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EXPLORATION OF 1,2-SUBSTITUTED BENZIMIDAZOLE DERIVATIVES: SYNTHESIS AND IN VITRO ANTHELMINTIC ACTIVITY AGAINST INDIAN EARTHWORMS

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ABSTRACT

The present study involves the synthesis and characterization of novel 1,2-substituted benzimidazole derivatives with potential anthelmintic activity. The benzimidazole core was synthesized by the reaction of *o*-phenylenediamine with chloroacetic acid in the presence of 4N hydrochloric acid. Subsequent condensation with 2-ethylphenylhydrazine yielded a series of 1,2-substituted derivatives, which were further converted to their corresponding Mannich bases using various secondary amines. The progress of the reactions was monitored by thin-layer chromatography (TLC), and the crude products were purified by recrystallization. Structural confirmation of the synthesized compounds was carried out using IR and ^{1}H NMR spectroscopy. The IR spectra (KBr) exhibited characteristic peaks at ~3042 cm^{-1} (aromatic C–H), 2984 cm^{-1} (aliphatic C–H), 3323 cm^{-1} and 3414 cm^{-1} (N–H stretching), and 1563 cm^{-1} (C=N stretching). The ^{1}H NMR spectra (DMSO-d$_6$) showed signals at δ 7.7 (s, 2H, Ar–CH), 3.76 (s, 2H, CH$_2$–NH), 4.06 (s, 1H, Ar–NH), 2.2 (s, 1H, N–H), and 1.15 (s, 6H, CH$_3$). Preliminary in vitro screening of the synthesized compounds revealed promising anthelmintic activity.

KEYWORDS: Benzimidazole derivatives, Mannich bases, Anthelmintic activity, 2-ethylphenylhydrazine.

INTRODUCTION

Benzimidazole derivatives have attracted considerable research interest in recent years due to their wide range of pharmacological and biological activities. As a privileged scaffold in both medicinal and agrochemical chemistry, the benzimidazole nucleus - comprising a fused bicyclic system of benzene and imidazole - exhibits versatile chemical reactivity and significant therapeutic potential.[1] This unique structure shares similarity with purine bases, which contributes to its ability to interact with various biological targets. Several benzimidazole-based compounds have demonstrated the capacity to selectively inhibit endothelial cell proliferation and suppress angiogenesis, both in vitro and in vivo, highlighting their relevance in anticancer and antiangiogenic research.[2]

Parasitic infections caused by nematodes continue to pose a serious threat to global public health and livestock productivity. Soil-transmitted helminths such as *Ascaris lumbricoides*, *Necator americanus*, *Ancylostoma duodenale*, and *Trichuris trichiura* affect more than one billion people worldwide, leading to significant morbidity. In veterinary settings, particularly in sheep, nematode species including *Teladorsagia circumcincta*, *Haemonchus contortus*, and *Trichostrongylus colubriformis* are known to cause substantial economic losses due to reduced productivity and increased mortality.[3]

Given the pharmacological significance of the benzimidazole scaffold and the need for effective anthelmintic agents, the present study is aimed at designing and synthesizing novel 1,2-substituted benzimidazole derivatives. These compounds were further evaluated for their anthelmintic potential through in vitro screening, with the goal of identifying promising candidates for further development.

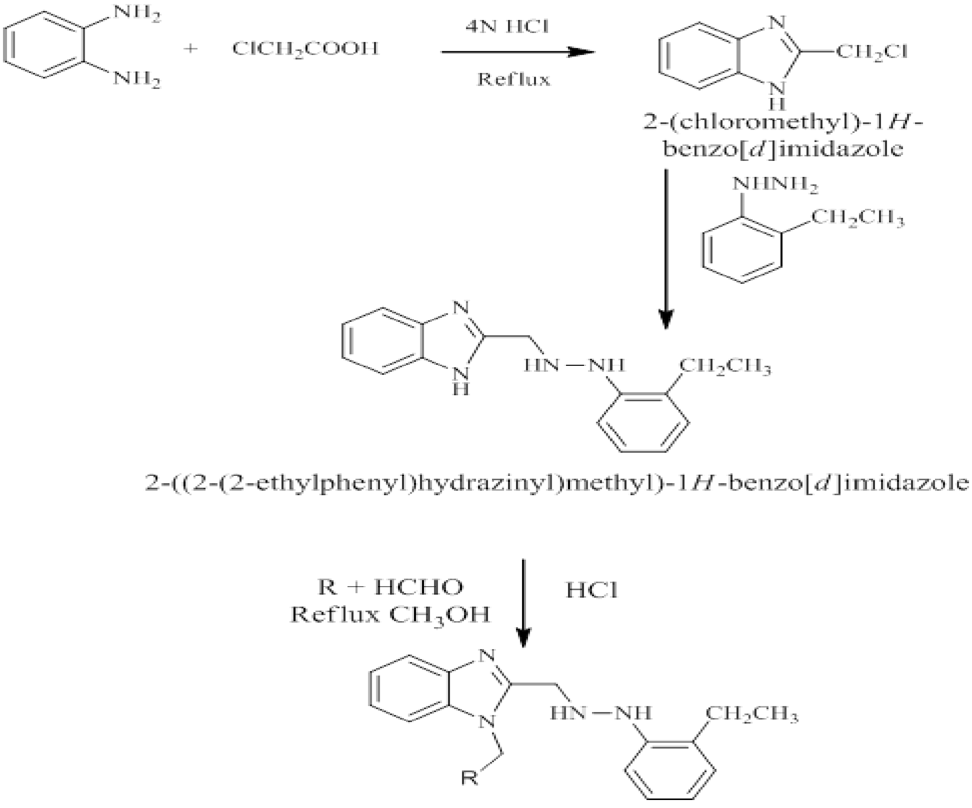
METHODOLOGY

All chemicals and reagents used in the present study were procured from standard commercial suppliers and used without further purification, unless otherwise stated. The progress of the reactions was monitored by thin-layer chromatography (TLC) on silica gel-G plates (Merck grade). Appropriate solvent systems were selected based on the polarity and nature of the reacting compounds. TLC spots were visualized under ultraviolet (UV) light and by exposure to iodine vapors.

Melting points of the synthesized compounds were determined using an open capillary method with a digital melting point apparatus and are reported without correction.

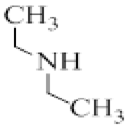
Structural characterization of the synthesized compounds was carried out using infrared (IR) spectroscopy and proton nuclear magnetic resonance (^{1}H NMR) spectroscopy. IR spectra were recorded using the KBr pellet method, and characteristic absorption peaks were analyzed to identify functional groups. ^{1}H NMR spectra were obtained using DMSO-d$_6$ as the solvent, with chemical shifts (δ) expressed in parts per million (ppm), referenced to tetramethylsilane (TMS) as an internal standard. The spectral data confirmed the structures and purity of the synthesized compounds.

Scheme of synthesis

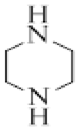


BI (a-e)

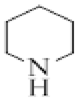
Various Secondary amines (R) used are



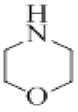
(a) diethylamine



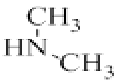
(b) piperazine



(c) piperidine



(d) morpholine



(e) dimethylamine

General Procedure for the Synthesis of 2-(Chloromethyl)-1H-benzimidazole

A reaction mixture comprising *o*-phenylenediamine (5.4 g, 0.05 mol), chloroacetic acid (7.1 g, 0.08 mol), and 4N hydrochloric acid (17.17 mL) was subjected to reflux for 45 minutes. After completion, the mixture was left to stand undisturbed overnight. The precipitated solid was then filtered, diluted with 100 mL of distilled water, and cooled. Neutralization was carefully carried out using an ammonia solution while maintaining a low temperature and ensuring vigorous stirring to avoid gum formation. The resulting solid product was collected by filtration, thoroughly washed with cold water, and dried in a vacuum desiccator. Final purification was achieved by recrystallization using ethanol. Yield**:** 5.1 g (68.3%); Melting Point: 172°C; Rf value: 0.42.

Procedure for the Synthesis of 2-((2-(2-ethylphenyl)hydrazinyl)methyl)-1H-benzo[d] imidazole

An equimolar reaction was carried out by refluxing the previously synthesized intermediate (0.02 mol) with 2-ethylphenylhydrazine (0.0217 mol) in methanol for 5 hours. Upon completion of the reaction, the hot solution was carefully transferred onto crushed ice with continuous stirring to precipitate the product. The solid was then collected by filtration, air-dried, and purified by recrystallization using absolute ethanol. Yield: 3.7 g (76%); Melting Point: 87°C; Rf value: 0.62.

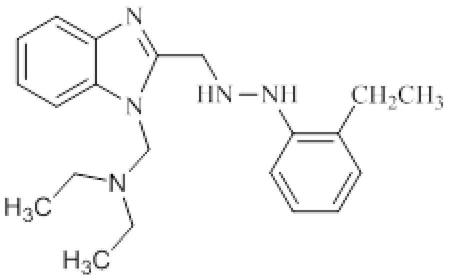
Procedure for the preparation of mannich bases with different secondary amines

A reaction mixture consisting of the previously synthesized compound, formaldehyde, different secondary amines, and hydrochloric acid was refluxed in methanol for 2.5 hours. After completion of the reaction, the hot mixture was filtered, and the clear filtrate was then cooled using cold water to facilitate crystallization. The resulting crystals were collected by filtration and further purified by recrystallization using absolute ethanol.

- **N-ethyl-N-((2-((2-(2-ethylphenyl)hydrazinyl)methyl)-1H-benzo[d]imidazol-1-yl) methyl) ethanamine (M1)**

A solution containing the previously synthesized compound (1 g, 0.004 mol), formaldehyde (5 ml, 0.004 mol), diethylamine (0.5 ml, 0.004 mol), and hydrochloric acid (2 ml) was refluxed in methanol for 2.5 hours. Upon completion, the hot reaction mixture was filtered, and the resulting clear solution was cooled using cold water to induce crystallization. The precipitated solid was collected by filtration and purified by recrystallization from absolute ethanol. Yield: 1.26 g (79%) Melting Point: 132 °C Rf value: 0.51

IR(KBr) cm^{-1}: 3041(CH, str, ar), 2984(CH, str, ali), 3321, 3414(–NH, str), 1563(C=N, str); 1H NMR (DMSO–d6): *δδ*ppm 7.5(1s, 2H, Ar–CH), 3.81(1s, 2H, CH_2–NH), 4.07(1s, 1H, Ar·N–H), 2.2(1s, 1H, N–H), 1.15(1s, 6H, CH_3).

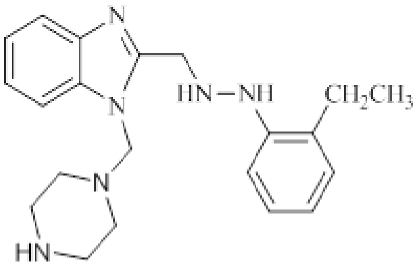


N-ethyl-*N*-((2-((2-(2-ethylphenyl)hydrazinyl)methyl)-1*H*-benzo[*d*]imidazol-1-yl)methyl)ethanamine
Chemical Formula: $C_{21}H_{29}N_5$
Molecular Weight: 351.49

- **2-((2-(2-ethylphenyl)hydrazinyl)methyl)-1-(piperazin-1-ylmethyl)-1H-benzo[d] imidazole (M2)**

A mixture consisting of the synthesized precursor (1 g, 0.004 mol), formaldehyde (5 ml, 0.004 mol), piperazine (360 mg, 0.004 mol), and hydrochloric acid (2 ml) was refluxed in methanol for 2.5 hours. After heating, the reaction mixture was filtered while still hot, and the filtrate was then cooled with cold water to facilitate crystallization. The resulting solid was collected by filtration and purified by recrystallization using absolute ethanol. Yield: 1.4 g (86.5%) Melting Point: 176 °C Rf value: 0.45

IR(KBr) cm^{-1}: 3173(CH, str, ar), 2977(C H, str, ali), 3356, 3342(–NH, str), 1661(C=N, str), 1255(–CN, str); 1H NMR (DMSO-d6) spectra: *δδ*ppm 2.2(1s,1H,N–H), 7.5(1s, 4H, Ar–CH), 3.82(1s, 2H, CH_2–N), 4.1(1s, 1H, Ar·N–H), 2.4(1s, 1H, amine N–H).

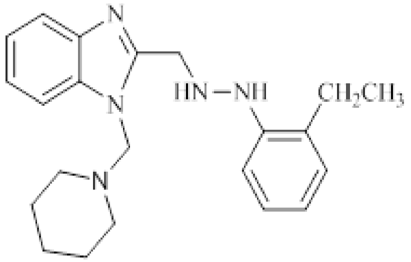


2-((2-(2-ethylphenyl)hydrazinyl)methyl)-1-(piperazin-1-ylmethyl)-1*H*-benzo[*d*]imidazole
Chemical Formula: $C_{21}H_{28}N_6$
Molecular Weight: 364.49

➢ 2-((2-(2-ethylphenyl)hydrazinyl)methyl)-1-(piperidin-1-ylmethyl)-1H-benzo[d] imidazole (M3)

A solution containing the synthesized intermediate (1 g, 0.004 mol), formaldehyde (5 ml, 0.004 mol), piperidine (0.42 ml, 0.004 mol), and hydrochloric acid (2 ml) was refluxed in methanol for 3 hours. Upon completion of the reaction, the hot mixture was filtered, and the clear filtrate was cooled by adding cold water to induce crystallization. The precipitated product was isolated by filtration and further purified through recrystallization using absolute ethanol. Yield: 0.72 g (58%) Melting Point: 233 °C Rf value: 0.52

IR(KBr) cm^{-1}: 3257(CH, str, ar), 2976 (CH, str, ali), 3382, 3415 (–NH, str), 1654(C=N, str), 1210(–CN, str); 1H NMR (DMSO-d6) spectra: δδppm 2.2(1s, 1h, N–H), 7.4(1s, 4H, Ar–CH), 3.86(1s, 2H, CH_2–N), 4.1(1s,1H, Ar·N–H), 1.05(1s, 4H, amine CH_2), 4.09(1s, 2H, meth CH_2).

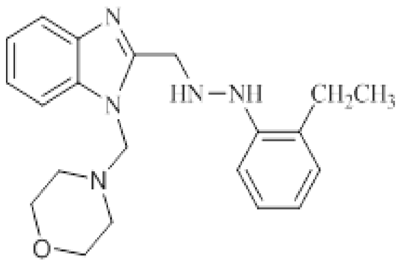


2-((2-(2-ethylphenyl)hydrazinyl)methyl)-1-(piperidin-1-ylmethyl)-1*H*-benzo[*d*]imidazole
Chemical Formula: $C_{22}H_{29}N_5$
Molecular Weight: 363.50

➢ 4-((2-((2-(2-ethylphenyl)hydrazinyl)methyl)-1H-benzo[d]imidazol-1-yl) methyl) morpholine (M4)

A mixture consisting of the synthesized precursor (1 g, 0.004 mol), formaldehyde (5 ml, 0.004 mol), morpholine (0.37 ml, 0.004 mol), and hydrochloric acid (2 ml) was refluxed in methanol for 3 hours. After completion, the reaction mixture was filtered while still hot, and the resulting clear filtrate was cooled by adding cold water to facilitate crystallization. The resulting solid was collected by filtration and purified by recrystallization from absolute ethanol. Yield: 0.82 g (69%) Melting Point: 121 °C Rf value: 0.61

IR(KBr) cm^{-1}: 3151(CH, str, ar), 2984 (CH, str, ali), 3314(–NH, str), 1656(C=N, str), 1626(C–O, str), 1248(–CN, str); 1H NMR (DMSO-d6) spectra: δδppm 2.2(1s, 1H, N–H), 7.5(m, 4H, Ar–CH), 3.83(1s, 2H, CH_2–N), 4.3(1s, 1H, Ar·N–H), 3.6(1s, 4H, amine O–CH).

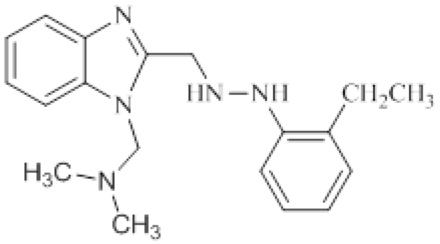


4-((2-((2-(2-ethylphenyl)hydrazinyl)methyl)-1*H*-benzo[*d*]imidazol-1-yl)methyl)morpholine
Chemical Formula: $C_{21}H_{27}N_5O$
Molecular Weight: 365.47

- **1-(2-((2-(2-ethylphenyl)hydrazinyl)methyl)-1H-benzo[d]imidazol-1-yl)-N,N-dimethylmethanamine (M5)**

A reaction mixture comprising compound 1 (1 g, 0.004 mol), formaldehyde (5 ml, 0.004 mol), dimethylamine (0.21 ml, 0.004 mol), and hydrochloric acid (2 ml) was refluxed in methanol for 3 hours. Upon completion, the hot solution was filtered, and the clear filtrate was poured into cold water to induce crystallization. The resulting solid was collected by filtration and further purified by recrystallization using absolute ethanol. Yield: 1.10 g (82%) Melting Point: 224 °C Rf value: 0.48

IR(KBr) cm^{-1}: 3173(CH, str, ar), 2981 (CH, str, ali), 3349(–NH, str), 1661(C=N, str); 1H NMR (DMSO–d6) spectra: $\delta\delta$ppm 2.1(1s, 1H, N–H), 7.3(m, 4H, Ar–CH), 3.81(1s, 2H, CH_2–N), 4.3(1s, 1H, Ar·N–H), 2.27(1s, 6H, amine N–CH_3).



1-(2-((2-(2-ethylphenyl)hydrazinyl)methyl)-1*H*-benzo[*d*]imidazol-1-yl)-*N*,*N*-dimethylmethanamine
Chemical Formula: $C_{19}H_{25}N_5$
Molecular Weight: 323.44

Anthelmintic activity[5-10]

Earthworm collection

Adult earthworms were collected from moist soil and thoroughly washed with normal saline to remove adhering soil and debris. Earthworms measuring 2–5 cm in length and 0.2–0.3 cm in width were selected for the study due to their anatomical and physiological similarity to human intestinal roundworm parasites, making them a suitable model for preliminary anthelmintic screening.

Preparation of test solutions

The synthesized benzimidazole derivatives and standard drug (Albendazole) were first dissolved in a minimal quantity of dimethyl sulfoxide (DMSO), followed by dilution with normal saline to a final volume of 25 mL. This yielded test solutions of varying concentrations (20, 40, and 80 mg/mL).

Experimental procedure

Fresh test and standard drug solutions were prepared immediately prior to the experiment. The study was conducted using six groups of earthworms of comparable size. Each group was placed in separate Petri dishes containing 25 mL of the respective test or standard solutions at different concentrations (20, 40, and 80 mg/mL). Normal saline served as the negative control, while Albendazole was used as the reference standard.

The time taken for the onset of paralysis and subsequent death of the worms was recorded. Paralysis was noted when the worms ceased all movement, except upon vigorous shaking. Death was confirmed when the worms did not respond even to intense physical stimulation or immersion in warm water (50°C), along with discoloration and fading of body segments.

RESULT

The synthetic pathway for the preparation of the target compounds is illustrated in the reaction scheme. The benzimidazole nucleus was synthesized by condensing *o*-phenylenediamine with chloroacetic acid in the presence of 4N hydrochloric acid under reflux conditions. A novel series of 1,2-substituted benzimidazole derivatives was subsequently synthesized by condensing the parent benzimidazole compound with 2,4-dinitrophenylhydrazine. These derivatives were further converted into their respective Mannich bases through reaction with various secondary amines.

The progress of each reaction was monitored by thin-layer chromatography (TLC), ensuring the formation of the desired products. Crude products were purified by recrystallization using suitable solvents, and the structures were preliminarily confirmed based on melting point and spectral data.

The physical characteristics, such as appearance, melting point, yield percentage, and Rf values of the synthesized compounds, are summarized in **Table 1**.

Table 1: Physical properties of compounds.

Compound	Molecular Formula	Molecular Weight	Yield %	Melting Point	Rf Value
M1	$C_{21}H_{29}N_5$	351.49	79%	132^o C	0.51
M2	$C_{21}H_{28}N_6$	364.49	86.5%	176^o C	0.45
M3	$C_{22}H_{29}N_5$	363.50	58%	233^o C	0.52.
M4	$C_{21}H_{27}N_5O$	365.47	69%	121^o C	0.61
M5	$C_{19}H_{25}N_5$	323.44	82%	224^o C	0.48

The synthesized 1,2-substituted benzimidazole derivatives were evaluated for their anthelmintic activity using Indian earthworms as a model. The test was performed at three different concentrations (20, 40, and 80 mg/mL), with Albendazole used as the reference standard and normal saline as the control. The time required for the onset of paralysis and death of the worms was recorded and is presented in **Table 2**.

A closer examination of the data revealed that compound **M2**, bearing a piperazine moiety, and compound **M4**, containing a morpholine side chain, exhibited comparatively higher anthelmintic activity. These compounds demonstrated shorter paralysis and death times, approaching the efficacy of the standard drug Albendazole. The remaining derivatives also displayed significant activity, indicating that substitution at the Mannich position influences the biological response.

These findings suggest that benzimidazole derivatives bearing electron-rich, heterocyclic secondary amines may enhance anthelmintic potential, warranting further investigation into their structure–activity relationships (SAR).

Table 2: Anthelmintic activity of compounds.

Sl. No.	Comp.	Time taken for paralysis			Time taken for Death		
		20 mg/ml	40 mg/ml	80 mg/ml	20 mg/ml	40 mg/ml	80 mg/ml
1	M1	5.18 ± 0.16	3.23 ± 0.26	2.49 ± 0.21	6.42 ± 0.33	5.32 ± 0.22	3.29 ± 0.26
2	M2	3.22 ± 0.18	2.08 ± 0.42	1.61 ± 0.32	4.52 ± 0.19	3.08 ± 0.33	2.16 ± 0.34
3	M3	5.53 ± 0.17	4.52 ± 0.09	2.19 ± 0.34	6.25 ± 0.21	5.46 ± 0.15	3.38 ± 0.39
4	M4	4.37 ± 0.25	3.42 ± 0.45	2.34 ± 0.35	5.22 ± 0.23	4.39 ± 0.33	2.42 ± 0.22
5	M5	6.36 ±	4.33 ±	2.46 ±	6.52 ±	5.36 ±	3.13 ±

		0.32	0.21	0.13	0.25	0.15	0.25
6	Albendazole	2.24 ± 0.11	1.34 ± 0.32	0.43 ± 0.27	3.54 ± 0.25	2.13 ± 0.24	1.25 ± 0.24

DISCUSSION

The 1,2-substituted benzimidazole derivatives were successfully synthesized following the designed synthetic routes, as depicted in the reaction scheme. The formation of the benzimidazole core and subsequent substitutions were confirmed through detailed spectroscopic analyses. Infrared (IR) spectroscopy revealed characteristic absorption bands corresponding to key functional groups, while proton nuclear magnetic resonance (^{1}H NMR) spectroscopy further validated the structural integrity of the synthesized compounds. Reaction progress was effectively monitored using thin-layer chromatography (TLC), and recrystallization ensured the isolation of pure derivatives.

The synthesized compounds were evaluated for their anthelmintic activity using Indian earthworms as the biological model, with results summarized in Table 2. Among the tested derivatives, compounds M2 and M4, containing piperazine and morpholine side chains respectively, demonstrated superior anthelmintic efficacy. The enhanced activity observed in these compounds may be attributed to the presence of nitrogen-containing heterocyclic moieties, which are likely to promote stronger interactions with parasitic targets, thereby increasing biological potency.

Other synthesized derivatives also exhibited significant anthelmintic activity, although with slightly lower potency compared to M2 and M4. This variation in activity underscores the influence of the nature and position of substituents on the benzimidazole scaffold. Electron-donating groups and sterically favorable side chains at the 1,2-positions appear to enhance binding affinity to parasite receptors, contributing to improved efficacy.

These findings highlight the critical role of molecular modifications in tuning the biological activity of benzimidazole derivatives. Future studies incorporating molecular docking and structure-activity relationship (SAR) analysis are warranted to elucidate the detailed mechanism of action and to guide the rational design of more potent anthelmintic agents within this chemical class.

CONCLUSION

In summary, a series of novel 1,2-substituted benzimidazole derivatives were successfully synthesized and characterized using spectroscopic techniques. The compounds exhibited promising anthelmintic activity against Indian earthworms, with derivatives bearing piperazine (M2) and morpholine (M4) side chains showing the most potent effects. The results indicate that structural modifications at the 1,2-positions of the benzimidazole nucleus significantly influence biological activity. These findings support the potential of benzimidazole-based compounds as effective anthelmintic agents and provide a foundation for further optimization through detailed SAR studies and molecular modeling to enhance their efficacy.

Future directions

The present study successfully synthesized and evaluated a series of 1,2-substituted benzimidazole derivatives for their anthelmintic activity. Building on these findings, several important avenues for future research are proposed:

1. **Structure-Activity Relationship (SAR) Studies:** Conducting comprehensive SAR analyses will help identify the critical structural features responsible for enhanced anthelmintic activity, guiding the rational design of more potent derivatives.
2. **Molecular docking studies:** Computational docking investigations can elucidate the binding interactions of the synthesized compounds with specific biological targets, facilitating structure-based optimization of benzimidazole derivatives.
3. **Synthesis of novel derivatives:** Further chemical modifications of the benzimidazole core, including incorporation of various heterocyclic moieties or electron-withdrawing/donating substituents, may yield derivatives with improved pharmacological profiles.
4. **In Vivo anthelmintic evaluation:** While the current work focused on in vitro testing using Indian earthworms, subsequent in vivo studies in appropriate animal models are essential to assess efficacy, pharmacokinetics, and safety parameters more comprehensively.
5. **Toxicity and pharmacokinetic studies:** Evaluations of cytotoxicity and ADME (Absorption, Distribution, Metabolism, and Excretion) properties will be crucial for determining the drug-likeness and therapeutic potential of the compounds.
6. **Comparative studies with existing anthelmintics:** Comparative efficacy and safety studies against established anthelmintic drugs can help establish whether the synthesized

benzimidazole derivatives offer advantages such as enhanced potency, selectivity, or reduced resistance.

Pursuing these future directions will provide deeper insights into the biological activity of benzimidazole derivatives and aid in the development of novel, clinically relevant anthelmintic agents.

REFERENCES

1. Walia R, Hedaitullah MD, Naaz SF, Iqbal K, Lamba HS. Benzimidazole derivatives: An overview. *Int J Res Pharm Chem,* 2011; 1(3): 565–574.
2. Shruthi SD, Padmalatha RS, Ramachandra YL. Isolation, characterization, antibacterial, anthelmintic, and *in silico* studies of polyprenol from *Kirganelia reticulata* Baill. *Med Chem Res,* 2013; 22(6): 2938–2945.
3. Farukh A, Mubashira A. Synthesis and characterization of dinuclear macrocyclic cobalt(II), copper(II), and zinc(II) complexes derived from 2,2,2',2'-S,S-[bis(bis-N,N-thiobenzimidazolyloxalato-1,2-ethane)]: DNA binding and cleavage studies. *Eur J Med Chem,* 2009; 44: 834–844.
4. Bradbury SP, Coats JR. Comparative toxicology of the pyrethroid insecticides. *Rev Environ Contam Toxicol,* 1989; 133: 133–177.
5. Vercruysse J, Albonico M, Behnke JM, Kotze AC, Prichard RK, McCarthy JS, et al. Is anthelmintic resistance a concern for the control of human soil-transmitted helminths? *Int J Parasitol Drug Resist,* 2011; 1: 14–27.
6. Shobhit S, Pandeya SN, Meena KY, Singh BK. Synthesis and analgesic activity of novel derivatives of 1,2-substituted benzimidazoles. *J Chem,* 2013; 2013: 1–6.
7. Thorn GW, Adams RD, Brundwal E, Isselbacher KJ, Petersdorf RG. *Harrison's Principles of Internal Medicine.* New York: McGraw-Hill, 1977.
8. Tambe VD, Nirmal SA, Jadhav RS, Ghogare PB, Bhalke RD. Anthelmintic activity of *Wedelia trilobata* leaves. *Indian J Nat Prod,* 2006; 22: 27–29.
9. Kaur H, Kumar S, Singh I, Saxena KK, Kumar A. Synthesis, characterization and biological activity of various substituted benzothiazole derivatives. *Dig J Nanomater Biostruct,* 2010; 5: 67–76.
10. Ghose AK, Crippen GM. Atomic physicochemical parameters for three-dimensional structure-directed quantitative structure–activity relationships. *J Chem Inf Comput Sci,* 1987; 27: 21–35.

11. Binkowski TA, Naghibzadeh S, Liang J. CASTp: Computed atlas of surface topography of proteins. *Nucleic Acids Res,* 2003; 31: 3352–3355.
12. Vidya SM, Krishna V, Manjunatha BK, Rajesh KP, Bharath BR, Manjunatha H. Antibacterial and molecular docking studies of entagenic acid, a bioactive principle from seed kernel of *Entada pursaetha* DC. *Med Chem Res,* 2012; 21(7): 1016–1022.
13. Himaja M, Sirisha B, Moonjit D, Munirasekhar D. Synthesis and anthelmintic activity studies of 1-substituted benzimidazole derivatives. *J Indian Chem Soc,* 2015; 92: 908–910.
14. Leena KP, Subin MZ, Deepthy CH. *In silico* design, synthesis and characterization of some novel benzothiazole derivatives as anticancer agents. *Asian J Pharm Clin Res,* 2017; 10: 50–55.
15. Woods DJ, Knauer CS. Discovery of veterinary antiparasitic agents in the century: A view from industry. *Int J Parasitol,* 2010; 21, 40: 1177–1181.
16. Kozo A, Koazuhiro A, Masayuki K, Yonhzhe Y. *Chem Abstr,* 2001; 134: 86247.
17. Rang HP, Dale MM, Ritter JM, Moore PK. *Rang and Dale's Pharmacology.* 5th ed. New York: Churchill Livingstone, 2003; 687–692.
18. Tripathi KD. Anthelmintics. In: *Essentials of Medical Pharmacology.* 5th ed. New Delhi: Jaypee Brothers Medical Publishers, 2004; 759–766.
19. Amrutkar SV, Umesh DB, Pargharmol P, Kotgire SS, Ranawat MS. Synthesis and antifungal activity of 1-alkyl/H-2-[4-(alkyl/aryl-piperazin-1-yl)-methyl]-benzimidazole derivatives. *Int J Pharm Pharm Sci,* 2010; 2(2): 84–92.
20. Maneshwar T, Krishna M, Vijetha N. Synthesis, characterization and biological evaluation of novel benzimidazole derivatives. *J Pharm Res,* 2015; 4: 188–192.