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ALBENDAZOLE ANALOGUES: A COMPREHENSIVE STUDY ON SYNTHESIS AND BIOLOGICAL ACTIVITY.

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ABSTRACT:

Albendazole, a benzimidazole is widely used to treat parasitic worm infestations. Recent research has focused on synthesizing novel derivatives of albendazole to enhance its therapeutic efficacy and explore new biological activities. The present work deals with the sequence of benzimidazole fused novel albendazole compounds synthesized by conventional methods and screened for anthelmintic, antibacterial, and antifungal activity studies. The purity of the compound was checked by TLC using precoated Silica Gel G plates. The melting points were determined by Labtronics apparatus and were presented uncorrected. IR and NMR analysis characterized all the newly synthesized compounds. Further, all the albendazole derivatives were screened for anthelmintic activity, compound D2 exhibited higher anthelmintic activity against earthworm species *Pheretima posthuma*. In the future, studies using various in-vivo models will establish a broad therapeutic spectrum of the compound.

KEYWORDS: Benzimidazole, Albendazole, Albendazole analogs, Anthelmintic activity, *Pheretima posthuman*.

I. INTRODUCTION:

Albendazole, methyl [5-(propylthio)-1*H*-benzimidazol-2-yl] carbamate, is a broad-spectrum anti-parasitic agent, first introduced in 1975 for the treatment of liver flukes, tapeworms, lung and gastrointestinal nematodes in sheep and cattle.[1, 2] It was approved for human use in 1982.[3] Albendazole is highly effective with limited toxicity, and it may have anti-tumor activity against human pancreatic cancer cells,[4] gastric cancer cells[5, 6], and colorectal cancer cells.[7] Therefore, albendazole has been recognized as a potential cancer treatment drug.

One of the main issues facing veterinary and human medicine is the control of helminth parasites. In the lack of effective vaccines and sufficient cleanliness, prophylaxis and therapy typically depend on anthelmintics. There are concerns about the development of drug resistance, side effects, lack of efficacy, and cost-effectiveness that drive the need for new classes of anthelmintics.[8]

Human helminth infections, mainly due to soil-transmitted helminths (STHs), lymphatic filariasis (LF), and schistosomiasis (SCH) belong to the class of neglected tropical diseases (NTDs) are the major targets of global elimination programs. [3] Parasitic infections are still a major health problem in developing countries, affecting mainly the infantile population. It has been reported that benzimidazole 2-carbamates (BZC), such as Albendazole (Abz) and Mebendazole (Mbz) are mainly used as anthelmintic agents. [9] Modes of action of anthelmintic drugs are described. Certain anthelmintic medications affect nematode neuromuscular transmission quickly and specifically. Diethylcarbamazine strengthens the innate, nonspecific immune system and inhibits host and potentially parasite enzymes involved in the metabolism of arachidonic acid. [10]

II. NEED OF THE STUDY:

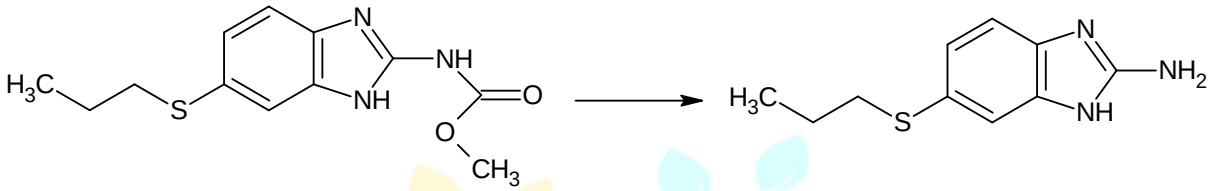
Analogs of albendazole must be studied to increase the medication's effectiveness and range of action against parasitic infections. The demand for new chemicals to address these issues is expanding as medication resistance rises. Optimization of pharmacological profiles of albendazole analogs can be achieved by investigating their production and biological characteristics. In many areas, parasites such as helminths are resistant to albendazole. By synthesizing analogs, therapeutic efficacy can be restored and resistance can be overcome. Investigating these analogs might also lead to discovering fresh treatment approaches for helminthic illnesses. Public health outcomes could be improved by study, particularly in environments with limited resources.

III. MATERIALS AND METHODS:

The synthetic chemicals and solvents were procured from different commercial suppliers and used without further purification. TLC utilizing precoated Silica Gel G plates was used to determine the purity of compounds. Labtronics equipment was used to determine the melting points, which were then shown without correction. The FTIR Spectrophotometer Model: Bruker ECO-ATR was used to record infrared (IR) spectra. Using TMS as an internal standard and CDCl3 solvent, the Bruker Ultra Shield Model DPX 500 MHz spectrometer was used to record the nuclear magnetic resonance spectrum. [11,12]

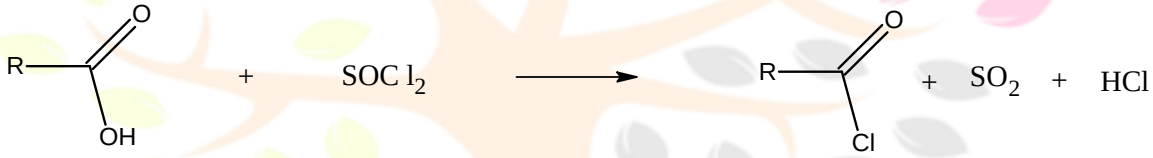
IV. EXPERIMENTAL WORK:

Preparation of 5-(propylsulfanyl)-1H-benzimidazole-2-amine from Albendazole:



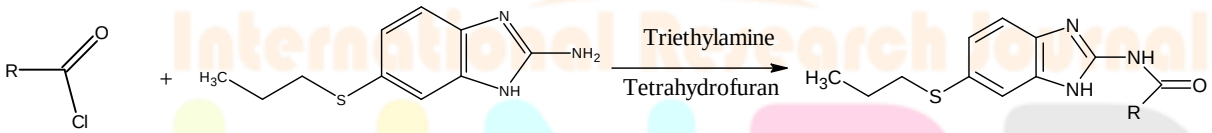
To make 5-(propylsulfanyl)-1H-benzimidazole-2-amine, albendazole was dissolved in 20 ml of 50% HCl solution. The medium was carefully stirred and maintained at 40–50°C for 24 hours. 5- (propylsulfanyl)-1H-benzimidazole 2-amine compounds were obtained by filtering the medium following the aforementioned treatment. The acquired product was recrystallized from the proper solvent (benzene) to obtain the equivalent pure product. The yield was about 55%.

Preparation of acid chloride derivative of some NSAID containing free -COOH group:



NSAID containing free -COOH group is refluxed with thionyl chloride until all free acid is converted into acid chloride. The completion of the reaction was monitored by TLC.

Coupling of acid chloride with 5-(propylsulfanyl)-1H-benzimidazole-2-amine to produce respective amide derivatives:



To a solution containing 1.0 mol of acid chloride of formula II and 1.0 mol of purified 5-(propylsulfanyl)-1H-benzimidazole-2-amine in 200 ml of anhydrous tetrahydrofuran, 1.0 mol of triethylamine has been slowly added (about 10 minutes) while stirring. The reaction mixture which became slightly warm was stirred for 45 minutes and then poured under agitation into 2L of distilled water. The stirring was continued until the precipitation of the desired compound was complete. Then the precipitate was dried, washed with water, and dried again and the obtained product was recrystallized from the appropriate solvent (i.e. methanol) to afford the corresponding pure product. The yields of desired compounds are between about 60-78%.

A) Physical and Spectral Data of Synthesized Compounds

1) N-(5-(propylsulfanyl)-1H-benzimidazol-2-yl)-2- {[3 (trifluoromethyl) phenyl] amino} pyridine-3-carboxamide] (D1)

Yield 70%, MP 150-155 °C; IR (KBr, cm^{-1}): 3331 (N-H str Amide), 2958, 2923(C-H str. Aromatic), 1633 (N-H deformation), 1596(C=O, 1551, 1527(C-C str. (in ring)), 1445 (CH_3 def), 1358-1269 (C-N Str), 1228, 1111(C-F str), 897 (Aromatic Out of plane def), 840 (N-H out of plane), 693 (C-S str). ; ^{1}H-NMR: (δ ppm): 11.258 (s, 1H NH-C=O), 8.148 (s 1H NH), 8.018 (m, Pyridyl), 7.301 (t, 3H, Ar-H), 7.033(m, Ar-H), 2.806 (t, 2H CH2-S), 1.518(m, CH2), 1.290(t, CH_3).

2) [N-(5-(propylsulfanyl)-1H-benzimidazol-2-yl) amino]-2-oxoethyl acetate-2, 6-di chloro-N-phenyl aniline (D2)

Yield 75%, MP 118-122 °C; IR (KBr, cm^{-1}): 3329 (N-H str Amide), 2957, 2916 (C-H str. Aromatic), 1732 (C=O str ester), 1620 (N-H deformation Amide), 1587(C-C str. (in a ring), 1443 CH3(sp^3) def, 1360-1268 (C- N str.), 1194, 1059 C-Cl str. (Aryl), 957-848 (Aromatic (Out of plane def), 846 (N-H out of plane), 695(C-S str.) ^{1}H-NMR: (δppm): 8.083 (s, 1H NH-C=O), 7.523(d 1H Ar-H), 7.338 (t, 2H, Ar-H), 7.154 (m, Ar-H), 7.075(m Ar-H) 6.553(d, CH2), 6.396(d, CH_2), 4.699(s NH), 3.783(d, CH_2), 2.967 (t, 2H CH2-S), 1.725 (m, CH_2), 1.092(t, CH_3).

3) N-(5-(propylsulfanyl)-1H-benzimidazol-2-yl)-2-[4-(2-methylpropyl) phenyl] propanamide (D3)

Yield 60%, MP 188-190 °C; IR (KBr, cm^{-1}): 3332 (N-H str Amide), 2958, 2919 (C-H str. Aromatic), 1621 (N-H deformation Amide) 1587, 1523 (C-C str. (in ring), 1465 CH_2(def), 1359-1268 (C-N str.), 957-847 (Aromatic Out of plane def), 791 (N-H out of plane), 696 (C-S str). ^{1}H-NMR: (δppm): 7.455 (s, 1H NH-C=O), 7.332 (m, 3H, Ar-H) 7.001 (d, 2H, Ar-H), 2.803 (t, 2H CH_2-S), 2.379(d, CH_2), 1.620 (m, CH_3), 1.379(m, CH-CH_3), 0.978(1, CH_3).

4) N-(5-(propylsulfanyl)-1H-benzimidazol-2-yl)-2-(6-acetylnaphthalen-2-yl) propanamide (D4)

Yield 65%, MP 90-95 °C; IR (KBr, cm^{-1}): 3331 (N-H str Amide), 2958, 2923 (C-H str. Aromatic), 1621 (N-H deformation Amide) 1587, 1523 (C-C str. (in ring), 1459 CH_2(def), 1376-1268 (C-N str.), 957-848 (Aromatic (Out of plane def), 791N-H (out of plane), 698 (C-S str).

5) N-(5-(propylsulfanyl)-1H-benzimidazol-2-yl)-{1-[(3-chlorophenyl)carbonyl]-5-methoxy-2-methyl-1H-indol-3-yl} acetyl amide (D5)

Yield 72%, MP 155-160°C; IR (KBr, cm^{-1}): 3331 (N-H str Amide), 2958, 2926 (C-H str. Aromatic), 2869 $CH_3(sp^3)$ str 1633 (N-H deformation Amide), 1588-1477 C-C str. (in the ring), 1460 (CH2 def), 1398-1268 (C-N str), 957-747 (Aromatic (Out of plane def), 791 (N-H out of plane), 696 (C-S str).

6) N-(5-(propylsulfanyl)-1H-benzimidazol-2-yl)-2-[(2,3-dimethylphenyl) amino] benzamide (D6)

Yield 78%, MP 180-190°C; IR (KBr, cm^{-1}): 3329 (N-H str Amide), 2958, 2921 (C-H str. Aromatic), 2867 $CH_3(sp^3)$ str, 1620 (N-H deformation Amide), 1586-1510 C-C str. (in ring), 1460 CH_2 def, 1359-1268 (C-N str), 957-847 (Aromatic (Out of plane def), 791 (N-H out of plane), 698 (C-S str). ^{1}H-NMR: (δppm): 7.766 (s, 1H NH-C=O), 7.454 (m, 2H, Ar-H), 7.334 (d, 1H, Ar-H), 7.086(t.2H Ar-H), 2.805 (t, 2H CH_2-S), 1.598 (m, CH_2), 0.979(t, CH_3)

7) N-(5-(propylsulfanyl)-1H-benzimidazol-2-yl)-2-(6-fluorobiphenyl-3-yl) propanamide (D7)

Yield 75%, MP 185-190°C; IR (KBr, cm^{-1}): 3330 (N-H str Amide), 2959, 2918 (C-H str. Aromatic), 1620 (N-H deformation Amide), 1586-1510 (C-C str. (in ring), 1460 (CH_2 def), 1359-1268 (C-N str), 957-847 (Aromatic (Out of plane def), 791 (N-H out of plane), 692 (C-S str).

8) 2-{[5-(propylsulfanyl)-1H-benzimidazol-2-yl] carbamoyl} phenyl acetate (D8)

Yield 68%, MP 175-180°C; IR (KBr, cm^{-1}): 3336 (N-H str Amide), 2959, 2922 (C-H str. Aromatic), 2868 $CH_3(sp^3)$ str, 1621 (N-H deformation Amide), 1587-1481(C-C str. (in ring), 1460 (CH_2 def), 1359-1269 (C-N str), 957-847(Aromatic (Out of plane def), 791 (N-H out of plane), 696 (C-S str). ^{1}H-NMR: (δppm): 7.598 (s, 1H NH-C=O), 7.460 (s, Ar-H), 7.329 (d, 1H, Ar-H), 7.096(m, 2H Ar-H) 2.800 (t, 2H CH_2-S), 1.595 (m, CH_2), 0.973(t, CH_3).

9) 2-hydroxy-N-[5-(propylsulfanyl)-1H-benzimidazol-2-yl] benzamide (D9)

Yield 70%, MP 188-190°C; IR (KBr, cm^{-1}): 3338 (N-H str Amide), 2958, 2926 (C-H str. Aromatic), 2868 $CH_3(sp^3)$ str, 1622 (N-H deformation Amide), 1589, 1523 (C-C str. (in ring), 1460 (CH_2 def), 1359-1225 (C-N str), 957-847(Aromatic (Out of plane def), 791 (N-H out of plane), 699 (C-S str).

Table no. 01: Important pharmacokinetic parameters for good oral bioavailability of the titled compound (D1-D9)

Compound	R	MF	TPSA (A^2)	n-ROTB	MW	Mi Log P	n-OHNH donor	n-ON acceptor	Lipinskis violation
Rule					**<500**	**<5**	**<5**	**<10**	**<1**
D1	Niflumic Acid amide	$C_{23}H_{22}F_3N_5OS$	103.38	9	473.51	3.99	4	5	0
D2	Aceclofenac amide	$C_{26}H_{26}Cl_2N_4O_3S$	116.79	12	545.48	4.5	4	3	1
D3	Ibuprofen amide	$C_{23}H_{31}N_3OS$	78.46	9	397.58	4.33	3	1	0
D4	Naproxen amide	$C_{24}H_{27}N_3O_2S$	87.69	8	421.56	3.79	3	2	0
D5	Indomethacin amide	$C_{24}H_{27}N_3O_2S$	109.69	10	549.08	4.48	3	3	1
D6	Mefenamic acid amide	$C_{29}H_{29}ClN_4O_3S$	90.49	8	432.58	4.5	4	1	0
D7	Flurbiprofen amide	$C_{25}H_{28}N_4OS$	78.46	8	435.56	4.9	3	2	0
D8	Aspirin amide	$C_{25}H_{26}FN_3OS$	104.76	8	371.45	3.08	3	3	0
D9	Salicylic acid amide	$C_{19}H_{21}N_3O_3S$	98.69	6	329.42	2.69	4	2	0

MF Molecular formula, TPSA topological polar surface area, n-ROTB number of rotatable bonds, MW molecular weight, n-OHNH number of hydrogen bond donors, n-ON number of hydrogen bond acceptors.

B) Biological Evaluation:

Experimental Animals: The anthelmintic activity of adult Indian earthworms (*Pheretima posthuma*) was investigated. After being removed from damp soil, the earthworms were cleaned to remove stool. For all experimental procedures earthworms measuring 3-6 cm in length and 0.1-0.1-2 cm in width were employed. Because of their morphological and physiological similarities to human intestinal roundworm parasites, earthworms can be utilized to investigate anthelmintic activity. [13-16]

Invitro Anthelmintic Activity:

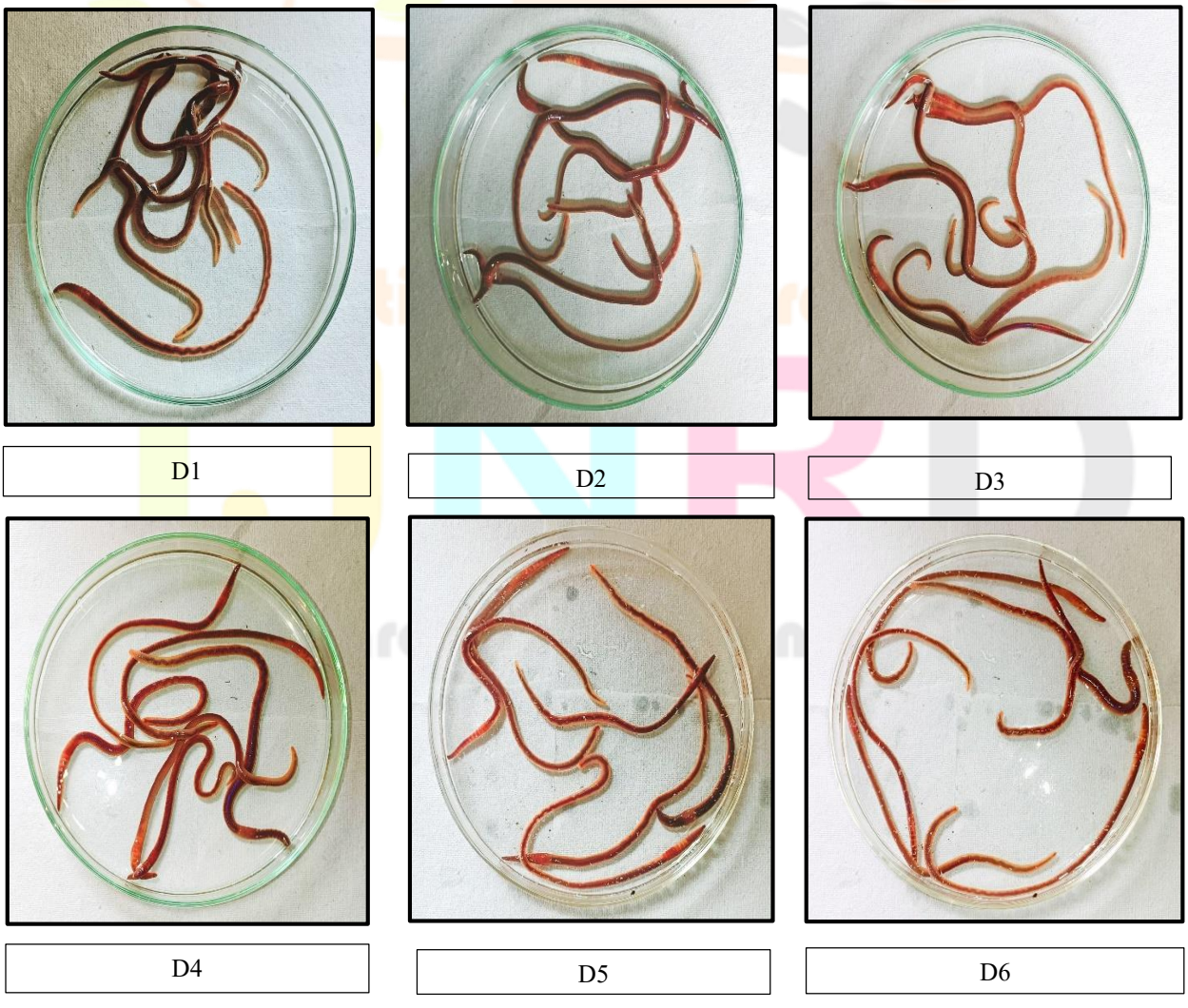
The anthelmintic activity of the recently synthesized compounds was examined. All newly synthesized prototypes were subjected to an in vitro anthelmintic bioassay using *Pheretima posthuma* (an earthworm from the Sangli region) of approximately identical size (6 cm ± 1). Before research, the worms were acclimated to the laboratory environment [17,18]. The worms were separated into groups, each of which had six earthworms. To create concentrations of 5, 10, and 20 mg/ml all of the prototypes were dissolved in a minimum of 2% v/v Tween80, and the volume was adjusted to 20 ml using regular saline. Before the experiments started all the prototypes and the standard medication solution were made from scratch. [19,20,21] After being cleaned in regular saline solution, each earthworm was put into 20 milliliters of solitary worm dying. When the worms do not resurrect even in regular saline, it was claimed that paralysis has taken place. The worms' loss of motility and subsequent fading of their body color marked their death. [22, 23,24]

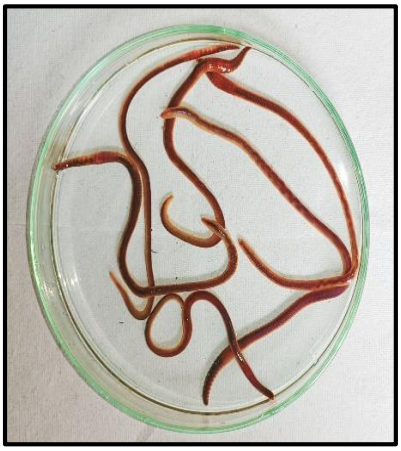
Statistical Analysis:

The findings were presented as MEAN ± SEM Dunnett's test and one-way analysis of variance (ANOVA) was used to establish statistical significance and level of significance.[25]

V. RESULT AND DISCUSSION:

Fig No. 01 Petri plates showing Antihelmintic Activity for Test Compounds & Standard





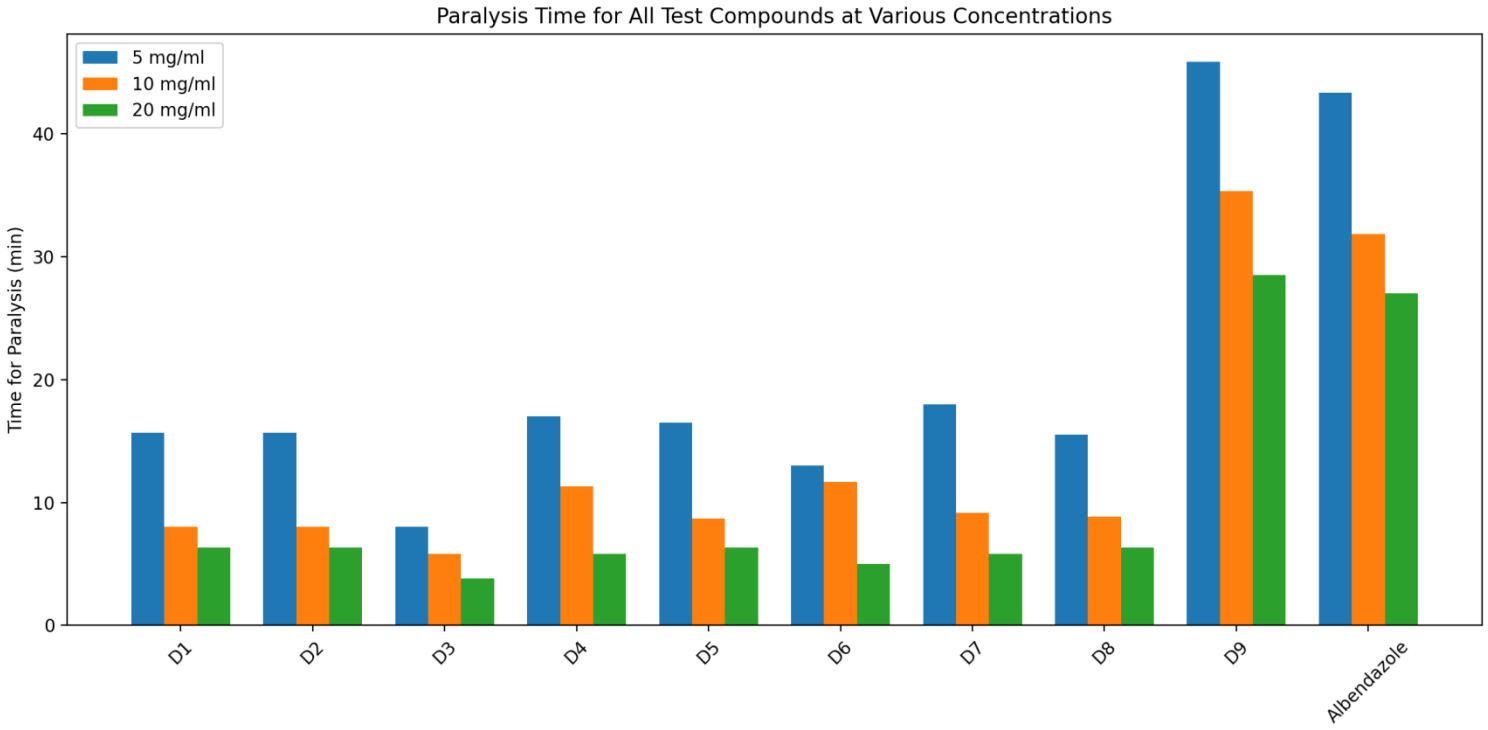
D7

Std (Albendazole)

Table No. 02: In vitro Antihelmintic activity of synthesized derivatives

Test compounds	Time taken for paralysis (p)		
	Paralysis time (min)		
	5mg/ml	10mg/ml	20mg/ml
Control	-	-	-
D1	15.67 ± 0.49	8 ± 0.36	6.33 ± 0.56
D2	8 ± 0.37	5.83 ± 0.31	3.83 ± 0.31
D3	17 ± 0.37	11.33 ± 0.33	5.83 ± 0.31
D4	16.5 ± 0.43	8.67 ± 0.42	6.33 ± 0.42
D5	13 ± 0.37	11.67 ± 0.33	5 ± 0.37
D6	18 ± 0.58	9.17 ± 0.48	5.83 ± 0.31
D7	15.5 ±0.43	8.87 ± 0.33	6.33 ± 0.42
D8	45.83 ± 0.31	35.33 ± 0.56	28.5 ± 0.43
D9	43.33 ± 0.50	31.83 ± 0.48	27 ± 0.37
Albendazole	35.33 ± 0.42	20.83 ± 0.30	7.83 ± 0.30

Showing Paralysis time of test compounds



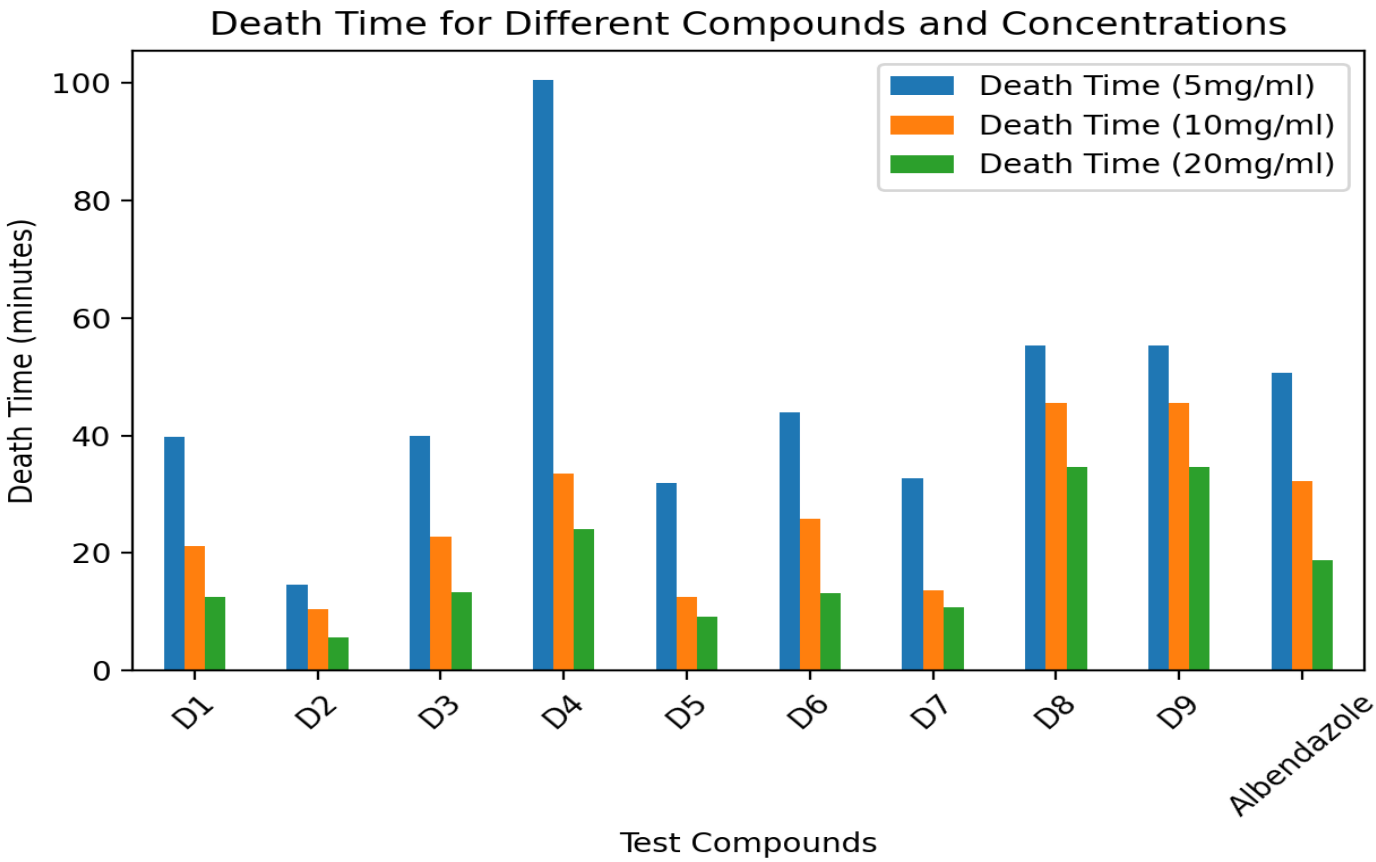
Results are expressed as Mean SEM. P < 0.01 is considered significant when compared with the control. (One-way ANOVA followed by Dunnet's Test)

Table No. 03: In vitro Anthelmintic activity of synthesized derivatives

Test compounds	Time taken for paralysis (p)		
	Paralysis time (min)		
	5mg/ml	10mg/ml	20mg/ml
Control	-	-	-
D1	39.83 ± 0.37	21.17 ± 0.31	12.5 ± 0.43
D2	14.67 ± 0.33	10.5 ± 0.43	5.67 ± 0.33
D3	40 ± 0.37	22.83 ± 0.48	13.33 ± 0.50
D4	100.5 ± 0.43	33.5 ± 0.42	24 ± 0.73
D5	32 ± 0.58	12.5 ± 0.43	9.17 ± 0.48
D6	44 ± 0.33	25.83 ± 0.31	13.17 ± 0.31
D7	32.67 ± 0.67	13.67 ± 0.50	10.83 ± 0.54
D8	55.33 ± 0.56	45.5 ± 0.62	34.67 ± 0.42
D9	55.33 ± 0.56	45.5 ± 0.62	34.67 ± 0.42
Albendazole	50.66 ± 0.21	32.30 ± 0.43	18.83 ± 0.60

Showing the Death time of test compounds

Results are expressed as Mean±SEM. P < 0.01 is considered significant when compared with the control. (One-way ANOVA followed by Dunnet's Test)



VI. CONCLUSION:

Different Albendazole derivatives were synthesized using a novel synthetic scheme using 5-(propylsulfanyl)-1H-benzimidazole-2-amine as the starting material. This can be further reacted with NSAIDs by using the Schotten Baumann Reaction from Albendazole derivatives. The reaction afforded desired derivatives in yield ranging from 55% to 85%. All synthesized compounds meet the expected spectral data. compound D2 exhibited higher anthelmintic against earthworm species *Pheretima posthuma*. Heterocyclic and fused heterocyclic substituents were exhibited and might be responsible for maximum anthelmintic activity.

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CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest regarding the publication.

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