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Research Article

SYNTHESIS, CHARACTERIZATION, AND ANTHELMINTIC ACTIVITY OF NOVEL BENZOTHIAZOLE DERIVATIVES CONTAINING INDOLE MOIETIES

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ABSTRACT

Objective: The objective of this study was to synthesize and evaluate the anthelmintic activity (AA) of novel benzothiazole derivatives containing indole moieties (BDIM).

Methods: The present works which involve the substituted isatin Schiff bases undergo acetylating and reacting with 2-aminobenzothiazole to give novel BDIM.

Results: All the newly synthesized molecules (5a-5o) were characterized by Fourier-transform infrared spectroscopy, H-nuclear magnetic resonance, and mass spectral analysis along with physical data. The biological potentials of the newly synthesized compounds are evaluated for their AA using an Indian earthworm (*Pheretima posthuma*), and albendazole was used as standard drug.

Conclusion: The synthesized compound 5f, 5n, and 5o showed good AA, whereas others exhibited significant activities.

Keywords: Substituted benzaldehyde, Isatin, p-Toluidine, 2-aminobenzothiazole, Anthelmintic activity.

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INTRODUCTION

Heterocyclic compounds are those which have a cyclic structure with two, or more, different kinds of atom in the ring. This two kinds of atoms are like carbon and the other elements (heteroatoms), most often N, O and S. Although the parent compound, benzothiazole is not widely used, many of its derivatives are found in commercial products or in nature[1-3]. Benzothiazole ring found to be possessing pharmacological activities such as antitumor, antitubercular, antimalarial, anticonvulsant, anthelmintic, analgesic, anti inflammatory and antifungal. Isatin or 1H-indole-2, 3-dione is an indole derivative. Indole derivatives have acquired conspicuous significance due to their wide spectrum of biological activities.

In view of the high degree of bioactivity shown by the benzothiazole derivatives mentioned above facts, we aimed to construct a system combining both these vital moieties such as benzothiazole and indole in a single molecular framework, together with an exploration of the additive effects of their biological activities. Hence, we describe here in the synthesis, characterization, and anthelmintic activity (AA) of novel benzothiazole derivatives containing indole moieties (BDIM).

METHODS

The synthesized compounds were screened for anthelmintic activities [4-7]. The IR spectra were recorded on a Shimadzu 8700 spectrometer using the ATR technique (Attenuated Total Reflectance) in the range of 400–4000 cm-1. and values are reported in cm^{-1} and the spectra were interpreted.^{1}H-nuclear magnetic resonance (NMR) spectra were recorded on DPX-200 MHz NMR spectrometer using dimethyl sulfoxide (DMSO)-d_6 and chemical shifts (δ) are reported in parts per million downfield from internal reference tetramethylsilane ,and the spectra were interpreted. Mass spectra were recorded on mass spectrophotometer (model Shimadzu) by Liquid chromatography–mass spectrometry (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 for the preparation of 2-aminobenzothiazole (2-ABT)

Aniline (4.6 g, 0.05 mol) and potassium thiocyanate (3.8 g, 0.05 mol) were dissolved in absolute ethanol containing 4 ml of concentrated hydrochloric acid (HCl). To this mixture, bromine in glacial acetic acid (6.75 ml, 0.125 mol) was added and the reaction mixture was refluxed for 1 h. 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 [8].

Synthesis of substituted isatin from aniline

In a round-bottomed flask are placed 9gm of chloral hydrate and 120ml of water. To this solution are then added, in order: 13gm of crystallized sodium sulphate, a solution of 4.5 gm of aniline in 30ml of water to which 5.12 gm of concentrated hydrochloric acid has been added to dissolve the amine and finally, a solution of 11gm of 5 hydrochlorid in 50ml of water. Flask was then heated vigorously until the reaction was completed. After it, the solution containing beaker was cooled in running water followed by the filtration of reminder crystallized product with suction pump and air dried. 18.4 g of concentrated sulfuric acid (10.0 ml) was warmed to 50°C and 2.5 g of dry isonitrosoacetanilide was added in such a rate so as to keep the temperature between 60°C and 70°C but not higher. External cooling was applied at this stage so that the reaction could be carried out more rapidly after the addition of isonitroso compound was finished. The solution was heated to 80°C and kept at this temperature for about 10 min to complete the reaction. Then, the reaction mixture was cooled to room temperature and poured it into 10 times its volume of cracked ice. After standing for 90 min, the final product was filtered with suction pump followed by washing with cold water to remove sulfuric acid and dried in air.

Synthesis of isatin schiff bases (ISB) (3a-3i)

A mixture of equimolar quantity of substituted aromatic aniline (0.01 mol) and compound 2a-2d was dissolved in 20 ml of ethanol and refluxed for 2–3h in the presence of few drops of 2 ml glacial acetic acid. The progress of the reaction was monitored by thin-layer chromatography (TLC) (n-Hexane:EtoAc 7:3). The reaction mixture was cooled to room temperature and kept in the refrigerator for overnight to get precipitate. A solid was obtained, which was filtered off and recrystallized from methanol or ethanol to give crystalline solid.[9]

Synthesis of 1-acetyl-3-(phenylimino) indolin-2-one derivatives

A mixture of equimolar (0.01) quantity of compound (3a-3i, ISB) and 5.1 ml of acetic anhydride was taken in to 250ml round bottom flask. Then, whole of the content was of refluxed for about 4 h, and then, the solution was poured into beaker containing crushed ice followed by the filtration and drying of the product.

General procedure for the synthesis of novel 1-((E)-1-(benzo[d] thiazol-2-yl) imino) ethyl)-3-(phenylimino) indolin-2-one (5a-5o)

A mixture of equimolar quantity of substituted N-acetyl isatin derivatives (0.01 mol) and compound 1a-1b was dissolved in 20 ml of ethanol and refluxed for 2–3h in the presence of few drops of 2 ml glacial acetic acid. The progress of the reaction was monitored by TLC (n-Hexane:EtoAc 8:2). The reaction mixture was cooled to room temperature and kept in the refrigerator for overnight to get precipitate. A solid was obtained, which was filtered off and recrystallized from methanol or ethanol to give crystalline solid (Fig. 1)

5a: (E)-1-((E)-1-(benzo[d]thiazol-2-yl) imino) ethyl)-3-(phenylimino) indolin-2-one

M.P. 219–221°C; Mol. formula: $C_{23}H_{16}N_4OS$, yield 78%, IR (ν cm^{-1}): 3143, 3054 (C-H *Str,* Ar), 2930, 2891, 2793 (C–H *Str*, Aliphatic), 2311 (C-S-C *Str*), 1684 (C=O *Str*, Indole), 1588 (C=N *Str*), 1515 (C=CH *Str*), 1431 (C=C *Str*, Ar). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 8.38–8.27 (d, 2H, Ar-H), 8.11–7.88 (d, 2H, Ar-H), 7.84–7.77(t, 3H, Ar-H), 7.69–7.67 (d, 2H, Ar-H), 7.55–7.54 (d, 2H, Ar-H), 7.51–7.41 (t, 3H, Ar-H). 3.33 (S, 3H, -CH_3); Mass (ESI-MS): m/z 396 (M), 397 (M+1, 100%).

5b: (E)-5-chloro-1-((E)-1-(benzo[d]thiazol-2-yl) imino) ethyl)-3-(phenylimino) indolin-2-on

M.P. 213-215°C; Mol. formula: $C_{23}H_{15}N_4OSCl$, yield 82%,IR (ν cm^{-1}): 3037, 2932 (C-H *Str,* Ar), 2872 (C–H *Str*, Aliphatic), 2346 (C-S-C *Str*), 1721 (C=O *Str*, Indole), 1555 (C=N *Str*), 1520 (C=CH *Str*), 1432 (C=C *Str*, Ar), 771 (C-Cl *Str*, Ar).^{1}H-NMR (DMSO) $\delta\delta$ ppm: 8.37–8.28 (t, 3H, Ar-H), 7.88–7.84 (t, 3H, Ar-H), 8.10 (s, 1H, Ar-H), 7.83–7.68 (d, 4H, Ar-H), 7.58–7.57 (d, 2H, Ar-H), 7.55–7.51 (d, 2H, Ar-H), 3.39 (s, 3H, -CH_3); Mass (ESI-MS): m/z 430 (M), 431 (M+1, 100%),432 (M+2, 30%).

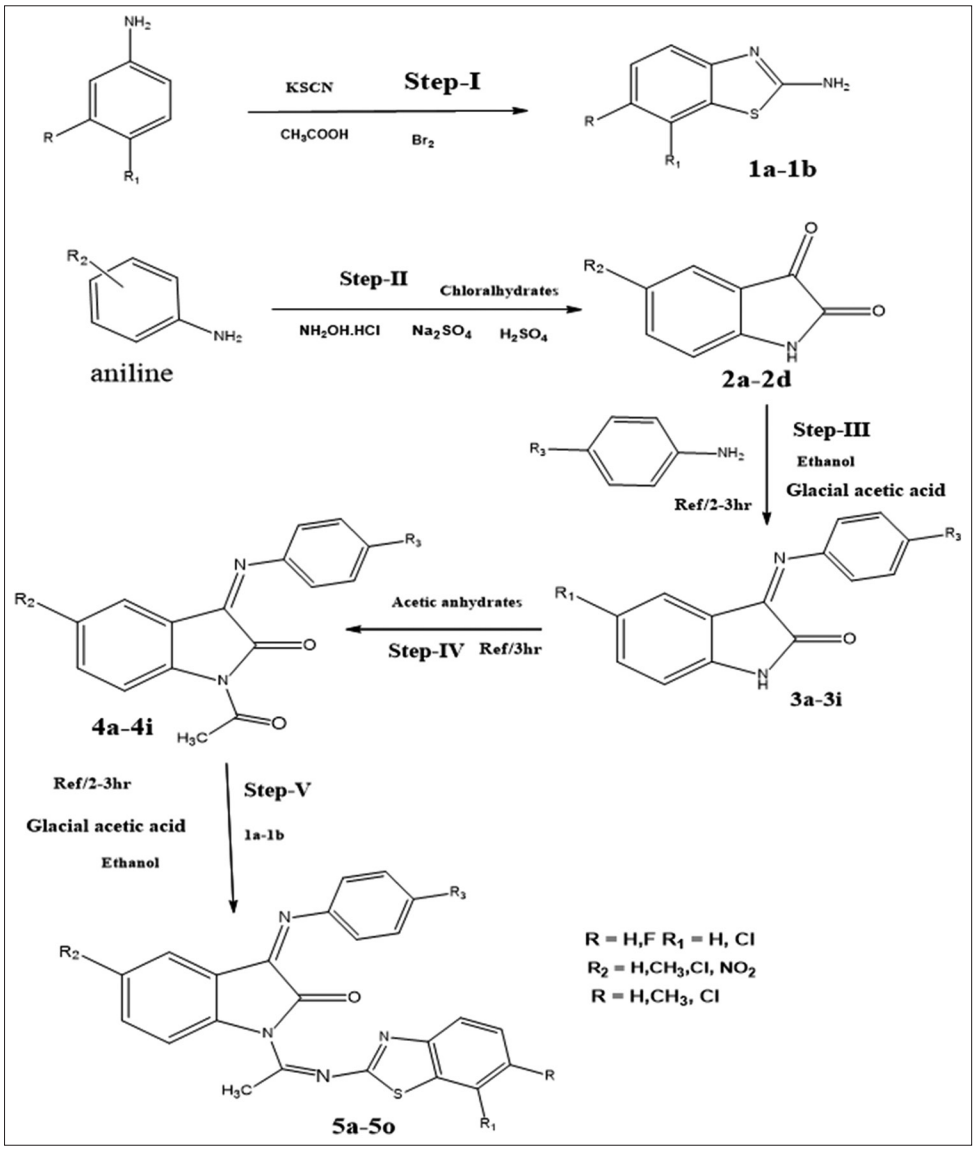

Fig. 1: Synthetic scheme

5c: (E)-1-((E)-1-(benzo[d]thiazol-2-yl) imino) ethyl)-3-(p-tolylimino) indolin-2-one

M.P. 203–205°C; Mol. formula: $C_{24}H_{18}N_4OS$, yield 77%. IR (ν cm^{-1}): IR (ν cm^{-1}): 3100 (C-H *Str,* Ar), 2987, 2882 (C–H *Str*, Aliphatic), 2336 (C-S-C *Str*), 1705 (C=O *Str*, Indole), 1663, 1546 (C=N *Str*), 1506 (C=CH *Str*), 1459 (C=C *Str*, Ar), 797 (C-Cl *Str*, Ar), 588 (C-F *Str*, Ar).^{1}H-NMR (DMSO) $\delta\delta$ ppm: 8.57–8.35 (d, 4H, Ar-H), 8.06–8.04 (d, 4H, Ar-H), 7.94–7.92 (t, 2H, Ar-H), 7.82–7.75 (t, 2H, Ar-H), 3.31 (s, 3H, -CH_3), 1.97–1.94 (s, 3H, -CH_3); Mass (ESI-MS): m/z 410 (M), 411 (M+1, 100%).

5d: (E)-5-chloro-1-((E)-1-(benzo[d]thiazol-2-yl) imino)ethyl)-3-(p-tolylimino)indolin-2-one

M.P. 231–233°C; Mol. formula: $C_{24}H_{17}N_4OSCl$, yield 66%. IR (ν cm^{-1}): 3093 (C-H *Str,* Ar), 2976, 2884 (C–H *Str*, Aliphatic), 2383 (C-S-C *Str*), 1699 (C=O *Str*, Indole), 1578 (C=N *Str*), 1565 (C=CH *Str*), 1476 (C=C *Str*, Ar), 803 (C-Cl *Str*, Ar).^{1}H-NMR (DMSO) $\delta\delta$ ppm: 8.47–8.37 (d, 2H, Ar-H), 7.95–7.88 (d, 4H, Ar-H), 7.40–7.39 (t, 2H, Ar-H), 7.35 (s, ^{1}H, Ar-H), 7.35–7.34 (d, 2H, Ar-H), 3.34–3.30 (s, 3H, -CH_3), 1.986–1.982 (s, 3H, -CH_3); Mass (ESI-MS): m/z 444 (M), 445 (M+1, 100%), 446 (M+2, 30%).

5e: (E)-1-((E)-1-((benzo[d]thiazol-2-yl) imino) ethyl)-3-(phenylimino) indolin-2-one

M.P. 191–193°C; Mol. formula: $C_{24}H_{18}N_4OS$, yield 67%. IR (ν cm^{-1}): 3065(C-H *Str,* Ar), 2987, 2894 (C–H *Str*, Aliphatic), 2365 (C-S-C *Str*), 171 6C=O *Str*, Indole), 1584 (C=N *Str*), 1543 (C=CH *Str*), 1467 (C=C *Str*, Ar),^{1}H-NMR (DMSO) $\delta\delta$ ppm: 7.98–7.90 (d, 2H, Ar-H), 7.78–7.80 (d, 2H, Ar-H), 7.68–7.67 (d, 2H, Ar-H), 7.40–7.39 (t, 2H, Ar-H), 7.29–7.28 (t, 3H, Ar-H), 7.14–7.10 (t, 2H, Ar-H), 2.14–2.10 (s, 3H, -CH_3); Mass (ESI-MS): m/z 410 (M), 411 (M+1, 100%).

5f: (E)-5-methyl-1-((E)-1-((benzo[d]thiazol-2-yl) imino) ethyl)-3-(p-tolylimino) indolin-2-one

M.P. 217–219°C; Mol. formula: $C_{25}H_{20}N_4OS$, yield 74%. IR (ν cm^{-1}): 3043 (C-H *Str,* Ar), 2978, 2875 (C–H *Str*, Aliphatic), 2312 (C-S-C *Str*), 1729 (C=O *Str*, Indole), 1576 (C=N *Str*), 1521 (C=CH *Str*), 1465 (C=C *Str*, Ar).^{1}H-NMR (DMSO) $\delta\delta$ ppm: 8.40–8.39 (d, 2H, Ar-H), 8.02–8.00 (d, 2H, Ar-H), 7.87–7.82 (d, 2H, Ar-H), 7.76 (s, 1H, Ar-H), 7.54–7.50 (d, 2H, Ar-H), 7.34–7.30 (t, 2H, Ar-H), 3.54–3.50 (s, 3H, -CH_3), 3.20–3.10 (s, 3H, -CH_3), 1.90–1.82 (s, 3H, -CH_3); Mass (ESI-MS): m/z 424 (M), 425 (M+1, 100%).

Pharmacological activity: AA[10-12]

The synthesized compounds are screened for anthelminthic activity using earthworms. Six earthworms of nearly equal size were placed in standard drug solution and test compound's solutions at room temperature. Normal saline was used as a control. The standard drug and test compounds were dissolved in minimum quantity of 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 (Figs. 2 and 3). 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. The death time of worm was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli. The mean lethal time and paralysis time of the earthworms for different test compounds and standard drug are tabulated in Table 1.

RESULTS AND DISCUSSION

The synthetic routes are outlined in Schemes 1. In the first case, aniline was heated with ammonium thiocyanate, to obtain 2-amnino

Table 1: Antihelmintic activity of BDIM

S. No	Name	Time (min) (mean±SEM)					
		For paralysis % concentration			For death % concentration		
	Concentration	0.1	0.2	0.5	0.1	0.2	0.5
	Control	-	-	-	-	-	-
	AB	15±0.121	12±0.053	8±0.043	44±0.043	34±0.119	26±0.125
1	5a	19±0.023	15±0.092	18±0.120	50±0.163	47±0.102	34±0.127
2	5b	31±0.129	23±0.120	19±0.124	52±0.120	45±0.134	33±0.178
3	5c	26±0.031	24±0.135	20±0.132	53±0.172	46±0.118	35±0.325
4	5d	23±0.051	21±0.171	18±0.141	50±0.153	47±0.121	38±0.120
5	5e	22±0.021	19±0.093	18±0.021	53±0.120	42±0.120	32±0.321
6	5f	23±0.134	16±0.122	15±0.031*	52±0.052	37±0.132	31±0.081
7	5g	21±0.028	24±0.120	20±0.154	52±0.051	46±0.065	59±0.031
8	5h	18±0.132	18±0.053	16±0.120	54±0.053	42±0.098	30±0.101
9	5i	25±0.032	19±0.024	19±0.032	52±0.121	47±0.120	35±0.162
10	5j	24±0.053	20±0.120	17±0.120	53±0.122	45±0.125	37±0.154
11	5k	28±0.125	21±0.065	14±0.054	50±0.187	46±0.145	39±0.131
12	5l	23±0.043	23±0.125	15±0.132	51±0.061	44±0.132	34±0.124
13	5m	19±0.063	19±0.122	15±0.120	47±0.163	37±0.192	32±0.120
14	5n	18±0.063	15±0.043*	10±0.131*	46±0.120*	37±0.120	29±0.234
15	50	17±0.145	14±0.043*	11±0.132	48±0.121*	38±0.165	28±0.097

All the results were shown in table and expressed as a mean±SEM of six worms in each group, BDIM: Benzothiazole derivatives containing indole moieties, SEM: Standard error of mean, AB: Albendazole

Table 2: Physical data of compounds 4a-4i

S. Code	M. for.	R	R_1	M. Wt.	M. P (°C)	% Yield	R_f value
4a	$C_{16}H_{12}N_2O_2$	H	H	264.09	137–139	69	0.73
4b	$C_{16}H_{14}N_2O_2$	Cl	H	298	181–183	72	0.82
4c	$C_{17}H_{14}N_2O_2$	H	CH_3	278	193–195	68	0.63
4d	$C_{17}H_{13}N_2O_2Cl$	Cl	H	312	153–155	75	0.59
4e	$C_{17}H_{14}N_2O_2$	CH_3	H	278	186–188	82	0.73
4f	$C_{18}H_{16}N_2O_2$	CH_3	CH_3	292	187–189	80	0.72
4g	$C_{16}H_{11}N_3O_4$	4-NO_2	H	309	225–227	76	0.57
4h	$C_{17}H_{13}N_3O_4$	3-NO_2	CH_3	323	183–185	74	0.63
4i	$C_{16}H_{11}N_2O_2Cl$	H	Cl	298	167–169	69	0.57

S. Code: Sample code, M. for.: Molecular formula, M. wt.: Molecular weight, M. P: Melting point

Table 3: Physical data of compounds 5a-5o

S. Code	M. for.	R	R_1	R_2	R_3	M. Wt.	M. P	% Yield	R_fV
5a	$C_{23}H_{16}N_4OS$	H	H	H	H	396	219–221	78	0.63
5b	$C_{23}H_{15}N_4OSCl$	H	H	Cl	H	430	213–215	82	0.76
5c	$C_{24}H_{18}N_4OS$	H	H	H	CH_3	410	203–205	77	0.57
5d	$C_{24}H_{17}N_4OSCl$	H	H	Cl	CH_3	444	231–233	66	0.61
5e	$C_{24}H_{18}N_4OS$	H	H	CH_3	H	410	191–193	67	0.53
5f	$C_{25}H_{20}N_4OS$	H	H	CH_3	CH_3	424	217–219	74	0.81
5g	$C_{23}H_{15}N_5O_3S$,	H	H	H	NO_2	441	251–253	76	0.67
5h	$C_{24}H_{17}N_5O_3S$,	H	H	NO_2	CH_3	455	231–233	81	0.51
5i	$C_{23}H_{15}N_4OSCl$	H	H	H	Cl	430	187–189	78	0.83
5j	$C_{23}H_{14}N_4OSClF$	Cl	F	H	H	448	231–233	80	0.59
5k	$C_{24}H_{16}N_4OSClF$	Cl	F	CH_3	H	462	193–195	78	0.75
5l	$C_{25}H_{18}N_4OS$	Cl	F	CH_3	CH_3	476	215–217	78	0.84
5m	$C_{23}H_{13}N_4OSFCl_2$	Cl	F	H	Cl	482	263–265	81	0.66
5n	$C_{23}H_{13}N_5SClF$	Cl	F	NO_2	H	493	243–245	68	0.71
5o	$C_{23}H_{13}N_4OSCl_2F$	Cl	F	Cl	H	482	225–227	76	0.77

S. Code: Sample code, M. for.: Molecular formula, M. wt.: Molecular weight, M. P: Melting point, R_fV: $R_f$value

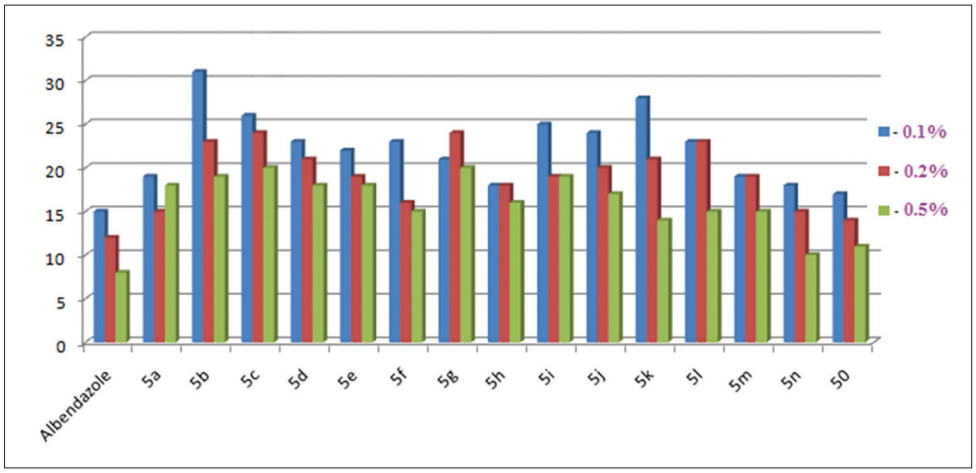

Fig. 2: Graphical representation of anthelmintic activity of compounds (5a-50) - Paralysis time (min)

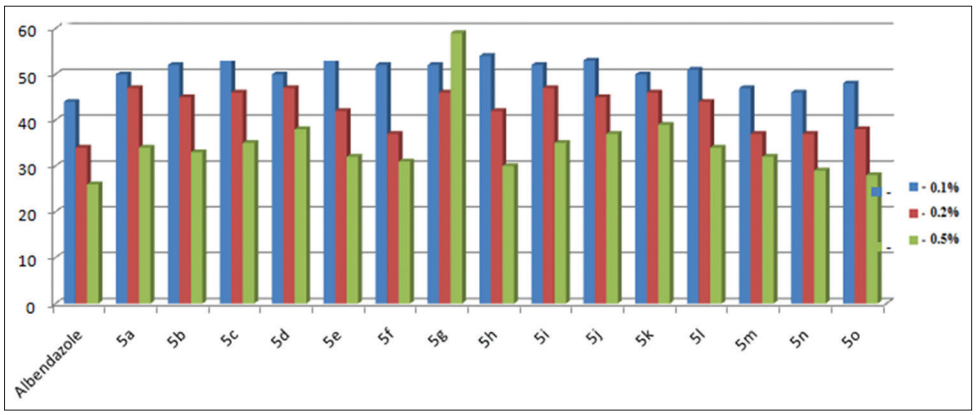

Fig. 3: Graphical representation of anthelmintic activity of compounds (5a-5o) - Death time (min)

benzothiazole (2-ABT). The substituted ISBs undergo acetylation and react with 2-ABT to give novel BDIM. The physical data results are shown in Tables 2 and 3. The synthesized compounds were screened for AA. The structures of all the newly synthesized compounds were characterized as 5a-5o on the basis of satisfactory analytical and spectral data including IR, LC-Mass, and ^{1}H NMR data.

AA

The synthesized compounds (5a-5o) were evaluated for AA on Indian earthworms (*Pheretima posthuma*). All compounds showed that AA is shown in Table 1. A closer inspiration of data from this table indicated that the synthesized compounds **5f, 5n,** and **5o** showed good AA, whereas others showed significant activities. After all, the synthesized compounds in overall estimation confirm the better activity against *P. posthuma.* The results are shown in Figs. 2 and 3.

The statistical analyses were carried out using one-way analysis of variance (Dunnett's test) at a 95% confidence interval, and all the activity data on comparison with vehicle control reach statistical significance with $p<0.05$ (Fig. 4).

Fig.4: Photographs of various benzothiazole derivatives - Anthelmintic activities

CONCLUSION

The present study highlights the importance of benzothiazole derivatives having various heterocyclic moiety features responsible for the AA and may serve as a lead molecule for further modification to obtain clinically useful novel entities. The AA of all the synthesized compounds showed moderate activity.

AUTHORS' CONTRIBUTIONS

The corresponding author has done all the work, interpreted the data, and written the manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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