# SYNTHESIS AND CHARACTERIZATION OF NOVEL SOME BENZIMIDAZOLE DERIVATIEVES AND ITS BIOLOGICAL EVALUATION

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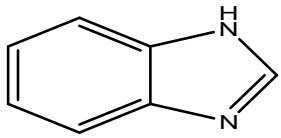
## ABSTRACT

**Due to the high synthetic versatility and potent bilological properties, benzimadozole and its derivatives form an intriguing class of heterocyclic compounds. The biological actions of benzoimidazole and its derivatives are quite diverse, including the ability to fight cancer, ulcers, and heliomphalitis. Given the biological significance of benzimadazoles, we planned to synthesise a number of novel benzimidazoles ($BI_1$–$BI_6$), characterise them through spectral analysis, screen them for anthelminthic activity and anti-ulcer activity. The equimolar mixture of glacial acetic acid and water in which o-phenylene diamine is dissolved is used in the synthesis process. When ammonia solution (10%) is added while stirring continuously, 2-methyl-1H-benzoimidazole(I) is produced. This compound then undergoes a nitration process to produce 2-methyl-6 nitro-1H-benzoimidazole(II). The 2-methyl-6 nitro-1H-benzoimidazole(II) was modified and then dissolved in methanol with various substituted aromatic aldehyde. Six new benzeimidazole derivatives ($BI_1$-$BI_6$) were produced by refluxing the reaction mixture for 1–3 hours after it had been agitated for 1–2 hours. In anthelmntic activity $BI_6$ compound taken less time to paralysis and antiulcer activity $BI_1$ shows potent activity with comparing with standard albendazole, aluminium hydroxide, and magnesium hydroxide, respectively.**


**KEY WORDS:** benzimidazole, anthelminthic activity, antiulcer activity, and albendazole

## INTRODUCTION

Benzimidazole is as Nitrogen-containing aromatic heterocyclic organic compound. Basically, benzimidazole is a bicyclic compound consisting of the fusion of benzene with imidazole which ultimately gives a privileged structure. The NH group present in Benzimidazole is relatively strongly acidic and weakly basic in nature. Benzimidazole is also known as benziminazoles or benzoglyoxalines or o-phenylene formamidine[1-4]. The most important positions or sites effecting drug actin of benzimidazole substituents is 1st and 2nd position. Due to their special structural features and electron-rich environment, Benzimidazole containing drugs bind to a variety of therapeutic targets, thereby exhibiting a broad spectrum of bioactivities[5-7]. Benzimidazole posses wide range of pharmacological actions such as anticancer, anti ulcer, antihypertensive, antimalarial, antihelmentic, antimicrobial , antiviral activity.




1*H*-benzo[*d*]imidazole

## MATERIALS AND METHODS

All the chemicals and solvents used were of synthetic grade from finer chemicals melting point were determined in open capillary tubes using melting point appratus .purity of the compounds was verified by single spot in TLC , 0.25mm aluminium plates with mobile phase benzene and acetone (7:3) .The IR spectra were recorded on SCHIMADZU FT-IR by using 1% potassium bromide discs.

**Experimental methodology[13-20]**

**Step:1 Synthesis of 2- methyl 1H-benzimidazole**

Weigh 5.43 grammes of o-phenylene diamine dissolved in 20 ml of water, add 5.4 grames of glacial acetic acid, and then reflux in a water bath for 1.5 hours. After cooling, add ammonia solution (10%) while vigorously shaking the mixture. With 10% aqueous ethanol and activated charcoal, the crude drug is re-crystallized.

**Step: 2 Preparation of 2-methyl-6 nitro-1H-benzoimidazole**

In a three-necked flask, 10.75ml of concentrated nitric acid was introduced, followed by the steady addition of an equal volume of concentrated sulfuric acid (1:1). After being held in ice-cold water, the combination was gradually combined with the aforementioned compound (6.72gm) over the course of a half-hour at room temperature. After being continually swirled for 12 hours and 45 minutes, the reaction mixture was progressively poured over crushed ice while being stirred. The substance that had precipitated was removed and cleaned with cold water.

**Step: 3 Preparation of benzimidazole derivatives**

A round bottom flask was filled with 0.01M of the 2-methyl 5-nitro benzimadazole produced above. The mixture was then transferred into the aforementioned round bottom flask after being added to 0.01M of substituted different aromatic aldehyde that had been dissolved in 30–40 ml of ethanol. For 1-2 hours, the reaction mixture was mixed, and it was left in reflux for 3 hours. The final product was assembled, allowed to cool at room temperature, and then

poured into ice water. Once the product has cooled, filter it and let it air dry. ethanol re-crystallized.

**Biological activities:**

**Anti-helmentic activity**

For all of the newly synthesised derivatives, adult earthworms measuring 4-5 cm in length and 0.1-0.2 cm in width were utilised. All of the earthworms were gathered in the AP Indian district of Anantapur. Six worms were placed in each of the groups that were formed from the worms. To assemble the concentrations of 25, 50, and 100 mg/ml, all of the recently synthesised derivatives were dissolved in a minimum of 2% v/v Tween 80 and the volume was increased to 10 ml with normal saline. All of the derivatives and standard medication solutions were newly made prior to the start of the tests. Before being discharged into 10 ml of the appropriate formulation, each earthworm was thoroughly cleaned in normal saline solution. The formulations were as follows: vehicle (2% v/v Tween 80 in normal saline), albendazole (20 mg/ml), and derivatives (25,50,100 mg/ml). Six observations were made for each petri dish containing six worms of the same size in order to determine the anthelmintic activity. They were practical since they moved naturally and elicited reactions. Individual worms' paralysis and deaths were tracked in terms of timing. When the worms failed to awaken even in regular saline, paralysis was thought to have occurred. Worms were said to have died when they stopped moving, which was followed by the fading of their body colour.

**Anti-ulcer activity**

The typical antacid, AHMH (aluminium hydroxide + magnesium hydroxide -500mg/ml), was compared to freshly synthesised prototypes at different concentrations (50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml, 250 mg/ml, and 500 mg/ml) for acid neutralising capacity. Water was added and well mixed with the 5ml amount of each extract to get the 70 ml total volume. Following the addition of 30 ml of 1N HCL and 15 minutes of stirring the standard and test preparation, 2–3 drops of phenolphthalein solution were added and combined. The excess HCL was immediately titrated with 0.5N sodium hydroxide solution dropwise until a pink colour appeared. The moles of acid neutralized is calculated by.

Moles of acid neutralized = ( vol. of HCL ×normality of HCL ) – ( vol. of NaOH × normality of NaOH )

Acid neutralizing capacity (ANC) per gram of antacid = moles of HCL neutralized / grams of antacid

**Synthetic scheme :**

SCHEME:

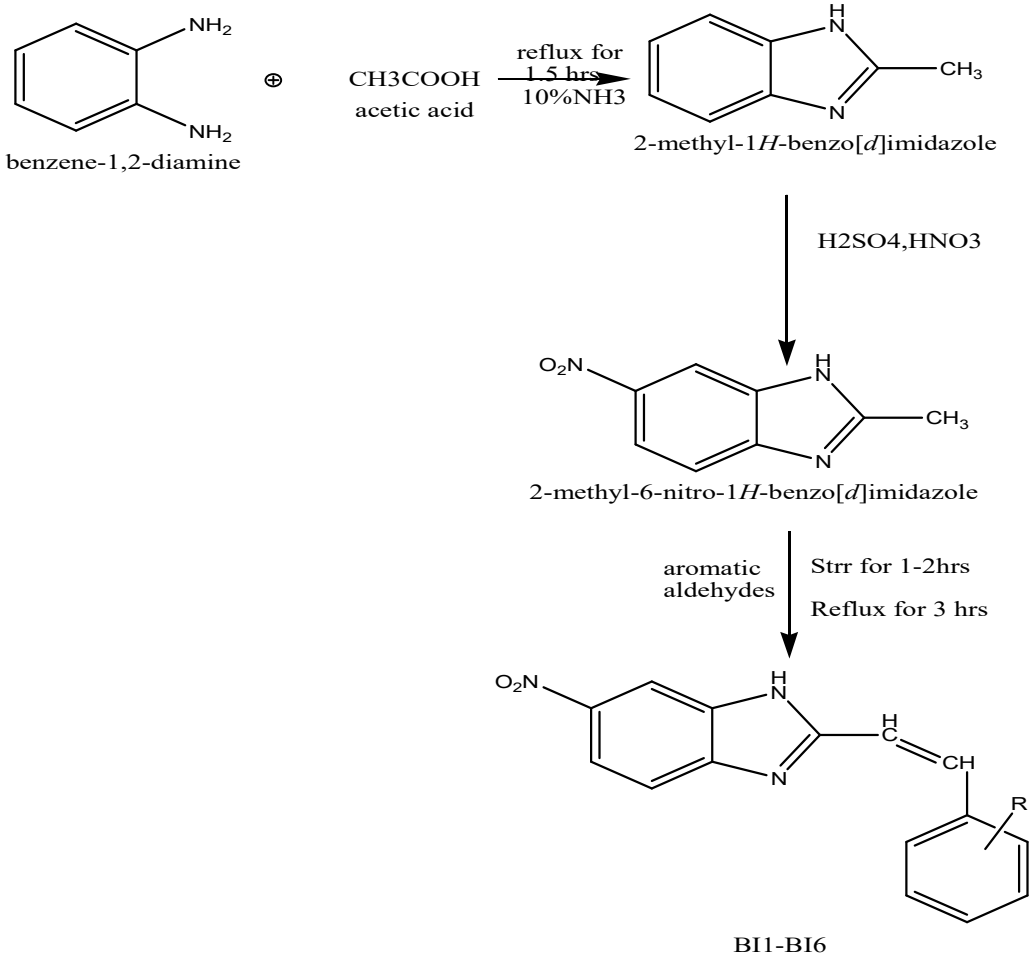



Fig:1 scheme for benzimidazole

Table no1:Benzimidazole derivatives ($BI_1$-$BI_6$)

| | |
|---|---|
| R= | 4- OH |
| | 4-Cl |
| | 3,4-$OCH_3$ |
| | 4-$N(CH_3)_2$ |
| | 3-$OCH_3$,4-OH |

| | |
|---|---|
| | 2- $NO_2$ |

## RESULTS AND DISCUSSION

**Characterization:**

**Compound ($BI_1$):**

**IR (KBr in $cm^{-1}$):** C=C stretch 2423.06, C-C Stretch 1600.37, Aromatic 1820.2, C-NH Stretch 3273.4, C-Cl Stretch 746.16

**NMR chemical shift (δ, ppm):** 2.2 (S,1H, CH, ArCH),7.2-7.8 (M, Ar CH,14H),8,4 (S, NH,1H)

**Mass:** Base peak 79.20000, molecular ion peak 299.71

**Compound ($BI_2$):**

**IR (KBr in $cm^{-1}$):** N-H Stretch 3319.3, C=C Stretch 1642.2, C-O Stretch 1143.1, C-N Stretch 1316.1, Aromatic Stretch 1600.6

**NMR chemical shift (δ, ppm):** 1.3 (S, CH,1H, Ar CH), 7.3-7.8 (M, Ar-H, 12H),8.6 (S,1H, NH)

**Mass:** Base peak 384.00, Molecular ion peak 311.29

**Compound ($BI_3$):**

**IR (KBr in $cm^{-1}$):** N-H Stretch 3419.3, C=C Stretch 1602.2**,** C=N Stretch 1543.1, C-HStretch1412.1, Aromatic 1602.6

**NMR chemical shift (δ, ppm):** 1.3 (S,1H, CH),1 (solvent peak),7.3-7.8 (M,12H), 7(S,1H, NH)

**Mass:** Base peak 79.30000, Molecular ion peak 308.33

**Compound ($BI_4$):**

**IR (KBr in $cm^{-1}$):** Aromatic1602.3, C=CStretch 1642.2 **,**C-H Stretch 1143.1, C-N Stretch 1216.1 ,O-H Stretch 3033.6

**NMR chemical shift (δ, ppm):** 1(S,1H, CH) , 5.5 (S,OH, 1H), 7-7.7 (M, Ar-H,12H), 8.6 (S,NH, 1H, Ar-CH).

**Mass:** Base peak 79.2500, Molecular ion peak 281.27

**Compound ($BI_5$):**

**IR (KBr in $cm^{-1}$):** N-H Stretch 3302.3, C=CStretch 1612.2, C-O Stretch1123.1, C=NStretch 1646.1, Aromatic C-H Stretch1622.6

**Compound ($BI_6$):**

**IR (KBr in $cm^{-1}$):** N-H Stretch 3349.3, C=CStretch 1622.2, C=N Stretch1516.1, Aromatic C-H Stretch 1603.6

**In-vitro anthelmentic activity**

The synthetic derivatives of antihelmintic activity were performed by using the adult earth worms 4-5 cm in length and 0.1-0.2 cm in width against the standard drug Albendazole , the results as shown in the table -2.

Table no 2: In vitro anthelminthic activity

| COMPOUNDS | Time taken for Paralysis (min) | | | Time taken for death (min) | | |
|---|---|---|---|---|---|---|
| | **25mg/ml** | **50mg/ml** | **100mg/ml** | **25mg/ml** | **50mg/ml** | **100mg/ml** |
| $BI_1$ | 40.09±1.25 | 22.47±1.04 | 16.4±1.5 | 69.21±2.74 | 33.13±1,17 | 25.41±1.10 |
| $BI_2$ | 37±2.50 | 28.43±1.87 | 18.01±2,46 | 64.09±1.03 | 22.33±2.37 | 19.25±1,92 |
| $BI_3$ | 29.19±2.34 | 22.58±2.03 | 17.46±2.37 | 47.18±2.47 | 19.06±1.94 | 16.53±2.07 |
| $BI_4$ | 28.17±2.23 | 22.32±2.46 | 12.32±1.74 | 39.29±2.35 | 14.42±2.78 | 11.34±1.38 |
| $BI_5$ | 42.52±1.08 | 36.56±1.98 | 19.33±1.09 | 70.45±1.35 | 34.28±1.76 | 28.36±2.78 |
| $BI_6$ | 24 .02±1.87 | 15.35±2.37 | 10.42±2.05 | 14.38±0.98 | 9.54±2.98 | 8.36±1.87 |
| Standard(albendazole (20mg/ml) | 23.26±1.5 | | | 62±6.8 | | |

**In- vitro anti-ulcer activity**

The synthesized compounds were evaluated for their in vitro anti-ulcer activity by Acid Neutralizing Capacity (ANC) method.

The synthesized derivatives of benzimidazole are performed the antiulcer activity against the standard aluninium hydroxide and magnesuim hydroxide.Table-3

Table no 3: In-vitro antiulcer activity by Acid Neutralizing Capacity (ANC) Method

| CONCENTRATION Mg/ml | ANC per gram of drug | | | | | |
|---|---|---|---|---|---|---|
| | $BI_1$ | $BI_2$ | $BI_3$ | $BI_4$ | $BI_5$ | $BI_6$ |
| 50 | 190 | 200 | 160 | 182 | 130 | 202 |
| 100 | 70 | 90 | 110 | 65 | 78.5 | 64 |
| 150 | 81 | 80 | 56.6 | 39.3 | 42.5 | 46.3 |
| 200 | 51.25 | 53.25 | 32.25 | 34.25 | 50.25 | 50.5 |
| 250 | 26 | 32 | 34.4 | 41 | 36.4 | 32.2 |
| 500 | 15.5 | 21.5 | 17.9 | 18.8 | 19.7 | 20.1 |
| $Al(OH)_2$ &$Mg(OH)_2$ (500mg/ml) | 10.7 | | | | | |

## Discussion

The present research work, involves the synthesis of series of 6 novel substituted compounds of benzimidazole. Here ortho-phenylenediamine taken as a starting material. Treatment of ortho-phenylenediamine and glacial acetic acid it forms 2-methyl-1H -Benzimidazole. To this compound undergoes a nitration process to form 5-nitro, 2-methyl-1H-Benzimidazole. This compound is treated with various aldehydes to form benzimidazole derivatives. Purification was done by recrystallization. Characterization of all derivatives were done by FT-IR.

All derivatives were screened for their *in-vitro* anthelminthic and anti-ulcer activities.

## Anthelminthic activity

In-vitro anthelminthic activity was performed by using earthworms, and Albendazole as the reference standard. All the titled compounds ($BI_1$-$BI_6$) were evaluated for *in-vitro* anti helminthic activity. The effect of the synthesized titled compounds was tested with different concentrations (25, 50, and 100 mg/ml). All derivatives were able to inhibit anti-helminthic activity. The most effective was $BI_6$, $BI_4$ shows more potent activity. The results were tabulated in table no.2 The order of anthelminthic activity of synthesized compounds as follows.

$$BI_6 > BI_4 > BI_3 > BI_2 > BI_1 > BI_5$$

**Anti ulcer activity**

In-vitro anti-ulcer activity was performed by using Acid neutralizing capacity (ANC), were compared with the standard antacid AHMH (aluminum hydroxide + magnesium hydroxide. All the titled compounds ($BI_1$-$BI_6$) were evaluated for *in-vitro* anti -ulcer activity. The effect of the synthesized titled compounds was tested with different concentrations ( 50, 100, 150, 200, 250, and 500 mg/ml). All derivatives were able to show anti-ulcer activity. The most effective was $BI_1$, $BI_3$ shows more potent activity. The results were tabulated in table no. 3 The order of anti-ulcer activity of synthesized compounds as follows.

$$BI_1 > BI_3 > BI_4 > BI_5 > BI_6 > BI_2$$

## CONCLUSION

Six substituted benzimidazole derivatives were synthesized and characterized by using physical data (solubility, melting point and TLC monitoring) and spectral data (FT-IR).

All the derivatives were evaluated for biological studies like *in-vitro* anthelminthic activity and *invitro* anti ulcer activity. The electron withdrawing groups such as chloro, Nitro, containing compounds ($BI_1$, $BI_6$) has shown the highest activity and the rest of compounds having electron donating groups ($BI_2$ $BI_3$, $BI_4$ $BI_5$) shows mild to moderate activity. In brief electron withdrawing groups containing compounds possess potent activity than that of compounds containing electron donating groups.

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