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Original Article

Evaluation of *in vitro* anthelmintic activities of novel 1,2,3 – benzotriazole derivatives synthesized in ultrasonic and solvent free conditions

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ABSTRACT

Objectives: To determine anthelmintic activity of the synthesized novel 1,2,3 – benzotriazole derivatives under ultrasonication in solvent free conditions.

Methods: Newer “1–(1H–benzo[d][1,2,3]triazole-1-carbonyl) derivatives” (5A–5P) were synthesized by using “1H–benzo[d][1,2,3]triazole” (1) as the starting material under ultrasonicated and solvent-free conditions. The resulting products were isolated and characterized by melting points and spectral studies. All the products were assayed for anthelmintic activity against *Pheretima posthuma* using albendazole and mebendazole as reference compounds.

Results: All the newer 1,2,3 – benzotriazole derivatives synthesized by ultrasound activation in solvent–free condition were obtained in moderate to good yields in the range of 71–82%. The data interpretation of the spectral values with reference to standard values confirmed the structures of the synthesized compounds. Out of the sixteen synthesized derivatives, four compounds (5B, 5F, 5J and 5N) showed anthelmintic activity in dose-dependent manner giving shortest time of paralysis and death with different concentrations of the derivatives. Among these four derivatives, 5J showed superior activity.

Conclusion: Out of the sixteen synthesized derivatives, four compounds (5B, 5F, 5J and 5N) containing *p*-nitrophenyl substituent attached to azo group of benzotriazole moieties exhibited equal or comparable anthelmintic activity with reference to albendazole. The superior activity of compound 5J might be due to attachment of additional *p*-nitrophenyl substituent to the cyano group.

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1. Introduction

The heterocyclic system containing benzotriazole moieties system is of wide interest because of their diverse biological

activities^{1,2} including anticonvulsant and anti-inflammatory activities,³ diuretic,⁴ analgesic,⁵ pesticidal.⁶ Recent publications reported synthetic protocols in solvent-less conditions^{7–9} and in presence of ultrasonic radiation.^{10–13} Anthelmintic

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infections are now being recognized as cause of much chronic ill health amongst the tropical people. More than half of population in the world suffers from worm infection of one or the other.¹⁴ Hence, newer 1,2,3 – benzotriazole derivatives were synthesized in ultrasonic and solvent-free conditions and their anthelmintic activities were studied.

2. Materials and methods

All organic solvents and chemicals were of analytical grade. Albendazole (Bandy Mankind Pharma Ltd., New Delhi) and Mebendazole (Mansukhlal Tribhovandas & Company, Mumbai) were used for anthelmintic activities.

For synthesis of benzotriazole derivatives, a 12 mm wide and 140 mm long probe (of an UP 400S ultrasonic processor) was immersed directly into the reaction mixture at room temperature. The operating frequency and the output power were 24 kHz and 240 W respectively. The synthesized compounds were characterized by spectral studies using Perkin Elmer 1600 series Fourier transformer-infrared spectrophotometer in KBr-pellet method; ¹H NMR, Bruker 400 MHz NMR spectrometer (Bruker Bioscience, Billerica, MA, USA) in MeOD using TMS as internal standard.

2.1. Scheme for the synthesis of the compounds

After suitable modifications to the classical synthesis carried out by other workers,¹⁵⁻¹⁷ sixteen new benzotriazole derivatives were synthesized under green conditions (viz., ultrasonication and solvent free conditions) by the addition of diazotization step (Fig. 1).

2.2. Methodology for in vitro anthelmintic activity

In vitro anthelmintic activity for the synthesized compounds was studied with minor modifications to the standard

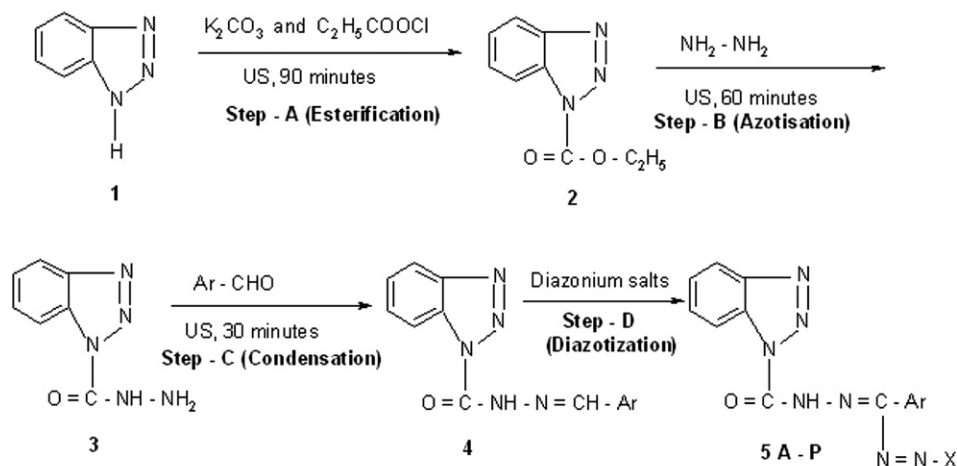
method.¹⁸ *Pheretima posthuma* (earthworm) obtained from Agricultural Department, Guntur, India, of nearly equal size (length: 9 ± 1.5 cm and width: 0.1–0.2 cm). Solutions of the all compounds and control drugs (albendazole and mebendazole) were prepared freshly. The drugs and synthesized compounds were dissolved in minimum quantity of DMF and adjusted to 15 ml volume with Tween-80 (3%) in normal saline. The test concentrations (1, 2.5 and 5% w/v) were taken in petri dishes (4 inches).

A group of six earthworms were released in to each of 15 ml of control drugs and the test suspensions (1, 2.5 and 5% w/v each). Observations were made for the time taken to paralysis and death of individual worms up to 4 h of the test period. Each petri dish was placed with 6 worms and observed for paralysis (or) death. The mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously. The death time of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli. Death was concluded when the worms lost their motility followed with fading away of their body colors.

3. Results

All the newer 1,2,3-benzotriazole derivatives synthesized by ultrasound activation in solvent-free condition were obtained in moderate to good yields in the range of 71–82%. The synthesized derivatives were characterized by FTIR and ¹H NMR values measured in cm^{-1} and δ (ppm) respectively. The data was interpreted with reference to standard values^{19,20} and given in Table 1 for some of the synthesized compounds.

All synthesized compounds were tested for anthelmintic activity and compared with the standard anthelmintic substances i.e., mebendazole and albendazole under the same conditions. The time taken for complete paralysis and death are reported in Table 2.



5A – D: Ar = C₆H₅; 5E – H: Ar = C₄H₃O; 5I – L: Ar = C₆H₄NO₂ and 5M – P: Ar = C₆H₄Cl

5A, E, I, M: X = C₆H₅; 5B, F, J, N: X = C₆H₄NO₂; 5C, G, K, O: X = C₆H₄Cl and 5D, H, L, P: X = C₆H₄Br

Note: US = Ultrasonication

Fig. 1 – Scheme of synthesis.

Table 1 – Spectral characterization of some of the synthesized compounds.

Compound code	Compound name	IR bands (in cm^{-1})	^1H NMR peaks δ (in ppm)
5A	1-(1H-benzo[d][1, 2, 3] triazole-1-carbonyl)-3, 5-diphenylformazan	1696.66 (Ar C=C, stretch); 1603.37 (N=N, stretch), 1542.28 (N-H, stretch), 1256.37 (Aryl C-N, stretch), 1007.57 (Aniline C-N, stretch) and 737.28 (CHO – deformation).	7.0 (1H, s, N-H), 7.06 (1H, d, C-H), 7.33 (2H, d, C-H), 7.40 (2H, d, C-H), 7.45–7.48 (2H, m, C-H), 7.56–7.59 (3H, m, C-H), 7.83 (2H, d, C-H) and 7.96 (2H, d, C-H).
5B	1-(1H-benzo [d][1, 2, 3] triazole-1-carbonyl)-5-(4-nitrophenyl)-3-phenyl formazan	1698.47 (Ar C=C, stretch); 1593.99 (N=N, stretch), 1495.50 (Ar-NO ₂ , stretch), 1301.09 (Aryl C-N, stretch), 1206.78 (Aniline C-N, stretch), 843.07 (p – disubstitution, deformation) and 739.50 (CHO – deformation).	7.0 (1H, s, N-H), 7.18 (2H, d, C-H), 7.40 (2H, d, C-H), 7.52–7.59 (3H, m, C-H), 7.83 (2H, d, C-H), 7.96 (2H, d, C-H) and 8.10 (2H, d, C-H).
5G	1-(1H-benzo[d][1, 2, 3]triazole-1-carbonyl)-5-(4-chlorophenyl)-3-(furan-2-yl) formazan	3649.31 (Amide-CONH, stretch), 1594.63 (N=N, stretch), 1485.68 (Furan Ring, C=C, stretch), 1206.43 (Aniline C-N, stretch), 1007.96 (C-O-C, stretch), 820.27 (p – disubstitution, stretch), 740.62 (CHO deformation) and 539.68 (C – Cl, deformation)	6.52 (1H, m, Furan C-H), 6.54 (1H, d, Furan C-H), 7.0 (1H, s, N-H), 7.27 (2H, d, C-H), 7.40 (2H, d, C-H), 7.49 (2H, d, C-H), 7.75 (1H, d, Furan C-H) and 7.96 (2H, d, C-H)
5H	1-(1H-benzo[d][1, 2, 3] triazole-1-carbonyl)-5-(4-bromophenyl)-3-(furan-2-yl) formazan	3652.10 (Amide-CONH, stretch), 1722.17 (Furan Ring, stretch), 1622.31 (N=N, stretch), 1511.18 (Ar C=C, stretch), 1457.07 (Furan Ring C=C, stretch), 1202.93 (Aniline C-N, stretch), 1005.36 (C-O-C, stretch), 875.16 (p – disubstitution, deformation), 773.52 (CHO deformation) and 515.35 (C-Br, deformation).	6.52 (1H, m, Furan C-H), 6.54 (1H, d, Furan C-H), 7.0 (1H, s, N-H), 7.22 (2H, d, C-H), 7.40 (2H, d, C-H), 7.75 (1H, d, Furan C-H), 7.76 (2H, d, C-H) and 7.96 (2H, d, C-H).
5M	1-(1H-benzo[d][1, 2, 3] triazole-1-carbonyl)-3-(4-chlorophenyl)-5-phenyl formazan	3650.62 (Amide-CONH), 1706.94 (Ar, C=C, stretch), 1593.76 (N=N, stretch), 1513.71 (N-H, stretch), 1264.35 (Aryl C-N, stretch), 1204.85 (Aniline C-N, stretch), 820.56 (p – disubstitution, stretch), 772.75 (CHO – deformation) and 605.30 (C-Cl, deformation).	7.0 (1H, s, N-H), 7.06 (1H, d, C-H), 7.33 (2H, d, C-H), 7.40 (2H, d, C-H), 7.45 (2H, d, C-H), 7.52 (2H, d, C-H), 7.77 (2H, d, C-H) and 7.96 (2H, d, C-H).
5O	1-(1H-benzo[d][1, 2, 3] triazole-1-carbonyl)-3,5-bis(4-chloro-phenyl) formazan	1710.45 (Ar, C=C, stretch), 1593.50 (N=N, stretch), 1486.45 (N-H, stretch), 1256.86 (Aryl C-N, stretch), 1143.95 (Aniline C-N, stretch), 827.70 (p – disubstitution, deformation), 773.01 (CHO – deformation) and 620.81 (C-Cl, deformation)	7.0 (1H, s, N-H), 7.27 (2H, d, C-H), 7.40 (2H, d, C-H), 7.49 (2H, d, C-H), 7.52 (2H, d, C-H), 7.77 (2H, d, C-H) and 7.96 (2H, d, C-H).

Table 2 – Anthelmintic activity of 1,2,3 – benzotriazole derivatives.

S. no.	Compound code	Concentration (% w/v)	Time taken in minutes (\pm SD)	
			Paralysis (P)	Death (D)
1	5A	1	26.12 \pm 0.52	75.12 \pm 0.46
		2.5	20.32 \pm 0.29	68.23 \pm 0.33
		5	15.23 \pm 0.30	61.22 \pm 0.38
2	5B	1	16.22 \pm 0.14	38.55 \pm 0.23
		2.5	14.23 \pm 0.14	30.21 \pm 0.29
		5	11.28 \pm 0.20	24.29 \pm 0.21
3	5C	1	19.44 \pm 0.35	69.23 \pm 0.43
		2.5	18.18 \pm 0.23	61.17 \pm 0.12
		5	17.35 \pm 0.41	52.34 \pm 0.19
4	5D	1	18.12 \pm 0.33	67.33 \pm 0.51
		2.5	17.48 \pm 0.41	59.27 \pm 0.18
		5	16.45 \pm 0.40	52.40 \pm 0.12
5	5E	1	26.36 \pm 0.36	66.22 \pm 0.32
		2.5	21.45 \pm 0.29	56.22 \pm 0.28
		5	17.56 \pm 0.33	48.23 \pm 0.39
6	5F	1	15.45 \pm 0.41	39.12 \pm 0.18
		2.5	14.11 \pm 0.29	31.21 \pm 0.20
		5	11.44 \pm 0.12	24.39 \pm 0.19
7	5G	1	18.38 \pm 0.33	75.43 \pm 0.58
		2.5	17.41 \pm 0.23	63.28 \pm 0.26
		5	16.32 \pm 0.13	53.38 \pm 0.21
8	5H	1	18.11 \pm 0.21	70.13 \pm 0.23
		2.5	17.31 \pm 0.11	62.48 \pm 0.16
		5	16.12 \pm 0.17	55.38 \pm 0.11
9	5I	1	25.52 \pm 1.36	45.45 \pm 1.20
		2.5	20.35 \pm 0.37	39.33 \pm 0.33
		5	15.23 \pm 0.40	33.41 \pm 0.29
10	5J	1	15.25 \pm 0.14	36.12 \pm 0.12
		2.5	13.11 \pm 0.19	30.21 \pm 0.26
		5	10.44 \pm 0.12	23.39 \pm 0.11
11	5K	1	18.21 \pm 0.29	69.21 \pm 0.63
		2.5	16.11 \pm 0.23	58.46 \pm 0.19
		5	14.39 \pm 0.27	51.23 \pm 0.28
12	5L	1	19.40 \pm 0.31	68.23 \pm 0.31
		2.5	18.22 \pm 0.12	62.17 \pm 0.18
		5	17.39 \pm 0.18	56.34 \pm 0.23
13	5M	1	27.22 \pm 0.42	66.23 \pm 0.33
		2.5	21.35 \pm 0.28	59.46 \pm 0.39
		5	19.45 \pm 0.33	49.38 \pm 0.37
14	5N	1	16.52 \pm 0.17	36.55 \pm 0.20
		2.5	14.38 \pm 0.18	29.21 \pm 0.26
		5	11.29 \pm 0.28	20.29 \pm 0.11
15	5O	1	19.18 \pm 0.36	66.21 \pm 0.32
		2.5	17.12 \pm 0.23	58.38 \pm 0.27
		5	15.32 \pm 0.19	49.28 \pm 0.13
16	5P	1	18.21 \pm 0.17	68.11 \pm 0.21
		2.5	17.29 \pm 0.13	59.28 \pm 0.14
		5	15.28 \pm 0.21	50.39 \pm 0.32
17	Albendazole	1	15.21 \pm 0.14	34.55 \pm 0.23
		2.5	13.08 \pm 0.24	28.21 \pm 0.18
		5	10.23 \pm 0.19	20.29 \pm 0.21
18	Mebendazole	1	9.11 \pm 0.18	28.17 \pm 0.11
		2.5	7.56 \pm 0.14	21.56 \pm 0.28
		5	5.23 \pm 0.39	17.32 \pm 0.19

Note: results are expressed as mean \pm SD of six determinations; control worms were alive up to 4 h of observation.

4. Discussion

Newer 1,2,3 – benzotriazole derivatives were synthesized by following green procedure under ultrasonic and solvent free

conditions and characterized by spectral studies. All the synthesized compounds were tested for anthelmintic activity against adult earthworms (*P. posthuma*) due to their anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.^{21,22} Albendazole, one of the

reference compound in the present study is effective in a broad range of helminth infections, including round worms, hookworms, whipworms, pinworms and its mechanism of action involves inhibition of the glucose uptake system leading to a lethal depletion of energy reserves in the helminthes.²³ Another reference compound, mebendazole binds to the worm's microtubular-protein 'β-tubulin' and inhibits its polymerization by blocking glucose uptake in the parasite^{24–27} and also exhibits potent antitumor property both *in vitro* and *in vivo*.²⁸

From the observations made in the present study, higher concentration of the synthesized derivatives exhibited paralytic effect much earlier and the time to death was shorter for worms. Out of the sixteen synthesized derivatives, four compounds (5B, 5F, 5J and 5N) showed anthelmintic activity in dose-dependent manner giving shortest time of paralysis (P) and death (D) with all three concentrations of the derivatives. These four compounds contain *p*-nitrophenyl substituent attached to azo group of benzotriazole moieties and hence, displayed equal or comparable anthelmintic activity with reference to albendazole. Even earlier, best anthelmintic activity was reported for *p*-nitrophenyl substituted benzotriazoles like N¹-(*p*-nitrophenyl) aminomethylene benzotriazole by Pawar.²⁹ Among these four derivatives (5B, 5F, 5J and 5N), 5J showed superior activity which might be due to attachment of additional *p*-nitrophenyl substituent to the cyano group.

Though, mebendazole was found to be effective compared to albendazole against the selected worms for the present study (Table 2), for mass treatment of multiple infections with *Ascaris*, hookworm, and *Trichuris*, albendazole was the preferred benzimidazole derivative from the comparative efficacy study of albendazole and mebendazole carried out in Pattani Province–Thailand by Jongsuksuntigul.³⁰ As the four synthesized compounds showed comparable anthelmintic activity to albendazole, these compounds may also be tested for multiple infections.

Better anthelmintic activity of the four compounds (5B, 5F, 5J and 5N) can be attributed to the *p*-nitrophenyl substituent attached to azo group of benzotriazole moieties. Superior activity of 5J might be due to attachment of additional *p*-nitrophenyl substituent to the cyano group. As the four synthesized compounds showed comparable anthelmintic activity to albendazole, these compounds may also be tested for multiple infections. Finally in conclusion, out of the sixteen 1,2,3 – benzotriazole derivatives, compounds with nitro substituents have shown best anthelmintic activities against *P. posthuma*.

Conflicts of interest

All authors have none to declare.

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