ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Imidazole Derivatives as Anthelmintic Agents: Synthesis, Spectral Characterization and Molecular Docking Insights.

Swaroopa HM, Rashmi T, Vinutha S*, Muhammad Abrar Choudhari, Hemanth Gowda D, Varshini KN, and Umme Shafeen.

Department of Pharmaceutical Chemistry, Bharathi College of Pharmacy, Bharathinagara, Mandya, Karnataka – 571422, India.

ABSTRACT

The emergence of resistance to existing anthelmintic drugs necessitates the development of new therapeutic agents. In this study, ten imidazole derivatives were rationally designed and evaluated for their anthelmintic potential through an integrated molecular docking and experimental approach. Docking studies against Pokeweed Antiviral Protein (PDB ID: 1JFF) identified favorable protein-ligand interactions, guiding the selection of potent candidates for synthesis. The compounds were synthesized via a one-pot condensation method and structurally confirmed using IR, ^{1}H-NMR and mass spectrometry. *In-vitro* anthelmintic activity was assessed using Indian earthworms, the compounds T3 and T8 exhibiting significant activity comparable to albendazole. The results highlight the influence of substituents on biological efficacy and support the imidazole scaffold as a promising framework for anthelmintic drug development. This study provides a foundation for further optimization and *in-vivo* investigation of imidazole-based compounds.

Keywords: Imidazole derivatives, Molecular docking, alpha-beta tubulin (1JFF) and One-pot Synthesis, Anthelmintic activity.

https://doi.org/10.33887/rjpbcs/2025.16.5.8

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Helminth infections are a significant public health concern, particularly in developing countries, contributing to malnutrition, impaired cognitive development and reduced productivity [1]. The growing resistance to conventional anthelmintic drugs underscores the urgent need for novel therapeutic agents [2].

Heterocyclic compounds have played a pivotal role in medicinal chemistry, and among them, imidazole, a five-membered nitrogen-containing ring has attracted particular interest [3]. Imidazole derivatives are known for their structural versatility and diverse biological activities, including antibacterial, antifungal, antiviral, anticancer, anti-inflammatory and antiparasitic properties [4, 5]. Their ability to interact with various biological targets makes them valuable scaffolds for the design of new therapeutic agents. In this context, exploring imidazole derivatives for anthelmintic potential represents a promising strategy to address the current challenges in helminth control [6].

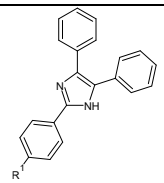
In the present study, a rational design approach was employed to generate some imidazole-based compounds with potential anthelmintic activity. Molecular docking studies were first conducted to predict the binding affinity and interaction patterns of the designed molecules with selected helminthic target proteins, thereby guiding the selection of promising candidates for synthesis. The shortlisted derivatives were then synthesized and structurally confirmed using spectroscopic techniques such as IR, NMR, and mass spectrometry. Finally, the synthesized compounds were evaluated for their *in-vitro* anthelmintic activity using standard models.

This integrated computational and experimental approach aims to establish a clear structure–activity relationship, contributing to the development of new imidazole-based anthelmintic agents with enhanced efficacy and potential for further preclinical development.

MATERIALS AND METHODOLOGY

Molecular docking

Dataset ligands and ligand optimization



A designed imidazole derivatives

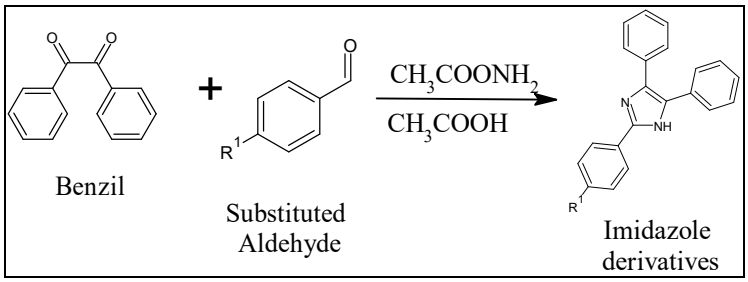
Structure Code	R
T1	H
T2	4-NO_2
T3	3-NO_2
T4	2-NO_2
T5	4-Cl
T6	3-Cl
T7	2-Cl
T8	3-CH_3
T9	4-CH_3
T10	4-OCH_3

Table 1: Designed imidazole derivatives

The 2D structures of 10 compounds were generated from the ACD/chemsketch software. The generated ligands cleaned and performed 3D optimization then saved in the MDL Molfile format. The ligands were then converted to a PDBQT file format using the open label chemistry toolbox [7, 8].

The three-dimensional (3D) structure of Pokeweed Anthelmintic Protein (PDB ID: 1JFF) was downloaded from Brookhaven protein data bank (https://www.rcsb.org) and saved as a Brookhaven protein data bank file [9] and the structure was optimized by deleting unbound water molecules which are over 1 Å, adding hydrogen atoms to satisfy the valences, adding missing amino acids to stabilize side chains and energy of the whole structure was minimized using AUTODOCK suite of MGL Tools [10]. Auto dock Vina was used for molecular docking studies. A grid was generated around the co-crystallized ligand. The co-ordinates (x = 31.59, y =24.19, z = 14.48) were generated with the help of MGL Tools & Pharmit:interactive exploration of chemical space (http://pharmit.csb.pitt.edu/) [11]. Prepared PDBQT files of target and ligands and docking performed in the absence of water molecules for all 10 molecules. After docking, the molecules were analyzed and visualized in the discovery studio for the interactions with the active site amino acids. Binding interactions and efficiency of the binding were calculated in terms of dock Score, which is a combination of hydrophilic, hydrophobic, metal binding groups, vander waals energy, freezing rotatable bonds and polar interactions with receptor.

Synthetic procedure



Scheme-I

Procedure: Benzil (1 gm), benzaldehyde (1ml) and ammonium acetate (1 g) were dissolved in glacial acetic acid (2ml) and then refluxed for 3 hr. After refluxing, cool the mixture add 150ml of water to that mixture. Then add 10% Ammonium hydroxide solution dropwise till precipitate formation then filter the product and recrystallize using ethanol.

Characterisation

Melting point of the synthesized compounds was determined by an open-end capillary tube method using electrically heated melting point apparatus. The respective values were expressed in °C and were uncorrected. Reaction progress and compounds purity was ascertained by thin layer chromatography (TLC). The structures of the synthesized compounds were elucidated by Fourier Transform IR spectrometer (Thermo Nicolet Nexus 670) in the range of 400-4000 cm^{-1} using KBr pellets and values are reported in cm^{-1} and the spectra were interpreted. ^{1}H-NMR spectra were recorded on Bruker-Topspin NMR spectrometer using DMSO-d6 and chemical shift (δ) are reported in parts per million down field from internal reference Tetramethylsilane (TMS). Mass spectra were recorded on Shimadzu by LC-MS-8030 mass spectrometer and the spectra were interpreted.

Anthelmintic activity

Indian earthworms were used to test the anthelmintic properties for each of the newly synthesized quinazolinone derivatives [12]. Six earthworms of remarkably similar dimensions were put in test and standard solution at room temperature. All of the compounds have been dispersed in a small amount of DMSO and diluted to 10 ml of standard saline solution to obtain quantities of 0.1%, 0.2%, and 0.5% w/v. Albendazole has been employed as a standard drug [13, 14].

The synthesized compounds (Scheme-I) are screened for anthelminthic activity by using Earth worms. Six earthworms of nearly equal size were placed in standard drug solution and test compound's solutions at room temperature [15]. Normal saline used as control. The standard drug and test compounds were dissolved in minimum quantity of dimethyl sulfoxide (DMSO) and adjusted the volume up to 10 ml with normal saline solution to get the concentration of 0.1% w/v, 0.2 % w/v and 0.5% w/v. Albendazole was used as a standard drug [16]. The compounds were evaluated by the time taken for complete paralysis

and death of earthworms. The mean lethal time for each test compound was recorded and compared with standard drug [17, 18]. The time taken by worms to become motionless was noted as paralysis time. To ascertain the death of the motionless worms were frequently applied with external stimuli, which stimulate and induce movement in the worms, if alive. The mean lethal time and paralysis time of the earthworms for different test [19, 20].

RESULT AND DISCUSSION

Molecular docking

Molecular docking studies were performed in order to find the possible protein ligand interactions of the dataset ligands. The potential active site amino acids of PDB ID: 1JFF complex were predicted using Computed Atlas of Surface Topography of Protein (CASTp). The target protein and inhibitors were geometrically optimized. All the 10 compounds were docked against active site of target protein using AUTODOCK VINA. Additionally, these also assisted in identifying the conformational changes of the ligand in the protein environment. About 100 different protein-ligand complex conformations for each docked complex were generated through AUTODOCK suite of MGL Tools, the confirmation with lowest binding energy was displayed as the best binding energy.

S. Code	Binding energy	No. of H Bonds	Interacting Amino acids	Bond length
T1	**-8.3**	1	THR:340	5.08
T2	-8.1	1	ARG:322	5.12
T3	**-8.3**	1	ARG:322	4.89
T4	**-8.2**	2	ALA:298, PHE:296	3.84, 3.72
T5	-8.1	-	-	-
T6	**-8.6**	-	-	-
T7	-8.2	-	-	-
T8	**-8.8**	1	THR:340	5.07
T9	-8.2	1	PRO:307	3.38
T10	7.9	-	-	-
native	-6.1	1	ASP:357	4.26

Table 2: Binding interactions of Imidazole derivatives against (PDB ID: 1JFF)

Figure 1: 3D Structure of structure of alpha-beta tubulin (PDB ID: 1JFF)

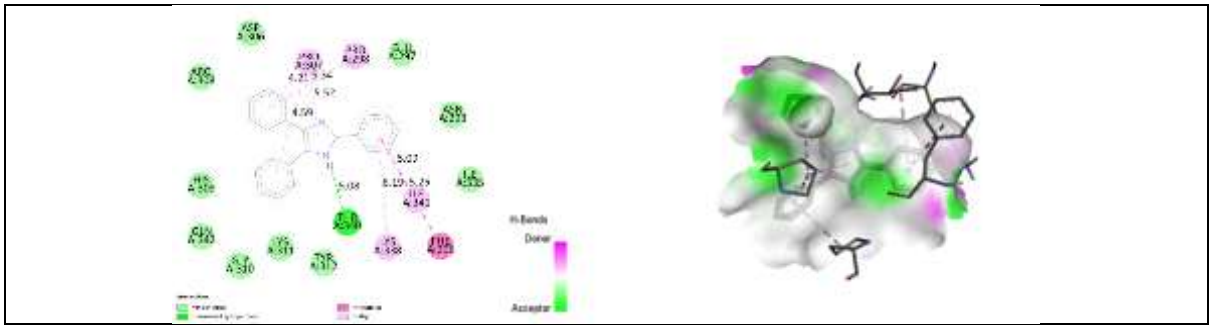


Figure 2: 2D and 3D Structure of the Compound T1 against PDB ID: 1JFF

ISSN: 0975-8585

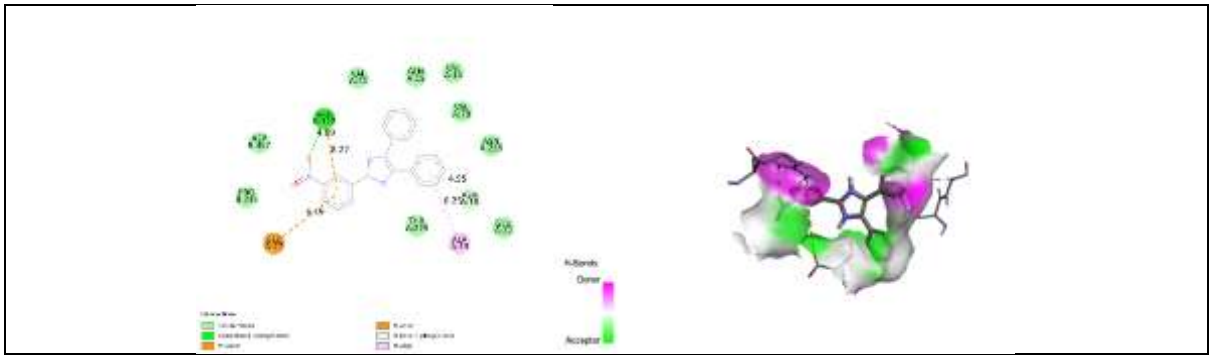


Figure 3: 2D and 3D Structure of the Compound T3 against PDB ID: 1JFF

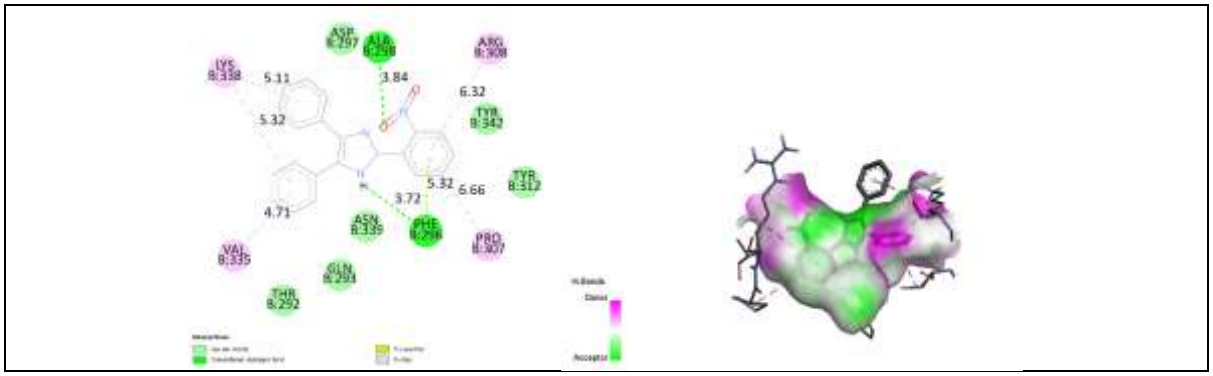


Figure 4: 2D and 3D Structure of the Compound T4 against PDB ID: 1JFF

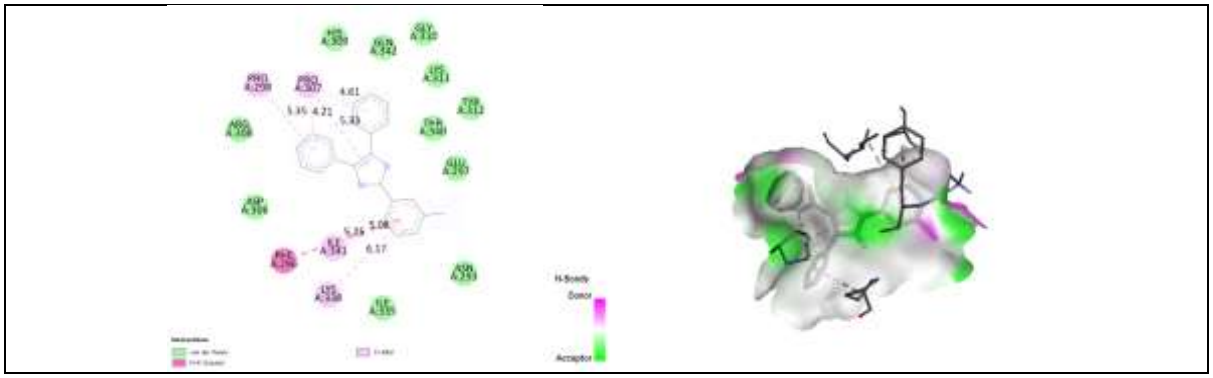


Figure 5: 2D and 3D Structure of the Compound T6 against PDB ID: 1JFF

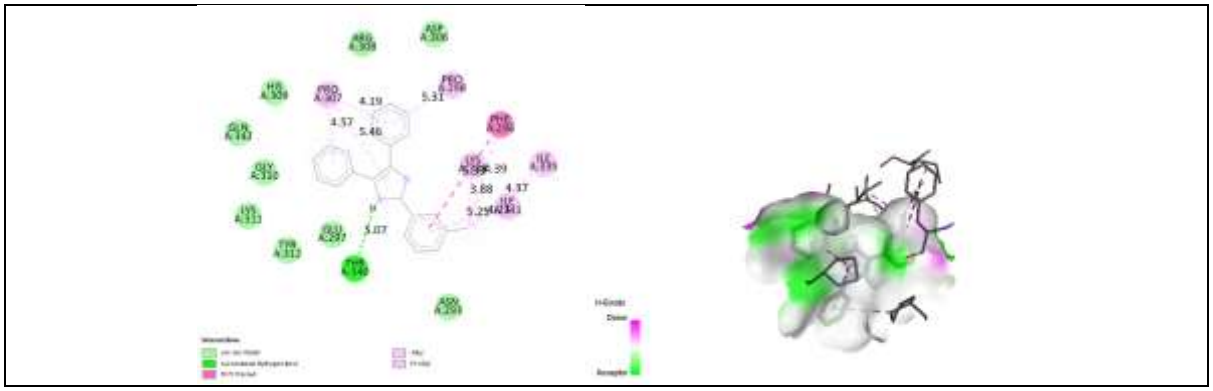


Figure 6: 2D and 3D Structure of the Compound T8 against PDB ID: 1JFF

ISSN: 0975-8585

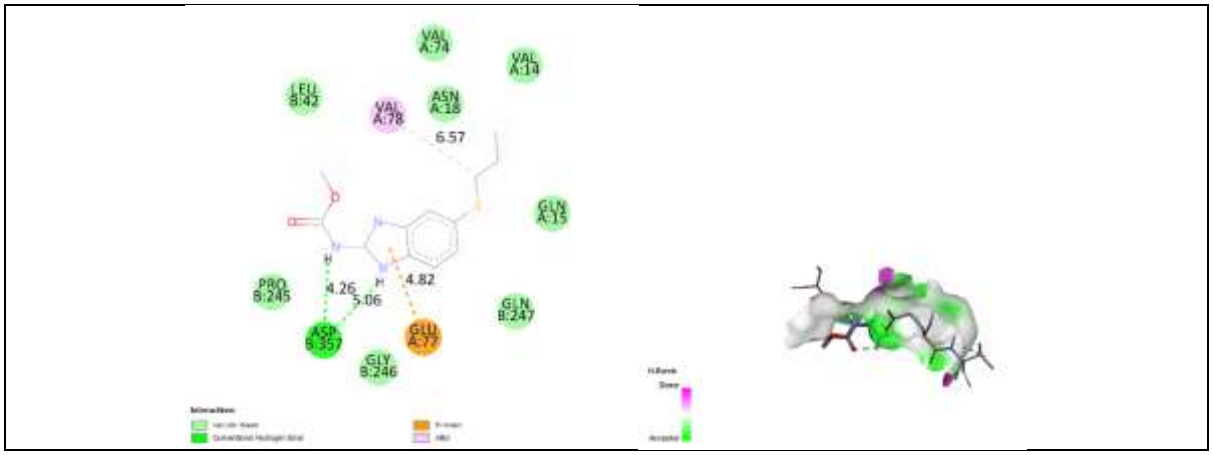


Figure 7: 2D and 3D Structure of the Albendazole against PDB ID: 1JFF

Synthesis

Code	R	Mol. Formula	Mol. Wt (g.mol^{-1})	Melting Point	% Yield
T1	H	$C_{21}H_{16}N_2$	296.37	242	78
T2	4-NO_2	$C_{21}H_{15}N_3O_2$	341.37	200	84
T3	3-NO_2	$C_{21}H_{15}N_3O_2$	341.37	160	84
T4	2-NO_2	$C_{21}H_{15}N_3O_2$	341.37	190	80
T5	4-Cl	$C_{21}H_{15}ClN_2$	330.81	97	83
T8	3-CH_3	$C_{22}H_{18}N_2$	310.40	210	79

Table 3: Physical Characterisation data of the Synthesized Compounds (T1, T2, T3, T4, T5 and T8)

Characterization

Compound T1: 2,4,5-triphenyl-1*H*-imidazole
IR (ν cm^{-1}): 3402 (N–H stretch, Imidazole), 3055 (C–H stretch, Aromatic), 1598 (C=N stretch, Imidazole ring), 1495 (C=C stretch, Aromatic), 1312 (C–N stretch), 756, 698 (Aromatic C–H bending).
^{1}H-NMR (DMSO, δ ppm): 12.45 (1H, s, NH of imidazole), 7.25–7.85 (15H, m, aromatic protons from three phenyl rings), 7.05 (1H, s, C–H proton at position 2 of imidazole).
Mass Spectrometry (ESI–MS): *m/z* 320.13 (M), *m/z* 321.14 (M+1, 100%).

Compound T2: 2-(4-nitrophenyl)-4,5-diphenyl-1*H*-imidazole
IR (ν cm^{-1}): 3405 (N–H stretch, Imidazole), 3098 (C–H stretch, Aromatic), 1595 (C=N stretch, Imidazole ring), 1510 and 1345 (N=O asymmetric and symmetric stretches, NO_2 group), 1490 (C=C stretch, Aromatic), 1310 (C–N stretch), 826, 702 (Aromatic C–H bending).
^{1}H-NMR (DMSO, δ ppm): 12.51 (1H, s, NH of imidazole), 8.24 (2H, d, Ar–H, ortho to NO_2), 7.72 (2H, d, Ar–H, meta to NO_2), 7.50–7.80 (10H, m, aromatic protons from two phenyl rings), 7.12 (1H, s, imidazole C–H at position 2).
Mass Spectrometry (ESI–MS): *m/z* 365.13 (M), *m/z* 366.11 (M+1, 100%).

Compound T3: 2-(3-nitrophenyl)-4,5-diphenyl-1*H*-imidazole
IR (ν cm^{-1}): 3412 (N–H stretch, Imidazole), 3090 (C–H stretch, Aromatic), 1602 (C=N stretch, Imidazole ring), 1521, 1340 (N=O asymmetric and symmetric stretches, NO_2 group), 1493 (C=C stretch, Aromatic), 1315 (C–N stretch), 823, 700 (Aromatic C–H bending).
^{1}H-NMR (DMSO, δ ppm): 12.48 (1H, s, NH of imidazole), 8.19–8.12 (1H, m, Ar–H adjacent to NO_2), 7.90–7.72 (2H, m, Ar–H), 7.58–7.45 (10H, m, aromatic protons from two phenyl rings), 7.10 (1H, s, imidazole C–H at position 2).
Mass Spectrometry (ESI–MS): *m/z* 365.13 (M), *m/z* 366.11 (M+1, 100%).

Compound T4: 2-(2-nitrophenyl)-4,5-diphenyl-1*H*-imidazole
IR (ν cm^{-1}): 3415 (N–H stretch, Imidazole), 3087 (C–H stretch, Aromatic), 1600 (C=N stretch, Imidazole ring), 1528 and 1337 (N=O asymmetric and symmetric stretches, NO_2 group), 1492 (C=C stretch, Aromatic), 1310 (C–N stretch), 825, 695 (Aromatic C–H bending).
^{1}H-NMR (DMSO, δ ppm): 12.44 (1H, s, NH of imidazole), 8.18–7.98 (3H, m, aromatic protons near ortho-NO_2 group), 7.55–7.40 (10H, m, aromatic protons from two phenyl rings), 7.06 (1H, s, C–H of imidazole ring).
Mass Spectrometry (ESI–MS): *m/z* 365.13 (M), *m/z* 366.11 (M+1, 100%).

Compound T5: 2-(4-chlorophenyl)-4,5-diphenyl-1*H*-imidazole
IR (ν cm^{-1}): 3410 (N–H stretch, Imidazole), 3085 (C–H stretch, Aromatic), 1595 (C=N stretch, Imidazole ring), 1490 (C=C stretch, Aromatic), 1260 (C–Cl stretch), 1310 (C–N stretch), 821, 698 (Aromatic C–H bending).
^{1}H-NMR (DMSO, δ ppm): 12.41 (1H, s, NH of imidazole), 7.55–7.45 (4H, d, Ar–H from 4-chlorophenyl ring), 7.40–7.80 (10H, m, aromatic protons from two phenyl rings), 7.05 (1H, s, imidazole C–H at position 2).
Mass Spectrometry (ESI–MS): *m/z* 336.10 (M), *m/z* 337.12 (M+1, 100%), *m/z* 338.10 (M+2, ~30%).

Compound T8: 2-(3-methylphenyl)-4,5-diphenyl-1*H*-imidazole
IR (ν cm^{-1}): 3408 (N–H stretch, Imidazole), 3080 (C–H stretch, Aromatic), 2923 (C–H stretch, Alkyl-CH_3), 1602 (C=N stretch, Imidazole), 1495 (C=C stretch, Aromatic), 1312 (C–N stretch), 822, 696 (Aromatic C–H bending).
^{1}H-NMR (DMSO, δ ppm): 12.40 (1H, s, NH of imidazole), 7.80–7.45 (10H, m, aromatic protons from two phenyl rings), 7.35–7.20 (3H, m, Ar–H from 3-methylphenyl), 6.98 (1H, s, imidazole C–H at position 2), 2.35 (3H, s, –CH_3 group on 3-methylphenyl ring).
Mass Spectrometry (ESI–MS): *m/z* 336.16 (M), *m/z* 337.18 (M+1, 100%).

Anthelmintic activity

Sl. no	Name	Time in minutes					
		For paralysis % Concentration			For death % Concentration		
	Concentration	0.1	0.2	0.5	0.1	0.2	0.5
	Control	-	-	-	-	-	-
	Albendazole	17	12	9	40	31	23
1.	T1	19	16	14	43	35	29
2.	T2	32	29	23	66	45	35
3.	T3	**17**	**14**	**11**	**42**	**33**	**27**
4.	T4	28	18	11	33	28	23
5.	T5	37	32	24	63	47	34
6.	T8	**17**	**13**	**11**	**41**	**32**	**25**

Table 4: Paralysis Time and Death Time of synthesized Compounds for Antihelmintic Activity.

The antihelmintic activity actual result is summarized in Table 3 and the Comparison of activity is shown in Figure 8. All of the studied drugs exhibited good-to-great activity, according to the data. Among the screened compounds, **Compound T8 and T3** showed excellent activity, and it was also comparable with the standard drug Albendazole.

ISSN: 0975-8585

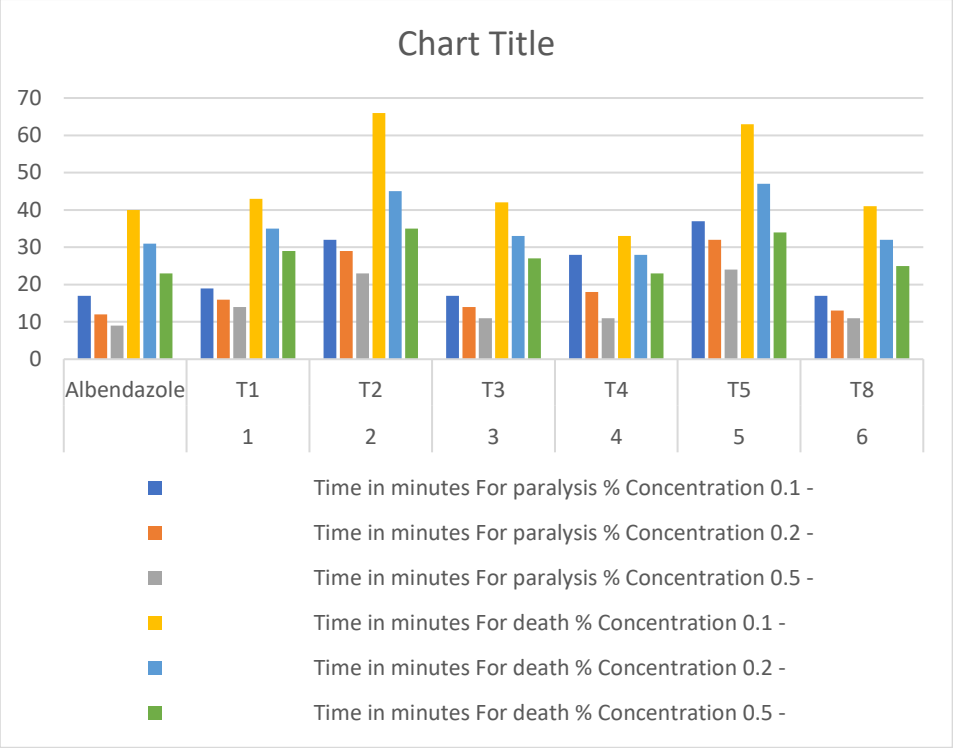


Figure 8: Graphical representation of Anthelmintic activity of Imidazole derivatives- Paralysis time and death time (min).

CONCLUSION

The present study successfully demonstrates an integrated computational and experimental approach for the development of some imidazole derivatives as potential anthelmintic agents. A series of ten imidazole-based compounds were rationally designed and subjected to molecular docking studies against the Pokeweed Antiviral Protein (PDB ID: 1JFF), a known helminthic target. The docking results revealed favorable binding interactions range from -8.8 to -8.1 kcal/mol. The compounds T8 exhibiting stronger binding affinity with -8.8 kcal/mol by Hydrogen bonding THR:340 compared to the native ligand, indicating promising biological potential.

Following computational analysis, the selected compounds were synthesized using a one-pot multicomponent reaction and subsequently characterized through IR, ^{1}H-NMR, and mass spectrometry to confirm their chemical structures. The synthesized derivatives were then screened for their *in-vitro* anthelmintic activity using Indian earthworms as the test organisms. Compounds T3 and T8 displayed the most potent anthelmintic effects, with paralysis and death times comparable to that of the standard drug, albendazole. These findings suggest a possible correlation between the substitution pattern on the imidazole ring and anthelmintic efficacy.

Overall, the study highlights the potential of imidazole scaffolds for further development as anthelmintic agents. The integration of molecular docking with synthetic and biological evaluation provides a cost-effective and efficient strategy for early-stage drug discovery. Future studies involving mechanism-based assays, toxicity profiling, and *in-vivo* validation are warranted to further explore the therapeutic potential of these derivatives.

REFERENCES

[1] Sultana S, Pandian P, Gupta JK, Rajkamal B, Barmavatu P, Mohanty D. Hybrids of imidazole with indoline derivatives: Microwave assisted synthesis, molecular docking studies, possible biological activities. Journal of the Indian Chemical Society. 2024 Apr 1;101(4):101143.

[2] Muheyuddeen G, Khan MY, Ahmad T, Srivastava S, Verma S, Ansari MS, Sahu N. Design, synthesis, and biological evaluation of novel imidazole derivatives as analgesic and anti-inflammatory agents: experimental and molecular docking insights. Scientific reports. 2024 Oct 4;14(1):23121.

[3] Musa AT, Abaid K. Theoretical Investigation on Biological Activity of Imidazole Derivatives [Carbenzim, Mebendazole] by using (DFT) and (PM3) Methods. Al-Nahrain Journal of Science. 2021 Jun 27;24(2):1-8.

[4] Mendogralo EY, Nesterova LY, Nasibullina ER, Shcherbakov RO, Myasnikov DA, Tkachenko AG, Sidorov RY, Uchuskin MG. Synthesis, antimicrobial and antibiofilm activities, and molecular docking investigations of 2-(1 H-Indol-3-yl)-1 H-benzo [d] imidazole derivatives. Molecules. 2023 Oct 14;28(20):7095.

[5] Khalaf MM, El-Lateef HM, Abdou A. Structural, DFT, Molecular Docking and Biological Activity of New Albendazole-Norfloxacin Mixed-Ligand Complexes: Promising Metal Complexes for Combating Microbial Resistance and Inflammation. Chemistry & Biodiversity. 2025 Apr;22(4):202402646.

[6] Campos-Fernandez L, Ortiz-Muniz R, Cortes-Barberena E, Mares-Samano S, Garduno-Juárez R, Soriano-Correa C. Imidazole and nitroimidazole derivatives as NADH-fumarate reductase inhibitors: Density functional theory studies, homology modeling, and molecular docking. Journal of computational chemistry. 2022 Sep 5;43(23):1573-95.

[7] Singh G, Patel R, Thakur AS, Singh MK. Synthesis, Computational Study of β-lactam Derivatives Bearing Quinazoline Schiff Bases as Anthelmintics. Letters in Drug Design & Discovery. 2024 Dec;21(19):4994-5001.

[8] Veerasamy R, Roy A, Karunakaran R, Rajak H. Structure–activity relationship analysis of benzimidazoles as emerging anti-inflammatory agents: An overview. Pharmaceuticals. 2021 Jul 11;14(7):663.

[9] Hamdi A, Daoudi W, Aaddouz M, Azzouzi M, Amhamdi H, Elyoussfi A, El Aatiaoui A, Verma DK, Abboud M, Ahari MH. Various synthesis and biological evaluation of some tri-tetra-substituted imidazoles derivatives: A review. Heliyon. 2024 May 30;10(10).

[10] Sicalo Gianechini L. Addressing anthelmintic resistance in parasitic nematodes by investigating the potential of a novel drug target and the dynamics of benzimidazole selection in a model parasitic nematode.

[11] Sadula A, Gaddhe L. Synthesis, computational studies and biological evaluation of novel Acenaphthoquinone-imidazole derivatives as dual inhibitors of HSP90 and Topo II in cancer therapy. Results in Chemistry. 2023;5(1):100796.

[12] Vainauskas V, Kavaliauskas P, Grybaite B, Petraitis V, Petraitienė R, Grigaleviciute R, Prakapaitė R, Acevedo W, Mickevicius V. Synthesis and Characterization of Sulfonamide-Imidazole Hybrids with In Vitro Antiproliferative Activity Against Anthracycline-Sensitive and Resistant H69 SCLC Cells. ChemMedChem.202500260.

[13] Mali G, Maji S, Chavan KA, Shukla M, Kumar M, Bhattacharyya S, Erande RD. Effective synthesis and biological evaluation of functionalized 2, 3-Dihydrofuro [3, 2-c] coumarins via an imidazole-catalyzed green multicomponent approach. ACS omega. 2022 Sep 29;7(40):36028-36.

[14] Mali G, Maji S, Chavan KA, Shukla M, Kumar M, Bhattacharyya S, Erande RD. Effective synthesis and biological evaluation of functionalized 2, 3-Dihydrofuro [3, 2-c] coumarins via an imidazole-catalyzed green multicomponent approach. ACS omega. 2022 Sep 29;7(40):36028-36.

[15] Siwach A, Verma PK. Synthesis and therapeutic potential of imidazole containing compounds. BMC chemistry. 2021 Feb 18;15(1):12.

[16] Valderas-Garca E, Haberli C, Alvarez-Bardon M, Escala N, Castilla-Gomez de Aguero V, de la Vega J, Del Olmo E, Balana-Fouce R, Keiser J, Martínez-Valladares M. Benzimidazole and aminoalcohol derivatives show in vitro anthelmintic activity against Trichuris muris and Heligmosomoides polygyrus. Parasites & Vectors. 2022 Jul 8;15(1):243.

[17] Kaliyaperumal S, Pattanayak P. Synthesis, Characterization and In Vitro Evaluation for Antimicrobial and Anthelmintic Activity of Novel Benzimidazole Substituted 1, 3, 4-Thiadiazole Schiff's Bases. FABAD Journal of Pharmaceutical Sciences. 2021 Dec 1;46(3):261-70.

[18] Pattanayak P, Kaliyaperumal S. Design, synthesis, characterization and in vitro antimicrobial and anthelmintic evaluation of metronidazole derivatives modified at position 1. Pharmaceutical Chemistry Journal. 2022 May;56(2):191-6.

[19] Yerra R. Synthesis, Chemical Characterization and Antibacterial Activity of Some Novel Triazole Substituted 5-Oxo-Imidazoline Derivatives. Journal of Tropical Pharmacy and Chemistry. 2024 Jun 30;8(1):10-6.
[20] Agrwal A, Juneja S, Dwivedi S, Kasana V. Molecular docking and antimicrobial analyses of synthesized imidazole derivatives in solvent less condition, adjacent to human pathogenic bacterial strains. Materials Today: Proceedings. 2022;57(1):2250-4.