conformations and exported in PDB format.
- Binding energies and protein–ligand interactions were analyzed to determine the docking efficiency of the synthesized derivatives.
- The docked complexes were visualized and analyzed using PyMOL software to study hydrogen bonding and other intermolecular interactions.
- The conformations exhibiting the lowest binding energy were considered the most favorable binding poses.

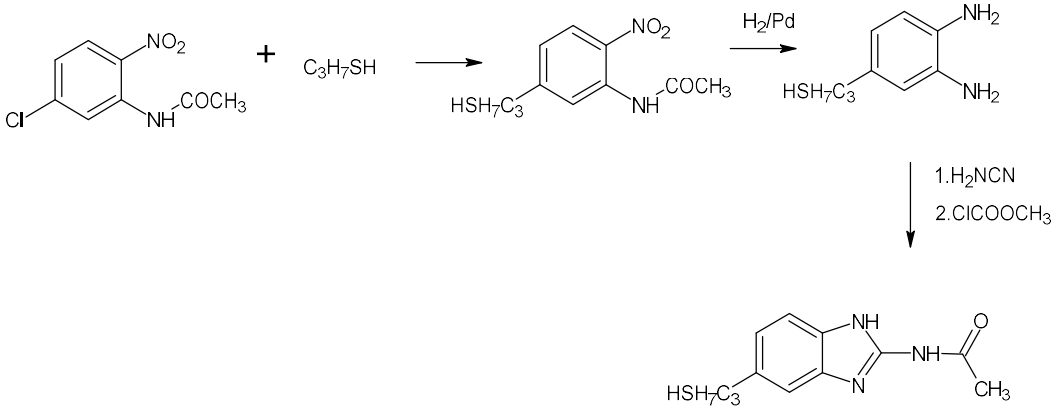
EXPERIMENTAL WORK:





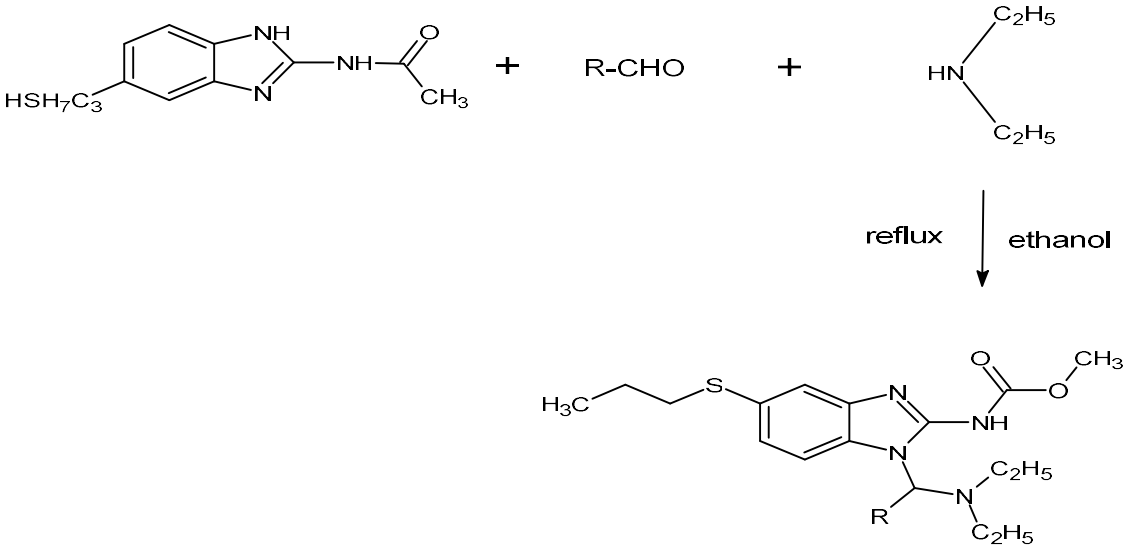
STEP 1st:

Synthesis of Methyl 5-(n-propylthio)-2-benzimidazolecarbamate (Albendazole)



3-propylthio-6-nitroacetanylide is formed by the reaction of 3-chloro-6-nitroacetanylide with propylmercaptane. Reducing the nitro group in this compound with hydrogen using a palladium on carbon catalyst gives 4-(propylthio)-o-phenylenediamine. Reacting the resulting derivative of o-phenylenediamine with cyanamide and then with the methyl chloroformate gives the desired albendazole.[8]

STEP 2nd :



Synthesis of methyl (1-((diethylamino) alky/aryl)-5-(propylthio)-1H-benzo[d]imidazole 2-yl) carbamate

An equimolar quantity (0.01 mol) of Albendazole (1 g) was accurately weighed and dissolved in ethanol (25 mL) or acetone (30 mL). To this solution, diethylamine (0.01 mol, 1 mL) was added under continuous stirring. Subsequently, formaldehyde or benzaldehyde (0.01 mol, 1 mL) was introduced dropwise into the reaction mixture. The resulting mixture was stirred for 1 h and then subjected to reflux for 5–6 h to facilitate completion of the reaction. After reflux, the reaction mixture was allowed to cool and kept in a freezer overnight to promote crystallization. The resulting yellow-colored solid product was isolated and collected for further analysis.

PHYSICAL AND SPECTRAL ANALYSIS OF SYNTHESIZED COMPOUNDS:

All reagents and solvents used in the present study were of analytical grade and procured from commercial sources. Unless otherwise specified, all chemicals were used as received without further purification.

The progress of the reactions and the purity of the synthesized compounds were monitored by thin-layer chromatography (TLC) using pre-coated silica gel G plates as the stationary phase. The chromatograms were visualized under appropriate conditions to confirm reaction completion and compound purity.

Melting points of the synthesized compounds were determined using a Labtronics melting point apparatus and are reported uncorrected.

Infrared spectra were recorded on a Bruker ECO-ATR FTIR spectrophotometer. The ^{1}H NMR spectra were acquired in $CDCl_3$ on a Bruker 400 MHz instrument at Spark Analytical, using tetramethylsilane (TMS) as an internal reference. The ^{13}C NMR spectra were recorded in $CDCl_3$ on Bruker spectrometers operating at 300, 400, or 500 MHz, with TMS as the reference standard.

Mass spectral analysis was performed using fast atom bombardment mass spectrometry (FAB-MS), which facilitates ion formation through bombardment with high-energy neutral atoms. The spectral data obtained were used to confirm the molecular weight and support the structural elucidation of the synthesized compounds.

PHARMACOLOGICAL SCREENING

Experimental Animals:

The anthelmintic activity was evaluated using adult Indian earthworms, *Pheretima posthuma*. The worms were collected from moist soil and thoroughly rinsed with water to remove adhering debris and fecal matter. Specimens of uniform size, approximately 3–6 cm in length and 0.1–0.2 cm in width,were selected for the study. *Pheretima posthuma* is widely employed as an experimental model due to its anatomical and physiological resemblance to human intestinal nematodes, making it suitable for preliminary screening of anthelmintic agents.

Evaluation of Anthelmintic Efficacy Using In Vitro Models:

The anthelmintic potential of the synthesized compounds was assessed using adult specimens of *Pheretima posthuma* obtained from the Sangli region. Worms of nearly uniform size (6 ± 1 cm) were selected and allowed to acclimatize under laboratory conditions prior to the experiment.[9] For the study, the worms were divided into groups, each containing six individuals. Test solutions were prepared at concentrations of 2, 4, and 6 mg/mL by dissolving the compounds in a small quantity of 2%

DMSO and diluting to a final volume of 20 mL with normal saline. Freshly prepared solutions of both the test compounds and the reference drug were used for the assay.

Each worm, after thorough washing with normal saline, was placed individually in a Petri dish containing 20 mL of the respective test solution. The time required to induce paralysis (P) and death (D) was recorded. Paralysis was defined as the absence of movement even after transfer to normal saline, while death was confirmed by complete cessation of motility along with gradual discoloration of the body.[10]

Statistical Evaluation and Data Analysis:

The experimental data were expressed as mean ± standard error of the mean (SEM). Statistical evaluation was performed using Two-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test to assess the significance of differences between treated groups and the control. [11]

RESULT AND DISCUSSION:

ADMET Analysis:

The physicochemical and pharmacokinetic properties of the synthesized albendazole derivatives (D1–D4) were evaluated using the SwissADME platform. All compounds were found to satisfy Lipinski's rule of five, suggesting favorable drug-likeness characteristics. The molecular weights of the derivatives were below 500 Dalton, while topological polar surface area (TPSA) values remained within the acceptable range (<140 Å). Additionally, the number of rotatable bonds did not exceed 10, indicating good molecular flexibility and potential oral bioavailability. The compounds also exhibited high predicted gastrointestinal absorption, supporting their suitability as orally active anthelmintic agents (**Table 1**).

Table 1 Important pharmacokinetic parameters for good oral bioavailability of the titled compound (D1-D4)

Compound	R	MF	TPSA (Å)	n-ROTB	MW	LogP	n-H bond donono	n-H bond acceptor	Lipinski violation
Rule					<500	<5	<5	<10	<1
D1	Cinnamaldehyde	$C_{26}H_{34}N_4O_2S$	84.69	12	466.64	4.60	1	4	1
D2	Anisaldehyde	$C_{24}H_{32}N_4O_3S$	93.92	12	456.60	3.83	1	5	0
D3	4-Bromobenzaldehyde	$C_{23}H_{29}BrN_4O_2S$	84.69	11	505.47	3.81	1	4	1
D4	Vanillin	$C_{24}H_{32}N_2O_4S$	114.15	12	472.60	3.75	2	6	0

(MF – Molecular formula, TPSA - topological polar surface area, n-ROTB - number of rotatable bonds, MW – Molecular weight)

Antihelmintic Activity Docking Interaction

Table No.2 Docking scores of synthesized compounds with receptor 5D73

Derivatives	Docking Scores of compounds with 5D73 receptor (kcal/mol)
D1	-7.1
D2	-6.9
D3	-6.6
D4	-6.5

(kcal/mol - kilocalories per mole)

DOCKING INTERACTION:

Interaction details of Methyl [1-((diethylamino)(2-phenylethenyl)methyl)-5-(propylthio)-1H-benzimidazol-2-yl]carbamate (D1) with the 5D73 receptor's active site

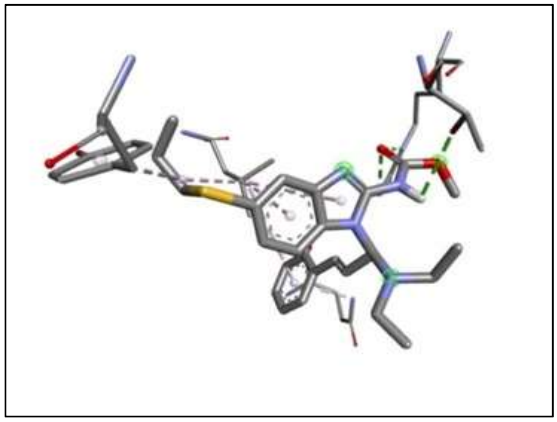


Fig.1(a) 3D interaction of compound D1 with 5D73 receptor

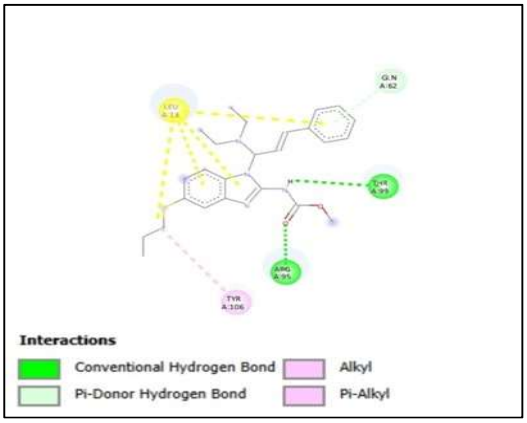


Fig.1(b) 2D interacton of compound D1

Int ox -
2-yl]carbamate (D2) with the 5D73 receptor's active site

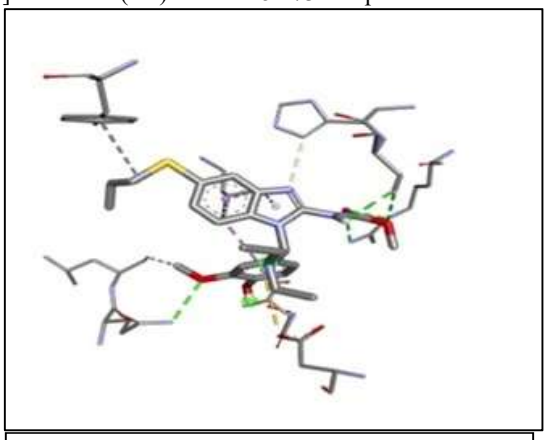


Fig.2(a) 3D interaction of compound D2 with 5D73 receptor

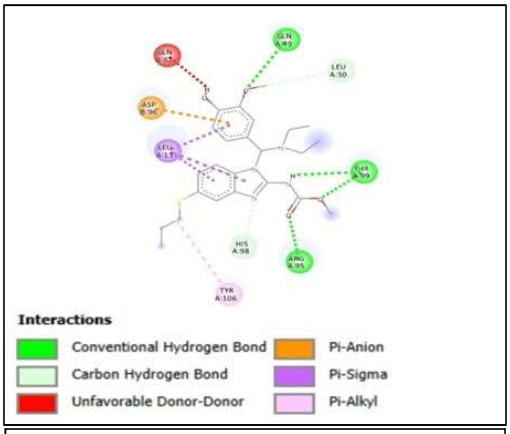


Fig.2(b) 2D interacton of compound D2

Interaction details of Methyl [1-((diethylamino)(4-bromophenyl)methyl)-5-(propylthio)-1H-benzimidazol-2-yl]carbamate (D3) with the 5D73 receptor's active site

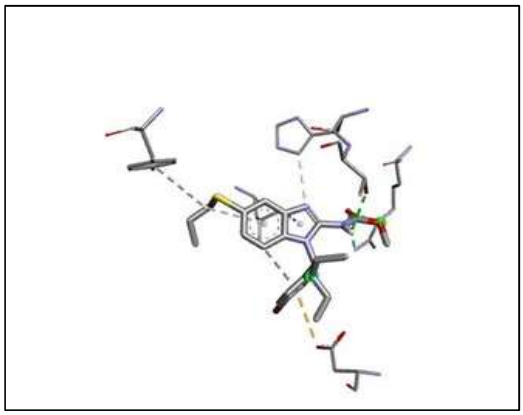


Fig.3(a) 3D interaction of compound D3 with 5D73 receptor

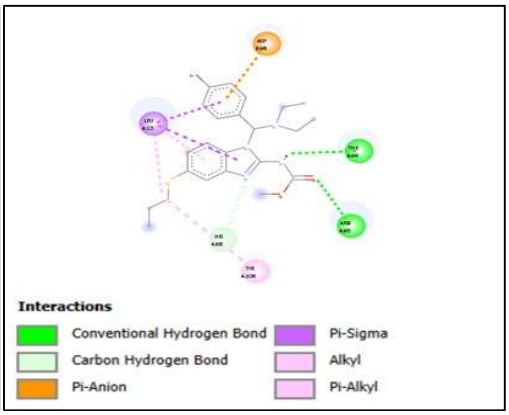


Fig.3(b) 2D interacton of compound D3

Interaction details of Methyl [1-((diethylamino)(4-hydroxy-3-methoxyphenyl)methyl)-5-(propylthio)-1H-benzimidazol-2-yl]carbamate (D4) with the 5D73 receptor's active site

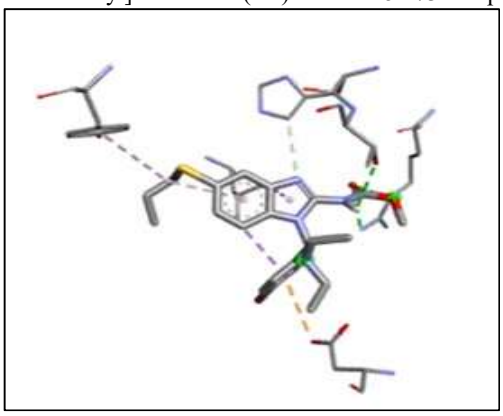


Fig.4(a) 3D interaction of compound D4 with 5D73 receptor

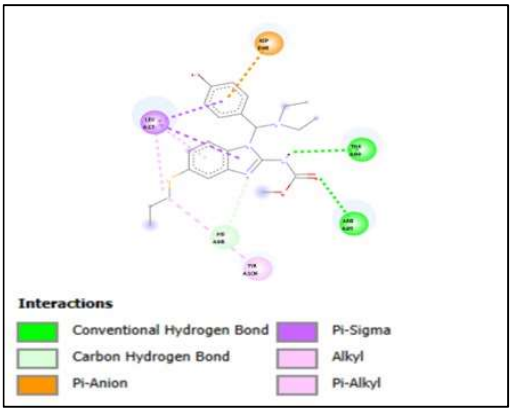


Fig.4 (b) 2D interacton of compound D4

Interaction details of Methyl 5-(n-propylthio)-2-benzimidazolecarbamate (Standard-Albendazole) with the 5D73 receptor's active site.

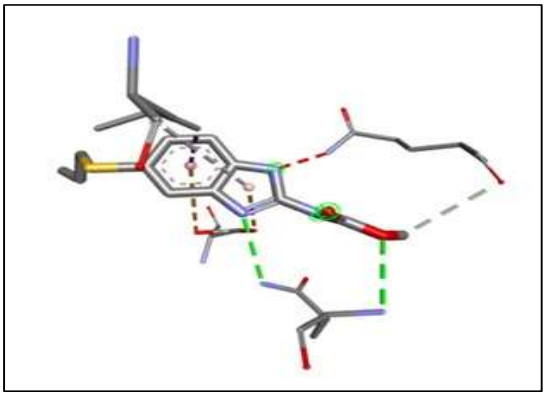


Fig. 5(a) 3D interaction of compound D5 with 5D73 receptor

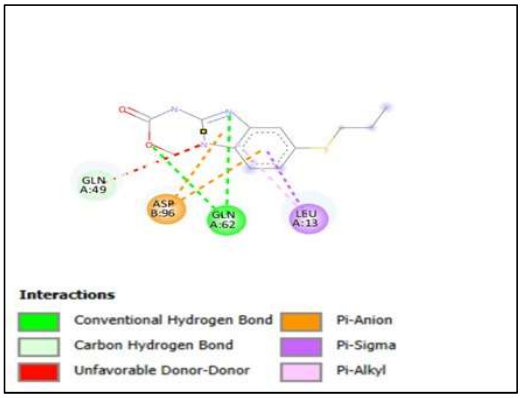


Fig.5(b) 2D interacton of compound D5

Mole[illegible]d derivatives possess significant affinity toward the target protein, with binding energies observed in the range of −6.5 to −7.1 kcal/mol relative to Albendazole. The obtained values suggest stronger interaction potential than the parent compound, which may translate into improved biological effectiveness. Among the tested molecules, derivatives D1 and D2 exhibited the most favourable docking scores −7.1 and −6.6 kcal/mol, respectively.

Detailed interaction analysis revealed that the introduction of Mannich base functionality contributed to enhanced ligand–protein binding. This improvement is likely associated with the formation of stabilizing interactions such as hydrogen bonding, hydrophobic contacts, and π–π interactions within the active site of the protein. Overall, these results underline the importance of structural modification of the albendazole framework in improving its activity profile. The enhanced binding characteristics of the synthesized compounds indicate their potential as promising candidates for further pharmacological evaluation as novel anthelmintic agents.

Physical and Spectral Data of Synthesized Compounds:

- **Methyl[1-((diethylamino)(2-phenylethenyl)methyl)-5-(propylthio)-1H-benzimidazol-2-yl]carbamate (D1):** Yield: 76%; M.P.: 186°C.

IR (cm^{-1}): 3300–3500 (N–H stretching), 1696–1730 (carbamate C=O stretching), 1300–1000 (aromatic C=C/C=N stretching), 1000–1400 (C–N stretching), 1300–1000 (C–O stretching), 700–600 (C–S thioether stretching).
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): ^{1}H NMR (δ, ppm): 10.40–10.80 (s, 1H, NH), 7.20–7.35 (m/s, Ar–H), 4.00 (s, 3H, OCH_3), 2.90–3.00 (t/m, CH_2 adjacent to heteroatom), 1.60–1.75 (m, aliphatic CH_2), 1.00–1.10 (t, 3H, terminal CH_3).
MS (m/z): 465 (M+1).

- **Methyl [1-((dimethylamino)(4-methoxyphenyl)methyl)-5-(propylthio)-1H-benzimidazol-2-yl]carbamate (D2):** Yield: 74%; M.P.: 178°C.
  IR (cm^{-1}): IR (cm^{-1}): 3300–3500 (N–H stretching), 1696–1730 (carbamate C=O stretching), 1300–1000 (aromatic C=C/C=N stretching), 1000–1400 (C–N stretching), 1300–1000 (C–O stretching), 700–600 (C–S thioether stretching).
  ^{1}H NMR ($CDCl_3$, δ ppm): ^{1}H NMR (δ, ppm): 10.20–10.80 (s, NH), 7.05–7.35 (m/s, aromatic protons), 3.85–4.00 (s, methoxy protons), 2.75–3.05 (t/m, CH_2 adjacent to heteroatom), 1.45–1.75 (m, aliphatic CH_2), and 1.00–1.10 (t, terminal CH_3).
  MS (m/z): 458(M+1).

- **Methyl [1-((diethylamino)(4-bromophenyl)methyl)-5-(propylthio)-1H-benzimidazol-2-yl]carbamate (D3):** Yield: 78%; M.P.: 184°C.
  IR (cm^{-1}): 3300–3500 (N–H stretching), 1696–1730 (carbamate C=O stretching), 1300–1000 (aromatic C=C/C=N stretching), 1000–1400 (C–N stretching), 1300–1000 (C–O stretching), 700–600 (C–S stretching, thioether), 690–515 (C–Br stretching).
  ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 10.40–10.80 (s, 1H, NH), 7.20–7.35 (m, Ar–H), 4.00 (s, 3H, OCH_3), 2.75–2.95 (t/m, CH_2 adjacent to heteroatom), 1.60–1.75 (m, aliphatic CH_2), 1.00–1.10 (t, CH_3).
  MS (m/z): 506 (M+1).

- **Methyl [1-((diethylamino)(4-hydroxy-3-methoxyphenyl)methyl)-5-(propylthio)-1H-benzimidazol-2-yl]carbamate (D4):** Yield: 70%; M.P.: 188°C.
  IR (cm^{-1}): 3200–3700 (O–H stretching), 3300–3500 (N–H stretching), 3000–2840 (aliphatic C–H stretching), 1696–1730 (carbamate C=O stretching), 1300–1000 (aromatic C=C/C=N stretching), 1000–1400 (C–N stretching), 1300–1000 (C–O stretching), and 700–600 (C–S stretching, thioether).
  ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 10.10–10.50 (s, 1H, Ar–OH), 10.40–10.80 (s, 1H, NH), 7.20–7.35 (m, Ar–H), 4.00 (s, 3H, OCH_3), 2.90–3.00 (t/m, CH_2 adjacent to heteroatom), 1.60–1.75 (m, aliphatic CH_2), 1.00–1.10 (t, 3H, CH_3).
  MS (m/z): 473(M+1).

PHARMACOLOGICAL EVALUTION

Antihelmintic activity:

D1 D2

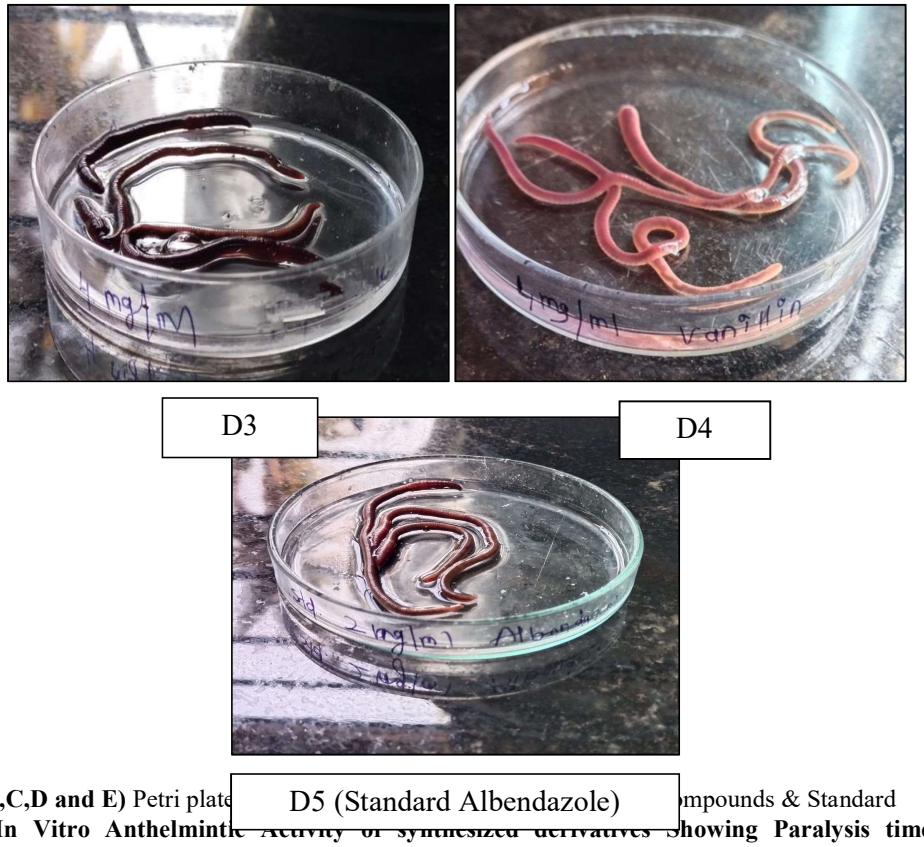


Fig.6 (A,B,C,D and E) Petri plate ompounds & Standard

Table 3 In Vitro Anthelmintic Activity of synthesized derivatives Showing Paralysis time of test compounds.

Test compounds	Time taken for paralysis (p) Paralysis time (min) 2mg/ml	4mg/ml	6 mg/ml
Control	-	-	-
D1	20.83±0.52****	12.80±0.40****	8.38±0.15****
D2	38.66±0.63****	20.98±0.62****	7.06±0.35****
D3	33.95±0.53****	26.88±0.29****	23.66±0.31****
D4	42.87±0.54****	32.60±0.35****	16.18±0.80****
Albendazole	52.35±45	44.17±0.18	38±0.46

Values are expressed as Mean ± SEM (n = 3). Statistical analysis was performed using two-way ANOVA followed by Dunnett's multiple comparison test. Values of $p < 0.05$ were considered statistically significant. The symbol **** indicates a highly significant difference compared with the standard drug Albendazole ($p < 0.0001$).

Present data indicates replication of 3 readings

Table 3 shows that the synthesized derivatives (D1–D4) showed dose dependent anthelmintic activity, with paralysis time decreasing as concentration increased (2–6 mg/mL). At 2 mg/mL, D1 showed the highest activity (20.83 ± 0.52 min), followed by D3 (33.95 ± 0.53 min), D2 (38.66 ± 0.63 min), and D4 (42.87 ± 0.54 min). At 4 mg/mL, D1 (12.80 ± 0.40 min) and D2 (20.98 ± 0.62 min) were more active than D3 and D4. At 6 mg/mL, D2 (7.06 ± 0.35 min) and D1 (8.38 ± 0.15 min) showed highest potency. Albendazole exhibited higher paralysis times (52.35 → 38 min), indicating lower activity than synthesized compounds.

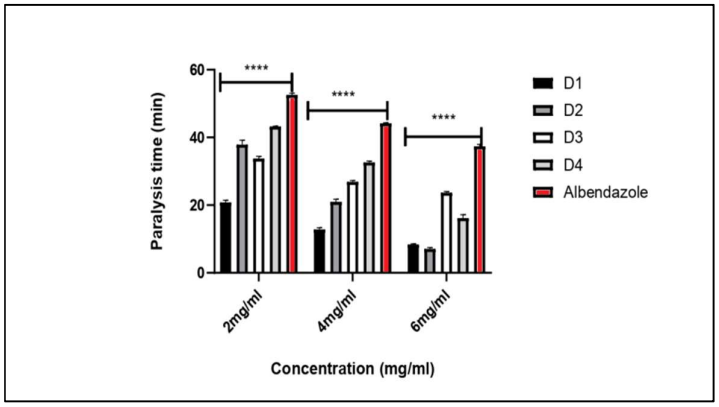


Fig.7. Graph Showing Concentration vs Paralysis Time of test compounds

Table 4 In Vitro Antihelmintic Activity of synthesized derivatives Showing death time of test compounds

Test compounds	Time taken for death (d)		
	Death time (min)		
	2mg/ml	4mg/ml	6mg/ml
Control	-	-	-
D1	43.52±0.46****	30.93±0.66****	18.90±0.56****
D2	64.97±0.54****	50.56±0.53****	43.31±0.90****
D3	57.62±0.46 ****	43.18±0.48****	17.79±0.52****
D4	69.23±0.89****	67.94±0.56****	38.25±0.74****
Albendazole	64.76±0.54	52.44±0.41	49.05±0.40

Values are expressed as mean ± SEM (n = 3). Statistical analysis was performed using two-way ANOVA followed by Dunnett's multiple comparison test. Differences were considered statistically significant at $p < 0.05$. ****$p < 0.0001$ versus the standard drug (Albendazole).

Present data indicates replication of 3 readings

Table 4 shows that Compound D1 showed the fastest activity at 2 mg/ml (43.52 ± 0.46 min), while D4 was slowest (69.23 ± 0.89 min); albendazole (64.76 ± 0.54 min) was comparable to D2. At 4 mg/ml, D1 remained most active (30.93 ± 0.66 min), followed by D3 (43.18 ± 0.48 min) and D2 (50.56 ± 0.53 min), while albendazole recorded 52.44 ± 0.41 min. At 6 mg/ml, D3 (17.79 ± 0.52 min) and D1 (18.90 ± 0.56 min) showed highest potency, outperforming albendazole (49.05 ± 0.40 min), whereas D2 (43.31 ± 0.90 min) and D4 (38.25 ± 0.74 min) demonstrated moderate activity.

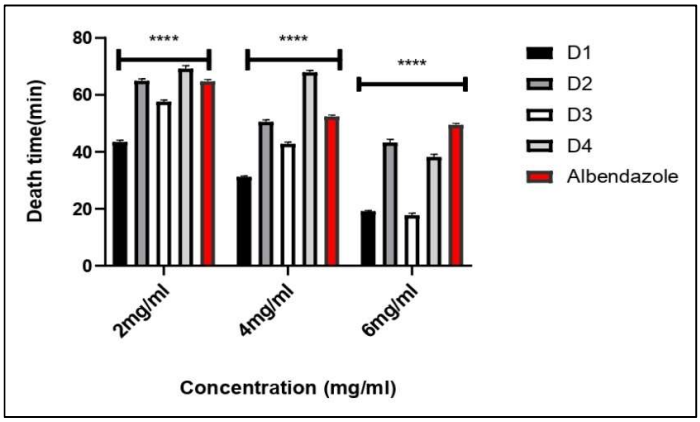


Fig.8 Graph Showing Concentration vs Death Time of test compounds

DISCUSSION:

The present study demonstrates that the synthesized derivatives (D1–D4) possess notable anthelmintic activity, as evidenced by both in silico and in vitro evaluations. Molecular docking analysis revealed favourable binding interactions with the target protein, with docking scores ranging from −6.5 to −7.1 kcal/mol. Among the tested compounds, D1 (−7.1 kcal/mol) and D2 (−6.6 kcal/mol) exhibited comparatively stronger binding affinity than Albendazole. The in vitro findings further supported these results, showing a clear dose-dependent response. Compounds D1 and D2 demonstrated enhanced paralytic effects, while D1 and D3 exhibited reduced time to mortality at higher concentrations. In comparison to the standard drug, all synthesized derivatives displayed improved efficacy, as indicated by shorter paralysis and death times.

Overall, compound D1 showed the highest level of activity, followed by D2 and D3, highlighting their potential as promising candidates for the development of more effective anthelmintic agents.

CONCLUSION:

Structural modification of albendazole through the incorporation of Mannich base moieties resulted in enhanced pharmacokinetic and biological properties. In silico ADMET analysis revealed that all synthesized derivatives complied with Lipinski's Rule of Five, suggesting favorable drug-likeness and oral bioavailability. Molecular docking studies demonstrated improved binding affinities and favorable protein–ligand interaction profiles compared to the parent compound, which correlated well with the observed in vitro anthelmintic activity. Furthermore, all synthesized derivatives (D1–D4) exhibited significant paralysis and death times against the tested helminths, indicating promising anthelmintic efficacy. These findings suggest that the synthesized Mannich base derivatives of albendazole may serve as potential lead candidates for the development of novel anthelmintic agents.

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