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SYNTHESIS AND ANTHELMINTIC ACTIVITY STUDY OF BENZIMIDAZOLE DERIVATIVES

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ABSTACT

The benzimidazole nucleus has a significant importance in medicinal chemistry and many benzimidazole containing compounds exhibit important biological activities. In the present study, synthesis, spectral studies and biological evaluation of three benzimidazole derivatives were investigated. The structures of the synthesized compounds were characterized by IR, ^{1}H-NMR, ^{13}C-NMR, Mass spectroscopy and CHN elemental analyzer and the synthesized compounds were screened for anthelmintic activity by using Indian earthworms, *Pheretima posthuma*. Albendazole 25mg/ml had taken as a standard drug for the experiment. 25, 50 and 100mg/ml concentrations of the three synthesized compounds (1a – 1c) had taken as tests sample.

KEYWORDS: Albendazole, Anthelmintic, benzimidazole, Spectroscopy, *Pheretima posthuma*.

INTRODUCTION

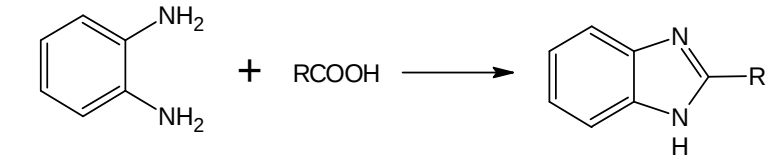
The benzimidazole ring system is an important pharmacophore in medicinal chemistry and modern drug discovery. Compound bearing benzimidazole nucleus have been of great interest to synthetic and medicinal chemists from a long time due to their unique chemical and biological properties mainly related to traditional anthelmintics like Albendazole and Oxibendazole. Albendazole, a benzimidazole carbamate (methyl-5-propylthio-1Hbenzimidazole-2-yl carbamate) with extensive clinical use as an anthelmintic drug can also inhibit hepatocellular carcinoma cell proliferation under both *in-vitro* and *in-vivo* experimental conditions.[1-2]

Benzimidazole derivatives have also been found to possess biological activities such as antiviral, antibacterial and anticancer.[2] Human and animal diseases caused by helminthes parasites have great impact on public health. Toxocariasis is an infection caused by the nematode Toxocara commonly found in the intestines of puppies and older dogs (*Toxocara canis*) and cats (*Toxocara cati*). Humans become infected either by ingesting embryonated eggs accidentally or eating contaminated food with soil containing the eggs (such as unwashed raw vegetables). In an infected person the worms can remain encysted in tissue so infection can persist for years. Treatment with Praziquantel or Albendazole is recommended alternative to these drugs are now being sought.[3] The continuous and longterm reliance on a small range of compounds has led to the development of drug resistance in many helminthic strains. In addition, after treatment with Albendazole or Mebendazole several side effects have been reported in hosts such as gastrointestinal symptoms (epigastric pain, diarrhea, nausea, vomiting); nervous system symptoms (headache, dizziness) and allergic phenomena (edema, rashes, urticaria). Some anthelmintic drugs such as Praziquantel and Albendazole are contraindicated for certain groups of patients like pregnant and lactating woman. The global burden of both, human and domestic animal parasitic diseases coupled with the emergence of drug resistance has made the development of new chemotherapy a critical need.[4]

Synthetic Study

Present study was undertaken to synthesize three novel 2-substituted benzimidazole derivatives and investigate their Anthelmintic activity. Target compounds were obtained by reacting O-phenylenediamine with three various carboxylic acid derivatives within the temperature range of 90-100^{o}C (1a – 1c).[5-6] The structures of the obtained compounds were elucidated by spectral data. Significant stretching bands in the FT/IR spectra were observed at expected regions. All of the aromatic and aliphatic protons in the 300 MHz ^{1}H NMR spectra were also recorded at estimated areas. Synthesis procedure of the 2-subsituted benzimidazole derivatives is outlined in Scheme 1. The synthesized compounds

were identified and characterized by using different physical, chemical and analytical tests. After characterization and identification the compounds were subjected to evaluate the Anthelmintic activity study by using Indian earthworms (*Pheretima posthuma*).



O-phenylenediamine Benzimidazole derivatives

R ➢ a = CH_3, b = $CH_2C_6H_5$ & c = C_6H_5

Scheme 1: Schematic synthesis of compounds (1a – 1c).

Biological Study

Indian adult earthworms (*Pheretima posthuma*) were used to study anthelmintic activity. The earthworm resembles both anatomically and physiologically to the intestinal roundworm parasites of the human beings, hence can be used to study anthelmintic activity. Albendazole as standard drug was procured from Sigma Aldrich for anthelmintic studies. All the test solutions (newly synthesized compounds) and standard drug solution (Albendazole) were prepared freshly before starting the experiment. All the test compounds were relatively insoluble in water. They were soluble in organic solvent. 1% CMC solution was prepared by using normal saline with various concentrations.[7]

MATERIALS AND METHODS

A. Synthesis And Characterization

Synthesis and characterization of different benzimidazole derivatives

Synthesis of Compound 1a (2-Methyl-1*H*-benzimidazole)

5.43gm (0.03mole) of o-phenylenediamine, 20ml of water and 5.4gm (0.09mole) of acetic acid were taken in a 100ml round bottom flaks. A reflux condenser was attached to the round bottle flaks. The mixture was heated in a water bath for 45 min at 100°C. The reaction mixture was slowly cooled and basified with aqueous sodium hydroxide. The solid compound was obtained at the bottom. The solid compound was filtered through the Buchner funnel and recrystallized from 10% aqueous ethanol. The product was dried and kept for further uses. Colour: White powder, Melting Point: 177-180°C, Yield: 89.8%, IR(KBr, cm^{-1}): 3295.16(N-H), 1500.15(C=C), 1593.09(C=N), 752.19(C-H), 1248.82(C-N), 1156.25(CH_3), ^{1}H NMR ($CDCl_3$, δ/ppm): 7.22 (m,2H), 7.48 (m, 5H), 7.58 (s, 1 H), 8.04 (dd, 2H); ^{13}C-NMR ($CDCl_3$, δ/ppm): 111.6 (benzimidazole, CH=), 122.1 (benzimidazole, CH=), 135.4 (benzimidazole, C), 149.3 (N-C=N), 134.3 (phenyl, C), 129.4 (phenyl, CH=); Anal. Calcd. for $C_8H_8N_2$: C, 80.33; H, 5.15; N, 14.42 %. Found: C, 80.12; H, 5.08; N, 14.22%; MS, *m/z*: 195 $[M+H]^+$.

Synthesis of Compound 1b (2-Benzyl-1*H*-benzimidazole)

5.43gm (0.03mole) of o-phenylenediamine, 20ml of water and 12.3gm (0.09mole) of phenyl acetic acid were taken in a 100ml round bottom flaks. A reflux condenser was attached to the round bottle flaks. The mixture was heated in a water bath for 45 min at 100°C. The reaction mixture was slowly cooled and basified with aqueous sodium hydroxide. The solid compound was obtained at the bottom. The solid compound was filtered through the Buchner funnel and recrystallized from 40% aqueous ethanol. The product was dried and kept for further uses. Colour: White to beige-grey powder, Melting Point: 235–2236°C, Yield: 86.1%, IR (KBr, cm^{-1}): 3416.66(N-H), 1486.05(C=C), 1645.17(C=N), 1185.18(C-N), 857.30(Ar-H), 2884.35(CH_3), ^{1}H NMR (DMSO-d_6, δ/ppm): 8.16–8.22 (m, 2H), 7.21–7.73 (m, 6H), 4.60 (d, 1H);[13] C-NMR ($CDCl_3$, δ/ppm): 111.6 (benzimidazole, CH=), 120.2 (benzimidazole, CH=), 137.8 (benzimidazole, C), 144.1 (N-C=N), 128.4 (phenyl, C), 127.8, 117.8 (phenyl, CH=); Anal. Calcd. for $C_{14}H_{12}N_2$: C, 74.28; H, 4.76; N, 13.33 %. Found: C, 74.01; H, 4.65; N, 13.18%; MS, *m/z*: 211 $[M+H]^+$.

Synthesis of Compound 1c (2-Phenyl-1*H*-benzimidazole)

6.0gm of o-phenylenediamine, 6gm of benzoic acid and 25ml of 4N dilute hydrochloric acid were taken in a 100ml round bottom flaks. A reflux condenser was attached to the round bottle flaks. The mixture was heated in a water bath for 2hrs at 100°C. The reaction mixture was slowly cooled. The solid compound was obtained at the bottom. The solid compound was filtered through the Buchner funnel and recrystallized from boiled water using charcoal. The product was dried and kept for further uses. Colour: Whitish-grey powder, Melting Point: 238-239°C, Yield: 76.8%, IR (KBr.cm^{-1}): 3062.75(N-H), 1454.23(C=C), 1584.41(C=N), 1128.28(C-N), 934.45(C-H), ^{1}H NMR (DMSO-d_6, δ/ppm): 8.00–8.08 (m, 2H), 7.20–7.60 (m, 6H); ^{13}C-NMR ($CDCl_3$, δ/ppm): 112.2 (benzimidazole, CH=), 119.2 (benzimidazole, CH=), 138.6 (benzimidazole, C), 143.5 (N-C=N), 150.1 (phenyl, NO_2), 144.4 (phenyl, C), 125.8, 128.6 (phenyl, CH=); Anal. Calcd. for $C_{13}H_{10}O_2$: C, 65.27; H, 3.76; N, 17.57 %. Found: C, 65.14; H, 3.69; N, 17.43%; MS, *m/z*: 240 $[M+H]^+$.

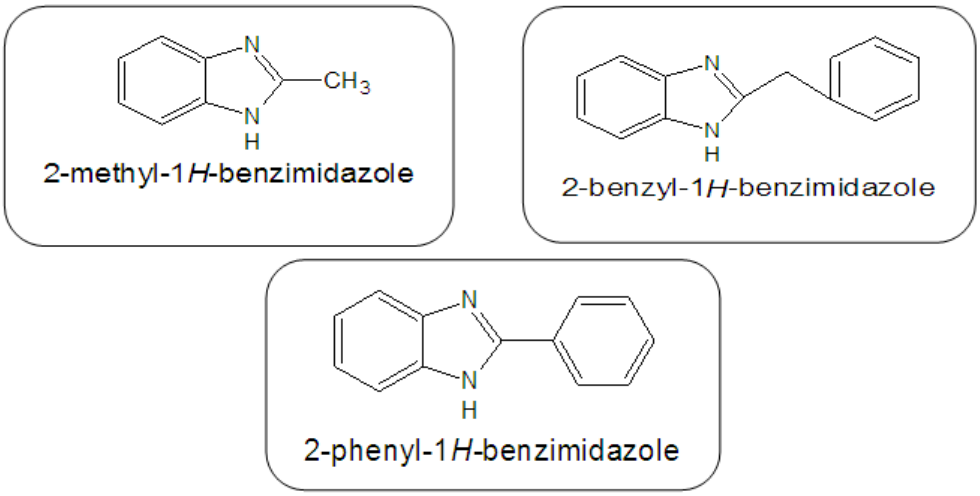


Fig 1: Structures of synthesized compounds (1a – 1c).

B. Biological Evaluation

Anthelmintic Activity Study of Compounds (1a & 1b)

All the chemicals and solvents used for this work were obtained from E. Merck Ltd., Mumbai and Loba Chemie Pvt. Ltd. The reagents were of analytical grade purity. Melting points of the synthesized compounds were determined in open capillary tubes using ROLEX melting point apparatus expressed in degree centigrade and were uncorrected.

Synthesis of compounds

Three benzimidazole derivatives **(1a – 1c)** were synthesized from o-phenylenediamine treated with three different carboxylic acids derivatives by refluxing with a range of temperature of 90-100^{o}C. For the first two synthesis 45mins refluxing were continued where as for third synthesis 2hrs refluxing was done. The precipitates were filtered, washed, dried and recrystallized. Then the products were used for Anthelmintic activity studies.

Anthelmintic activity

The Anthelmintic activity was evaluated on adult Indian earth worm *Pheretima posthuma* due to its anatomical resemblance with the intestinal roundworm parasites of human beings. The activity was carried out using Ajaiyeoba *et al* method [7]. Five groups of Indian earth worms each containing six earthworms approximately of equal size was used for the study. Each group of earth worms were treated with vehicle (1% CMC), three different synthesized compounds 1a, 1b & 1c (25, 50 and 100mg/ml conc.) and Albendazole (25mg/ml). Observations were made for the time taken for paralysis and death of individual worms **(Table 1)**. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lost their motility, followed with fading away of their body color.

RESULTS AND DISCUSSION

Table 1: Anthelmintic potency of synthesized Benzimidazole derivatives (1a – 1c).

Compounds	Concentration	*Pheretima posthuma*	
		Paralysis (P)	Death (D)
Control	-	-	-
Compound 1a	25mg/ml	74.35 ± 2.805	125.83 ± 5.23
	50mg/ml	51.34 ± 1.24	112.33 ± 5.87
	100mg/ml	37.86 ± 3.03	76.21 ± 1.751
Compound 1b	25mg/ml	85.32 ± 3.212	150.11 ± 2.929
	50mg/ml	69.21 ± 3.981	142.45 ± 8.91
	100mg/ml	50.32 ± 4.156	113.02 ± 3.39
Compound 1c	25mg/ml	78.16 ± 3.656	146.0 ± 2.828
	50mg/ml	62.33 ± 4.131	137.5 ± 9.75
	100mg/ml	44.01 ± 4.382	96.66 ± 3.26
Standard (Albendazole)	25mg/ml	55.66 ± 4.59	124.83 ± 6.99

All Values represent Mean± SD; n=6 in each group. Comparisons made between standard versus treated groups, P<0.05 was considered significant

Three different Benzimidazole derivatives were synthesized. For synthesis of first compound o-phenylenediamine treated with acetic and refluxed for 45mins in reflux condenser. The reaction mixtures were cooled, filtered and washed. The product was recrystallized and kept in dried condition for further uses.

For synthesis of second compound o-phenylenediamine treated with phenyl acetic acid and refluxed for 45mins in reflux condenser. The reaction mixtures were cooled, filtered and washed. The product was recrystallized and kept in dried condition for further uses.

For synthesis of third compound o-phenylenediamine treated with benzoic acid and refluxed for 2hrs in reflux condenser. The reaction mixtures were cooled and poured into crushed ice. The products were filtered and washed. The product was recrystallized and kept in dried condition for further uses.

It is the most important part that the compounds had been synthesized to be characterized by using physical, chemical and analytical parameters. The synthesized compounds were undergone through some physical parameters like Color, Yield percentage, Melting point etc. The synthesized compounds were then undergone various analytical study like, FTIR, ^{1}H-NMR, ^{13}C-NMR, Mass spectroscopy etc to confirm the presence of different functional groups, molecular weight and finally the structures. The synthesized compounds were found to be as 2-Methyl-1*H*-Benzimidazole, 2-Benzyl-1*H*-Benzimidazole and 2-Phenyl-1*H*-benzimidazole

All the three synthesized compounds showed significant Anthelmintic activity. Among these three synthesized compounds; **compound 1a** with concentration of 100mg/ml had shown potential Anthelmintic activity for paralysis and death respectively when compared with all other compounds with different concentrations. The overall results had confirmed that the paralysis and the death of the earth worms are dose dependent, where in increasing in dose the paralysis and death is taking less time.

CONCLUSION

The present study synthesis and characterization of Benzimidazole derivative from o-phenylenediamine had carried out by one way synthetic procedure, and then physical characterization had been evaluated by using different physical, chemical and analytical methods. The biological evaluation, Anthelmintic activity study had been carried out by using the earth worms and Albendazole is used as standard drug.

The studies had confirmed the identification of three different Benzimidazole derivatives and also had shown a significant Anthelmintic activity which is dose dependent.

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