



Research Article

SYNTHESIS, CHARACTERIZATION AND ANTHELMINTIC ACTIVITY OF SOME NOVEL BENZOTHAZOLE DERIVATIVES

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ABSTRACT

The N-(benzothiazol-2-yl)-2-(3,5-diphenyl-4,5-dihydro-1H-pyrazole-1-carbothioamido) acetamide was synthesized, purified, characterized and evaluated for the biological activity of newly synthesized structural analogs of Benzothiazole derivatives. All these molecules (5a-5l) were characterized by FTIR, ¹H NMR and mass spectral analysis along with physical data. The functional groups in the title compounds were indicated by their IR spectra. The number of protons in the compounds were confirmed by their ¹H NMR spectra. The structure of title compounds were confirmed by their Mass Spectra. Among all the compounds tested on earthworms (Anthelmintic activity), all compounds were (5a-5l) showed significant paralytic and death time of earthworms, compared to the standard drug Albendazole.

KEYWORDS: Substituted benzaldehyde, Thiosemicarbazide, 2-amino benzothiazole, Chloroacetylchloridelsatine and Anthelmintic activity.

INTRODUCTION

Heterocyclic compounds containing a ring made up, in addition to carbon atoms, other elements (heteroatoms), most often nitrogen, oxygen, and sulfur, and less frequently phosphorus, boron, and silicon.

Although the parent compound, benzothiazole is not widely used, many of its derivatives are found in commercial products or in nature.^[1-3]

Thiazole rings are planar and aromatic. Thiazoles are characterized by larger pi-electron delocalization than the corresponding oxazoles and have therefore greater aromaticity. This aromaticity is evidenced by the chemical shift of the ring protons in proton NMR spectroscopy (between 7.27 and 8.77 ppm), clearly indicating a strong diamagnetic ring current. The calculated pi-electron density marks C5 as the primary site for electrophilic substitution, and C2 as the site for nucleophilic substitution. Since most of the benzothiazole derivatives were reported for their diversified activity such as antitumor, anti-tubercular, antimalarial, anticonvulsant, anthelmintic, analgesic, anti-inflammatory and antifungal^[4-6].

MATERIALS AND METHODS^[7-9]

The synthesized compounds were screened for anthelmintic activities. Fourier Transform IR spectrometer (model Shimadzu 8700) in the range of 400-4000 cm⁻¹ Using KBr pellets and values are reported in cm⁻¹ and the spectra were interpreted. ¹H-NMR spectra were recorded on DPX-200 MHz NMR spectrometer using DMSO-d₆ and chemical shifts (δ) are reported in parts per million down field from internal reference Tetramethylsilane (TMS) and the Spectra were

interpreted. Mass spectra were recorded on Mass spectrophotometer (model Shimadzu) by LC-MS and the spectra were interpreted. Precoated Silica gel G plates were used to monitor the progress of reaction as well as to check the purity of the compounds: n-Hexane: Ethyl acetate (8:2).

General procedures:

Step: 1: Preparation of 2-aminobenzothiazole:

Aniline (4.6g, 0.05mol) and ammonium thiocyanate (3.8g, 0.05 mol) were dissolved in absolute ethanol containing 4 ml of con. HCl. To this mixture bromine in glacial acetic acid (6.75ml, 0.125 mol) was added and the reaction mixture was refluxed for 1 hr. Then it was cooled in ice bath. The precipitate obtained was filtered, washed with cold water and dried. The crude product was recrystallized from ethanol.

Step: 2: Preparation of compound 2: (N-(1, 3-benzothiazol-2-yl)-2-chloroacetamide):

To a solution of compound 1. (2.5 g, 0.016mol) in (30ml) glacial acetic acid, chloroacetyl chloride (3.7g, 0.032mol) was added drop wise with constant stirring. The reaction mixture was refluxed for 5 hrs then it was powered onto crushed ice. The precipitated solid that obtained was filtered off, washed with cold water, dried and recrystallized from aqueous ethanol.

Product was filtered, dried and recrystallized from ethanol to give N-(4-(1H-benzothiazol-2-yl)phenyl)acetamide.

Step-3: Synthesis of Chalcones derivatives:

Acetophenone (0.01M) was dissolved in ethanol (25ml) and Aldehyde (0.01M) was added and add aq. sodium hydroxide solution (40%, 10 ml) to the above solution. The mixture was stirred mechanically at room temperature for about 6-12hrs and kept overnight. The solid separate was filtered and washed with ice cold water. The crude product was crystallized from ethanol.

Step-4: Synthesis of Pyrazole derivatives:

Dif.Chalcone (0.01M) dissolved in ethanol (25 ml) were added to Thiosemicarbazide (0.01M). To this aq. KOH solution (0.02M) was added (prepared from KOH in small amount of distilled water). The reaction mixture was refluxed for 8 hours, cooled, diluted with water

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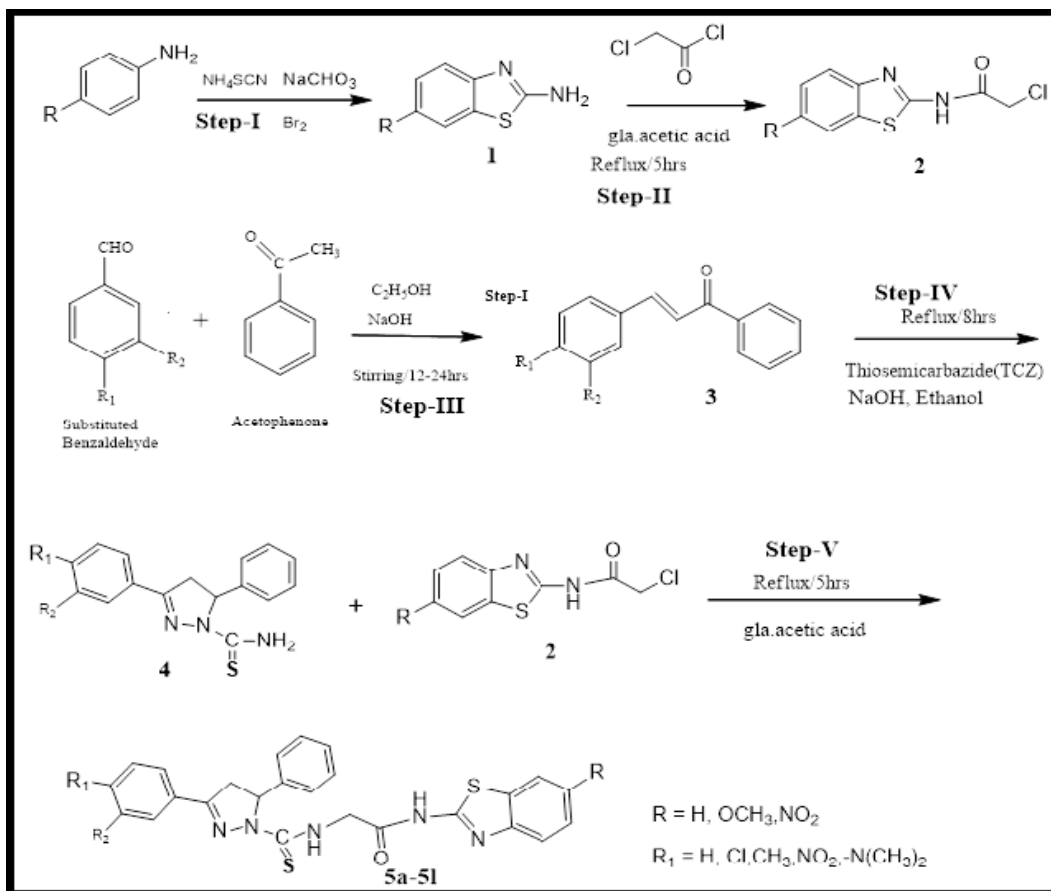
and acidified with conc. HCl. The product was filtered, dried and crystallized from ethanol.

Step-5: Synthesis of novel Benzothiazole derivatives (5a-5l):

Compound 4a-4f (0.01 mol) was taken in a mixture of Substituted Aldehyde (0.01 mol) and glacial acetic acid (5 mL) and

Ethanol 30ml, then the reaction mixture was refluxing for 1-3hrs. The progress of the reaction was monitored by TLC (Hexane: EtoAc 1:4). The reaction mixture was cooled to room temperature. A solid was obtained, which was filtered off and washed with hexane and recrystallized from Ethanol to give crystalline solid. The physical data are reported in **Table1**.

Scheme of synthesis:



Spectral Data:

5a-IR Cm^{-1} (KBr):3476(-NH Str, thiamide), 3413(-NH Str,acetamide), 3072(-CH Str, Aromatic), 2987(-CH Str, acetamide), 2931(-CH₂ Str, Pyrazole), 2828(-CH Str, Pyrazole), 1705(C=O Str), 1546(C=N Str), 1317(C-N Str), 1240(C=S Str). **¹H-NMR (DMSO δ ppm)**:11.05(1H,-NH Thiamide), 7.82-6.66(14H, Ar-H), 5.01(13H, acetamide), 4.55-4.52(2H, CH₂in Pyrazole), 3.05-3.00(2H, -CH₂ in acetamide), 2.28(1H, CH in Pyrazole). **Mass (EI-MS)**: 471(M), 472(M+1, 100%), 470(M-1,100%).

5b-IR Cm^{-1} (KBr):3489(-NH Str, thiamide), 3400(-NH Str,acetamide), 3019(-CH Str, Aromatic), 2920(-CH Str, acetamide), 2958(-CH Str, acetamide), 2937(-CH₂Str,Pyrazole), 2861(-CH Str, Pyrazole), 1733(C=O Str), 1591(C=N Str), 1359(C-N Str), 1283(C=S Str), 871(C-ClStr). **¹H-NMR (DMSO δ ppm)**: 3489(-NH Str, thiamide), 3400(-NH Str,acetamide), 3019(-CH Str, Aromatic), 2920(-CH Str, acetamide), 2958(-CH Str, acetamide), 2937(-CH₂Str,Pyrazole), 2861(-CH Str, Pyrazole), 1733(C=O Str), 1591(C=N Str), 1359(C-N Str), 1283(C=S Str), 871(C-ClStr). **Mass (EI-MS)**:506(M), 507(M+1, 100%), 504(M-1,100%).

5c-IR Cm^{-1} (KBr):3427(-NH Str, thiamide), 3405(-NH Str,acetamide), 3038(-CH Str, Aromatic), 2989(-CH Str, acetamide), 2917(-CH₂ Str,Pyrazole), 2874(-CH Str, Pyrazole), 1716(C=O Str), 1644(NO₂ Str), 1555(C=N Str), 1371(C-N Str), 1216(C=S Str). **¹H-NMR (DMSO δ ppm)**:3427(-NH Str, thiamide), 3405(-NH Str,acetamide), 3038(-CH Str, Aromatic), 2989(-CH Str, acetamide), 2917(-CH₂ Str,Pyrazole), 2874(-CH Str, Pyrazole), 1716(C=O Str), 1644(NO₂ Str),

1555(C=N Str), 1371(C-N Str), 1216(C=S Str). **Mass (EI-MS)**: 516(M), 517(M+1, 100%), 514(M-1,100%).

5d-IR Cm^{-1} (KBr):3412(-NH Str, thiamide), 3403(-NH Str,acetamide), 3083(-CH Str, Aromatic), 2998(-CH Str, acetamide), 2934(-CH₂ Str,Pyrazole), 2845(-CH Str, Pyrazole), 1702(C=O Str), 1586(C=N Str), 1347(C-N Str), 1232(C=S Str). **¹H-NMR (DMSO δ ppm)**: 11.34(1H,-NH Thiamide), 8.00-6.90(12H, Ar-H), 5.32(1H,-NH acetamide), 4.40-4.38(2H, CH₂in Pyrazole), 3.70-3.56(6H, -OCH₃), 3.25-3.17(2H, -CH₂ in acetamide), 2.35(1H, CHin Pyrazole). **Mass (EI-MS)**: 531(M), 532(M+1, 100%), 530(M-1,100%).

5e-IR Cm^{-1} (KBr):3458(-NH Str, thiamide), 3419(-NH Str, acetamide), 3081(-CH Str, Aromatic), 2958(-CH Str, acetamide), 2901(-CH₂ Str,Pyrazole), 2812(-CH Str, Pyrazole), 1711(C=O Str), 1530(C=N Str), 1359(C-N Str), 1220(C=S Str). **¹H-NMR (DMSO δ ppm)**:1HNMR (DMSO, δ ppm): 11.38(1H,-NH Thiamide), 7.55-6.80(13H, Ar-H), 5.30(1H,-NH acetamide), 4.38-4.36(2H, CH₂in Pyrazole), 3.37-3.31(2H, -CH₂ in acetamide), 2.28(1H, CH in Pyrazole), 1.39-1.35(6H, CH₃). **Mass (EI-MS)**: 514(M), 515(M+1, 100%), 513(M-1,100%).

5f- IR Cm^{-1} (KBr):3467(-NH Str, thiamide), 3417(-NH Str, acetamide), 3096(-CH Str, Aromatic), 2960(-CH Str, acetamide), 2905(-CH₂ Str,Pyrazole), 2895(-CH Str, Pyrazole), 1710(C=O Str), 1576(C=N Str), 1329(C-N Str), 1216(C=S Str). **¹H-NMR (DMSO δ ppm)**: 11.15(1H,-NH Thiamide), 7.75-7.52(13H, Ar-H), 5.15(1H,-NH acetamide), 4.36-4.31(2H, CH₂in Pyrazole), 3.37-3.31(2H, -CH₂ in acetamide), 2.36(1H,

CHin Pyrazole), 1.09-1.02(3H, CH₃). **Mass (EI-MS):** 485(M), 486(M+1, 100%), 484(M-1,100%).

5g-IR *Cm*⁻¹ (KBr):3487(-NH Str, thiamide), 3407(-NH Str,acetamide), 3038(-CH Str, Aromatic), 2932(-CH Str, acetamide), 2911(-CH₂ Str, Pyrazole), 2873(-CH Str, Pyrazole), 1716(C=O Str), 1555(C=N Str), 1371(C-N Str), 1216(C=S Str).¹H-NMR (DMSO δ ppm):11.18(1H,-NH Thiamide), 7.74-6.92(13H, Ar-H), 5.15(1H,-NH acetamide), 4.35-4.30(2H, CH₂in Pyrazole), 3.66-3.52(3H, -OCH₃), 3.36-3.35(2H, -CH₂ in acetamide), 2.35(1H, CHin Pyrazole).**Mass (EI-MS):** 501(M), 502(M+1, 100%), 500(M-1,100%).

5h- IR *Cm*⁻¹ (KBr):3418(-NH Str, thiamide), 3400(-NH Str,acetamide), 3028(-CH Str, Aromatic), 2970(-CH Str, acetamide), 2934(-CH₂ Str,Pyrazole), 2865(-CH Str, Pyrazole), 1723(C=O Str), 1543(C=N Str), 1359(C-N Str), 1224(C=S Str), 897(C-Cl Str).¹H-NMR (DMSO δ ppm): 11.32(1H,-NH Thiamide), 8.01-6.86(12H, Ar-H), 5.32(1H,-NH acetamide), 4.43-4.48(2H, CH₂in Pyrazole), 3.70-3.65(3H, -OCH₃), 3.30-3.23(2H, -CH₂ in acetamide), 2.45(1H, CHin Pyrazole).**Mass (EI-MS):** 535(M), 536(M+1, 100%), 534(M-1,100%).

5i-IR *Cm*⁻¹ (KBr):3454(-NH Str, thiamide), 3410(-NH Str,acetamide), 3043(-CH Str, Aromatic), 2930(-CH Str, acetamide), 2923(-CH₂ Str,Pyrazole), 2883(-CH Str, Pyrazole), 1711(C=O Str), 1640(NO₂ Str),1562(C=N Str), 1361(C-N Str), 1223(C=S Str).¹H-NMR (DMSO δ ppm): 11.02(1H,-NH Thiamide), 7.99-6.67(12H, Ar-H), 5.28(1H,-NH acetamide), 4.13-4.03(2H, CH₂in Pyrazole), 3.68-3.60(3H, -OCH₃), 3.28-3.26(2H, -CH₂ in acetamide), 2.30(1H, CHin Pyrazole).**Mass (EI-MS):** 546(M), 547(M+1, 100%), 545(M-1,100%).

5j- IR *Cm*⁻¹ (KBr):3428(-NH Str, thiamide), 3403(-NH Str,acetamide), 3061(-CH Str, Aromatic), 2974(-CH Str, acetamide), 2901(-CH₂ Str,Pyrazole), 2893(-CH Str, Pyrazole), 1723(C=O Str), 1573(C=N Str), 1342(C-N Str), 1232(C=S Str).¹H-NMR (DMSO δ ppm): 11.98(1H,-NH Thiamide), 8.21-6.98(11H, Ar-H), 5.27(1H,-NH acetamide), 4.47-4.43(2H, CH₂in Pyrazole), 3.890-3.53(9H, -OCH₃), 3.24-3.20(2H, -CH₂ in acetamide) , 2.38(1H, CHin Pyrazole).**Mass (EI-MS):** 561(M), 562(M+1, 100%), 560(M-1,100%).

5k-IR *Cm*⁻¹ (KBr):3430(-NH Str, thiamide), 3412(-NH Str,acetamide), 3063(-CH Str, Aromatic), 2928(-CH Str, acetamide), 2932(-CH₂ Str,Pyrazole), 2878(-CH Str, Pyrazole), 1703(C=O Str),1570(C=N Str), 1356(C-N Str), 1243(C=S Str).¹H-NMR (DMSO δ ppm): 11.27(1H,-NH Thiamide), 8.02-7.01(12H, Ar-H), 5.35(1H,-NH acetamide), 4.39-4.35(2H, CH₂in Pyrazole), 3.68-3.54(3H, -OCH₃), 3.20-3.14(2H, -CH₂ in acetamide) , 2.48(1H, CHin Pyrazole), 2.12-2.01(6H, -CH₃).**Mass (EI-MS):** 544(M), 545(M+1, 100%), 543(M-1,100%).

5l-IR *Cm*⁻¹ (KBr):3440(-NH Str, thiamide), 3404(-NH Str, acetamide), 3046(-CH Str, Aromatic), 2933(-CH Str, acetamide), 2935(-CH₂ Str, Pyrazole), 2880(-CH Str, Pyrazole), 1703(C=O Str), 1534(C=N Str), 1342(C-N Str), 1243(C=S Str). ¹H-NMR (DMSO δ ppm):11.65(1H,-NH Thiamide), 7.99-6.87(12H, Ar-H), 5.49(1H,-NH acetamide), 4.30-4.29(2H, CH₂in Pyrazole), 3.68-3.49(3H, -OCH₃), 3.19-3.15(2H, -CH₂ in

acetamide), 2.39(1H, CHin Pyrazole), 2.20-2.12(3H, -CH₃).**Mass (EI-MS):** 515(M), 516(M+1, 100%), 514(M-1,100%).

Anthelmintic activity:^[10,11]

The synthesized compounds are screened for anthelmintic 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. 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. The compounds were evaluated by the time taken for complete paralysis and death of earthworms (Figure 1, 2). The mean lethal time for each test compound was recorded and compared with standard drug. 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 compounds and standard drug are tabulated in Table No.2.

RESULTS AND DISCUSSION

Synthesis:

The characterization data of all compounds 5a-5l are given the experimental section. All the synthesized compounds gave satisfactory analysis for the proposed structures, which were confirmed on the basis of their elemental analysis by FT-IR, LC-MASS, ¹H NMR data. The presentwork which involve reactionbetween Aniline and ammonium thiocyanat to give 2-aminobenzothiazole and it can be reacting with chloroacetyl chloride to form 1, 3-benzothiazol-2-chloroacetamide. The different aromatic substituted aldehyde condensation with Acetophenone in the presence of NaOHtoget Chalcones, whichonreactionwithThiosemicarbazide(TCZ) and isatinsin presenceof NaOHto givepyrazole derivatives, which on reacting with 1, 3-benzothiazol-2-chloroacetamide to form respectivetitle compounds.The synthesized compounds werescreenedfor *in vitro*Anthelmintic activity activities.

Spectroscopy:

The structures of all the newly synthesized compounds were characterized as 5a-4l on the basis of satisfactory analytical and spectral data including IR, LC-MASS, ¹H NMR data

Anthelmintic activity:The synthesized compounds (5a-5l) were evaluated for anthelmintic activity on Indian earthworms (*Pheretimaposthuma*). All compounds showed anthelmintic activity is shown in table. Among the compounds tested all the compounds were showed significant paralytic time of earthworms, compared to standard drug Albendazole at 0.1%, 0.2% and 0.5% concentrations of compounds. A closer inspiration of data from this table indicated that compound 5i, 5j, 5k, 5l having more activity and compounds 5c, 5d and 5b showed moderate activity. After all, the synthesized compounds in overall estimation confirm the better activity against *peritimaposthuma*.

Table 1: Physical data of (5a-5l)

Code	R	R ₁	R ₁	Mol. Formula	Mol. wt (g.mol ⁻¹)	M.P (°C)
5a	-H	-H	-H	C ₂₅ H ₂₁ N ₅ OS ₂	471	168-170
5b	-Cl	-H	-H	C ₂₅ H ₂₀ N ₅ OS ₂ Cl	506	241-243
5c	-NO ₂	-H	-H	C ₂₅ H ₂₀ N ₆ O ₂ S ₂	516	232-235
5d	-OCH ₃	-OCH ₃	-H	C ₂₇ H ₂₅ N ₅ O ₃ S ₂	531	200-204
5e	-N(CH ₃) ₂	-H	-H	C ₂₇ H ₂₆ N ₆ O ₂ S ₂	514	268-270
5f	-CH ₃	-H	-H	C ₂₆ H ₂₃ N ₅ OS ₂	485	208-210
5g	-H	-H	-OCH ₃	C ₂₆ H ₂₃ N ₅ O ₂ S ₂	501	178-180
5h	-Cl	-H	-OCH ₃	C ₂₆ H ₂₂ N ₅ O ₂ S ₂ Cl	535	218-220
5i	-NO ₂	-OCH ₃	-OCH ₃	C ₂₆ H ₂₂ N ₆ O ₂ S ₂	546	251-253
5j	-OCH ₃	-OCH ₃	-OCH ₃	C ₂₈ H ₂₇ N ₅ O ₄ S ₂	561	218-220

5k	-N(CH ₃) ₂	-H	-OCH ₃	C ₂₈ H ₂₈ N ₆ O ₂ S ₂	544	241-243
5l	-CH ₃	-H	-OCH ₃	C ₂₇ H ₂₅ N ₅ O ₂ S ₂	515	212-216

Table 2. Anthelmintic activity of novel Benzothiazole derivatives

S. No.	Name	Time in Minutes					
		For Paralysis % Conc.			For Death % Conc.		
		0.1	0.2	0.5	0.1	0.2	0.5
	Control	-	-	-	-	-	-
	Albendazole	15	12	8	44	34	26
1	5a	27	19	17	60	47	38
2	5b	31	27	19	57	45	35
3	5c	28	26	17	58	59	49
4	5d	20	18	17	55	49	40
5	5e	20	24	28	49	42	32
6	5f	22	22	18	53	59	61
7	5g	20	23	20	41	56	59
8	5h	18	15	12	46	36	29
9	5i	17	14	10	47	37	29
10	5j	18	21	22	51	45	41
11	5k	17	21	19	50	45	38
12	5l	19	20	20	48	47	40

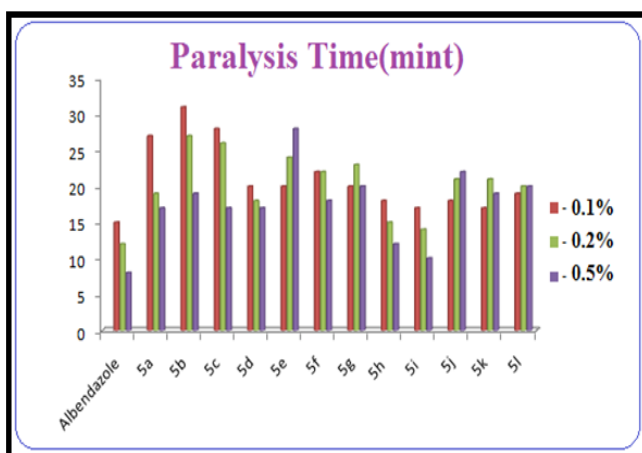


Fig. 1: Graphical representation of anthelmintic activity of compounds (5a-5l) - Paralysis time (min)

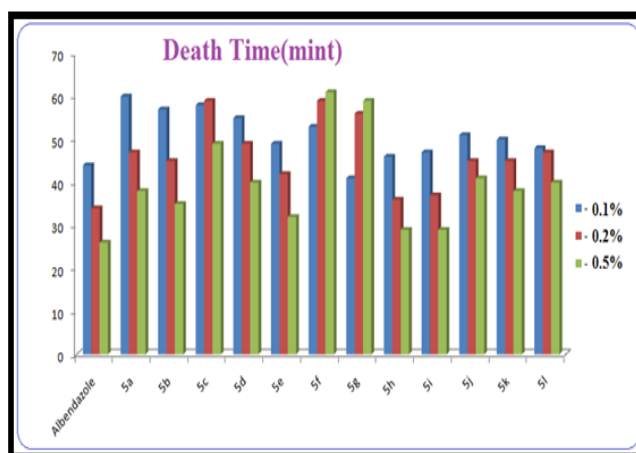


Fig. 2: Graphical representation of anthelmintic activity of compounds (5a-5l) - Death time (min)



Fig.3: Photographs of various Novels Benzothiazole derivatives –Anthelmintic activity

CONCLUSION

The objective of the present work was to synthesize, purify, characterize and evaluate the biological activity of newly synthesized

structural analogs of Benzothiazole. The yield of the synthesized compound was found to be in the range from 60-87%. In conclusion, the present study highlights the importance of Benzothiazole derivatives having various heterocyclic moiety features responsible for the anthelmintic activities and may serve as a lead

molecule for further modification to obtain clinically useful novel entities.

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