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Research Article

SYNTHESIS AND EVALUATION OF NOVEL ANTHELMENTIC BENZIMIDAZOLE DERIVATIVES

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ABSTRACT

2-substituted benzimidazole derivatives were synthesized, purified and characterized by means of TLC, Melting point, IR, ^{1}H NMR, Mass spectral analysis respectively. The study aimed at screening synthetic compound for anthelmintic activity. The anthelmintic activity of 2-substituted benzimidazole (**2a-2d**) compounds was evaluated for mean paralysis and mean death time.

Keywords: Albendazole, Anthelmentic, benzimidazole, Helminthes

INTRODUCTION

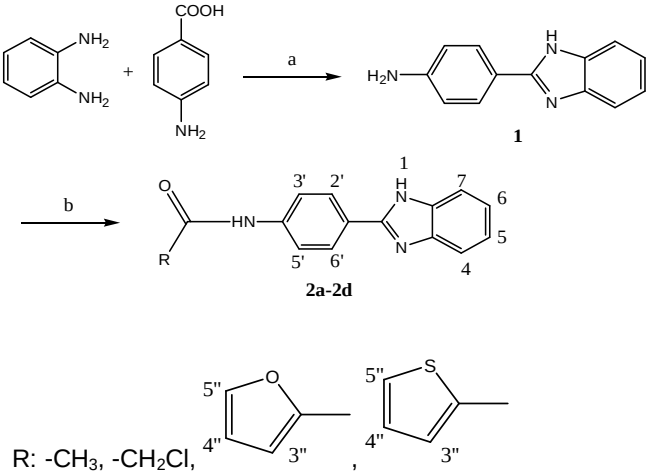
The benzimidazole ring system is an important pharmacophore in medicinal chemistry and modern drug discovery[1]. Compound bearing benzimidazole nucleus have been of great interest to synthetic and medicinal chemists from a long time due to their unique chemical and biological properties mainly related to traditional anthelmintics like Albendazole and Oxibendazole[2]. Albendazole, a benzimidazole carbamate (methyl-5-propylthio-1H-benzimidazole-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]. 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). Hymenolepiasis is caused by (*Hymenolepis nana* or *Hymenolepis diminuta*) the dwarf tapeworm which is the most common cause of all intestinal cestode infections. 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 long-term 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[4]. 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[3].

Synthetic chemistry

Present study was undertaken to synthesize some novel 2-substituted benzimidazole derivatives and investigate their anthelmintic activity. Target compounds were obtained in two steps. First of all, o-Phenylenediamine was condensed with p-Aminobenzoic acid in the presence of o-Phosphoric acid at higher

temperature (200°C) giving the 2-(4 aminophenyl) benzimidazole **1**. Reaction of the **1** was carried out with different commercially available acid chlorides in anhydrous THF and pyridine to achieve corresponding 2-substituted benzimidazole derivatives (**2a-2d**). 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. Some physicochemical properties of the compounds are given in Table1.



Scheme 1: Synthesis route of 2-substituted benzimidazole derivatives (**2a-2d**)
Reagents and conditions: a: o-Phosphoric acid, 200°C reflux 2h b: anhydrous THF and pyridine r.t, 16h.

Table 1: Characterization data of the compounds (2a-2d)

Compounds	R	Molecular Formula (Mol. wt.)	Yield (%)	m.p. (°C)
2a	-CH_3	$C_{15}H_{13}N_3O$ (251.28)	83.33%	314
2b	-CH_2Cl	$C_{15}H_{12}ClN_3O$ (285.73)	24.69%	347
2c	-furan	$C_{18}H_{13}N_3O_2$ (303.31)	69.76%	308
2d	-thiophene	$C_{18}H_{13}N_3OS$ (319.38)	76.92%	354

Biological evaluation

➢ Experimental model:

Indian adult earthworms (*Eisenia fetida*) 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[5].

➢ Worm collection and authentication:

The earthworms were collected from Kalptaru Vermi Culture Center Mumbai, India. Earthworms were authenticated in Department of Zoology, C.K.Thakur Arts, Commerce and Science College, New Panvel, Navi Mumbai, India, to confirm the identity of worms and were used for experimental protocol.

➢ Chemicals:

Albendazole as standard drug was procured from Sigma Aldrich for anthelmintic studies.

➢ Preparation of test and standard doses:

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. Thus solvent used for test and standard solution was 10%v/v Dimethylsulphoxide (DMSO) in distilled water. The synthesized compounds and Albendazole were dissolved in 1ml DMSO and diluted upto 10ml to prepare three concentrations i.e. 0.1%w/v, 0.5%w/v and 1.0%w/v for each compound. 10%v/v DMSO in distilled water served as control.

Table 2: Anthelmintic activity (Study of Paralysis time) (2a-2d)

Sr. No.	Compound	Time in minutes (mean ± SEM) For Paralysis %w/v of concentration		
		0.1	0.5	1.0
1.	Control	-	-	-
2.	2a	4.67±0.353	3.99±0.440	3.65±0.385
3.	2b	3.94±0.312	3.39±0.160	2.34±0.801
4.	2c	3.57±0.374	2.84±0.341	1.93±0.406
5.	2d	5.05±0.999	4.07±0.992	3.11±0.976
6.	Albendazole	5.23±0.990	4.50±0.831	3.14±0.965

Table 3: Anthelmintic activity (Study of death time) (2a-2d)

Sr. No.	Compound	Time in minutes (mean ± SEM) For Death %w/v of concentration		
		0.1	0.5	1.0
1.	Control	-	-	-
2.	2a	38.80±0.615	36.97±0.677	35.93±0.652
3.	2b	30.72±0.609	29.06±0.665	26.93±0.656
4.	2c	32.79±0.616	31.84±0.638	30.93±0.626
5.	2d	27.82±0.606	25.83±0.656	22.93±0.619
6.	Albendazole	49.56±0.603	47.42±0.637	44.54±0.665

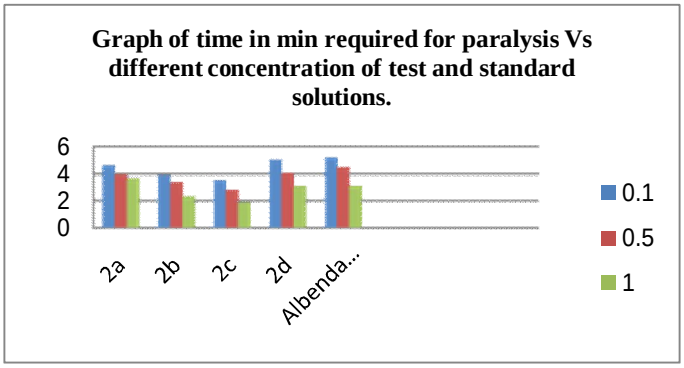


Fig. 1: Graph of time in min required for paralysis Vs. different concentration in % w/v of test and standard solutions.

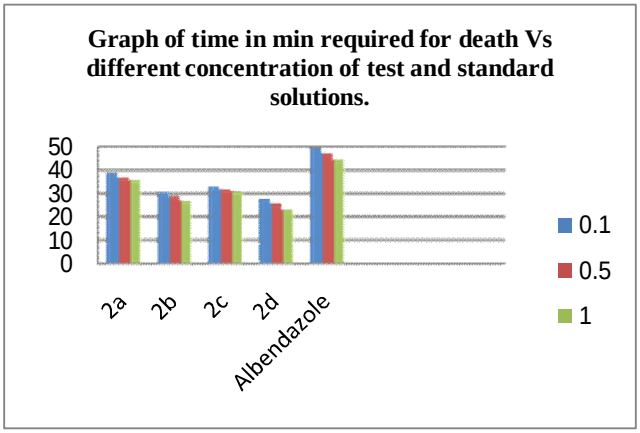


Fig. 2: Graph of time in min required for death Vs. different concentration in %w/v of test and standard solutions.

Result and discussion

The anthelmintic screening of the 2-substitiuted benzimidazole derivatives **2a-2d** showed an excellent activity than standard Albendazole. A closer inspection of data from tables and figures indicates that compounds **2a**, **2b** and **2c** showed better paralytic activity than standard Albendazole. Compound **2d** showed equivalent paralysis activity with standard Albendazole. The transformation order of screened compounds for paralysis time is **2c**>**2b**>**2a**>**2d**. Compounds **2a-2d** showed an excellent anthelmintic activity with respect to death of worms than standard Albendazole. The transformation orer of screened compounds for death time is **2d**>**2b**>**2c**>**2a**.

CONCLUSION

The present study describes the synthesis of four novel 2-substituted benzimidazole derivatives. The synthesized compounds exhibited excellent anthelmintic activity than standard Albendazole. From the present research work it is concluded that these 2-substituted benzimidazole derivatives would be the promosing anthelmintic candiates for in vivo studies in future.

Materials and methods

➢ Chemistry:

Melting points were determined by Differential Scanning Calorimetry (DSC). The IR spectra (in KBr pellets) were recorded on a JASCO FT/IR-8400S spectrophotometer. ^{1}H NMR spectra were recorded (DMSO-d_6) on a Varian (300 MHz) spectrometer using TMS as an internal standard. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; bs, broad singlet; dd, doublet of doublet; m, multiplet. Chemical shift values are given in δ (ppm) scales. The mass spectra were recorded on a JEOL JMS-T 100 spectrometer operating at 70 eV. The completion of the reaction was checked by thin layer chromatography (TLC) on silica gel coated aluminum sheets (Silica gel 60) obtained from Merck. Commercial grade solvents and reagents were used without further purification.

➢ General Procedure:

1. Synthesis of 2-(4-Aminophenyl)benzimidazole (**1**)

A mixture of p-Aminobenzoic acid (4.5g, 33mM) and o-Phenylenediamine (3.8g, 34mM) were stirred in a syrupy o-Phosphoric acid (45ml) at 200^{o}C for 2 hours. The reaction mixture was cooled and poured on crushed ice. The bulky white precipitate obtained was stirred in cold water (400ml) and sodium hydroxide solution (5M) was added until the $_P$H 7. The resulting solid was filtered and recrystallized from methanol; yield 51.43%; m.p. 246-248^{o}C; IR (KBr) cm^{-1} 3437.26 (N-H), 3360.11 (NH_2), 1498.74 (C=C), 1620.26 (C=N), 1180.47 (C-N), 833.28 (Ar-H). MS: m/z M+ 209.11.

2. Synthesis of N-[4-(1H-Benzimidazol-2-yl)-phenyl]-acetamide (**2a**)

Acetyl chloride (0.468g, 4.5mmol) was added to a stirried solution of **1** (0.6g 3mmol) in anhydrous THF (15ml) and pyridine (5ml). The reaction mixture was stirred at room temperature for 16h. The reaction mixture was poured into water and the solid was filtered and recrystallized from methanol, m.p. 314^{o}C; IR (KBr) cm^{-1} 3225.09 (N-H), 1450.52 (C-N), 1672.34 (C=O); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.1 (s, 3H, CH_3); 7.2 (qua, 2H, C 5,6-H); 7.5 (qua, 2H, C 4,7-H); 7.7 (d, 2H, C 2',6'-H); 8.1 (d, 2H, C 3',5'-H); 10.2 (bs, 1H, CONH); 12.8 (bs,1H, benzimidazole-NH).

3. Synthesis of N-[4-(1H-Benzimidazol-2-yl)-phenyl]-2-chloroacetamide (**2b**)

The title compound was obtained by the treatment of Chloroacetyl chloride instead of actyl chloride as described for **2a**. m.p. 347^{o}C; IR (KBr) cm^{-1} 3369.75 (N-H), 1448.59 (C-N), 1656.91 (C=O); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 4.4 (s, 2H, -CH_2Cl); 7.2 (qua, 2H, C 5, 6-H); 7.5 (qua, 2H, C 4, 7-H); 7.7 (d, 2H, C 2', 6'-H); 8.1 (d, 2H, C 3', 5'-H); 10.2 (bs, 1H, CONH); 12.8 (bs,1H, benzimidazole-NH).

4. Furan-2-carboxylic acid [4-(1H-benzimidazol-2-yl)-phenyl]amide (**2c**)

The title compound was obtained by the treatment of 2-Furoyl chloride instead of acetyl chloride as described for **2a**. m.p. 308^{o}C; IR (KBr) cm^{-1} 3311.89 (N-H), 1446.66 (C-N), 1639.55 (C=O); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 6.8 (dd, 1H, C 4"-H); 7.2 (qua, 2H, C 5, 6-H); 7.4 (d, 1H, C 3"-H); 7.5 (qua, 2H, C 4, 7-H); 7.9 (m, 3H, C 2', 6'-H + C 5"-H); 8.1 (d, 2H,C 3', 5'-H); 10.2 (bs, 1H, CONH); 12.8 (bs,1H, benzimidazole-NH).

5. Thiophen-2-carboxylic acid [4-(1H-benzimidazol-2-yl)-phenyl]amide (**2d**)

The title compound was obtained by the treatment of 2-Thiophen carbonyl chloride instead of acetyl chloride as described for **2a**. m.p. 354^{o}C; IR (KBr) cm^{-1} 3414.12 (N-H), 1450.52 (C-N), 1666.55 (C=O); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.2 (d, 1H, C 4"-H); 7.3 (dd, 2H, C 5, 6-H); 7.6 (dd, 2H, C 4, 7-H); 7.9

(m, 3H, C 2', 6'-H + C 3"-H); 8.1 (m, 3H, C 3', 5'-H + C 5"-H); 10.2 (bs, 1H, CONH); 12.8 (bs,1H, benzimidazole-NH).

In vitro anthelmintic activity

The anthelmintic assay was carried out as per the method of S.P. Theivendren et al[5]. The assay was performed in vitro, using adult earthworm (*Eisenia fetida*) owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human being for preliminary evaluation of anthelmintic activity. The worms were acclimatized to the laboratory condition before experimentation. The earthworms were divided into three groups of six earthworms in each. Six earthworms approximately equal size were placed in each petri dish containing 10ml of above test solution and standard drug solution and one group was treated as control with 10%v/v DMSO at room temperature. The time taken for complete paralysis and death was recorded. The mean paralysis time and mean lethal time for each compound was calculated (each reading taken in triplicate). The time taken for worms to become motionless was noted as paralysis time. To ascertain death, each worm was frequently applied with external stimuli which stimulates and induce movement in the earthworms, if alive. The test results were compared with standard drug Albendazole.

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