# SYNTHESIS AND BIOACTIVITIES OF SOME BENZIMIDAZOLES DERIVATIVES

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**Abstract -** Some new bisbenzimidazoles derivatives have been prepared by a classical Phillips method. Substituents have been placed on both benzene rings of the benzimidazole nuclei and the chain linking the two benzimidazole units has been varied from simple alkane chains to hydroxylated alkane chains. The biological valorisation has been achieved by antibacterial, antifungal and cytotoxicity tests.

**Keywords-** Bis-benzimidazoles, antibacterial, antifungal, cytotoxicity

## 1. INTRODUCTION

The chemistry of benzimidazole derivatives encompasses a wide field of interest ranging from vitamin $B_{12}$ [1], yeast antimetabolite [2], high polymers [3] to organic semi-and photo-conductors [4]. This unique ring system is structurally related to the biologically important purines and processes interesting absorption and emission characteristics and photoconductive properties. A number of benzimidazole derivatives are the structural isosters of naturally occurring nucleotides which allow them to interact easily with biopolymers of living systems, and different kinds of biological activity have been reported [5]. The synthesis of benzimidazoles has received much attention owing to the varied biological activity exhibited by a number of these compounds. Many derivatives of benzimidazole are well known as antimicrobial [6,7] and antifungal [8] agents and show a wide variety of biological activity as well as anthelmintic potency. As part of studies of properties of benzimidazole derivatives, we have investigated the synthesis and bioactivities of a series of bis-benzimidazole derivative.
In this study, we describes the synthesis and the biologicals activities of some bis-benzimidazoles. The biological valorization of synthesised compounds has been achieved by antibacterial test against Escherichia coli, Staphylococcus aureus, antifungal test against Candida albicans and cytotoxicity against Artemia salina larva.

## 2. MATERIAL AND METHODS

### 2.1 General procedures

All of melting points reported were determined on a Gallenkamp Melting Point Apparatus. The values are uncorrected.
Infrared spectra were determined with Bruker FTIR IFS 45. The compounds were studied in the solid state as potassium bromide disks.
Proton nuclear magnetic resonance spectra were obtained at ambient temperature using Bruker ACE 200 spectometer, chemical shift being quoted with respect to TMS as internal standard.

## 2.2. Chemistry

### 2.2.1. Synthesis of 2,2'-Ethylenebis-benzimidazole (**A**)

To a well-stirred solution of 2 mol of 1,2-phenylenediamine in 4N HCl was added the appropriate diacid (1 mol). The solution was heated to reflux for ten hours. The mixture was then cooled and the crystalline dihydrochloride which separated was removed by filtration. It was extracted with hot dilute ammonium hydroxide and washed with water. The product was recrystallized from ethanol with the aid of decolorizing carbon. Analogous compounds (**B, C**) where prepared by this method.

### 2.2.2. Synthesis of 2,2'-Ethylene bis(5-nitrobenzimidazole) (**D**)

This compound was odtained by nitration of 2,2'-Ethylenebis-benzimidazole (**A**). Thus, 4g (0.013 mol) of (**A**) were dissolved into concentrated $H_2SO_4$ (11 ml), and the solution was cooled in ice-water bath to maintain the temperature below 50°C. A mixture of fuming $HNO_3$ (1.385 ml) and concentrated $H_2SO_4$ (1.184 ml) was added dropwise in ca. 10 mn. Then the solution was stirred for 1 h and poured into an ice-water mixture. The raw product was filtered, washed with water, neutralized with sodium carbonate until pH 9, and washed with water to give raw (**D**). The recrystallization from ethanol gave pale yellow crystals. Analogous compounds (**E**, **F** ) where prepared by this method.

## 2.3. Biological's test technique

### 2.3.1 Antibacterial and antifungal tests

All compounds were tested in vitro for antibacterial activity against Gram positive *Staphylococcus aureus* (ATCC11230), Gram negative *Escerichia coli* (AtCC25922) bacteria and antifungal activity against *Candida albicans* (ATTC10231) by agar diffusion method [9]. Test and reference compounds (Ampicillin, Fluconazole and Miconazole) were solved in DMSO (2000µg/ml), 0.02 ml (one drop) of these solutions was dropped to paper disk (6 mm in diameter) and placed on an agar plate containing bacteria or fungi cells. DMSO as a control has no inhibition zone. The diameter of the growth inhibition zone around the paper disc was measured after incubation for 18-24 h at 37 ± 1°C.

### 2.3.2. Cytotoxicity test

A method, utilizing brine shrimp (*Artemia salina*), is proposed as a simple bioassay [10]. The procedure gives $LC_{50}$ values in µg/ml of active compounds in the brine medium.Samples were prepared by dissolving compounds in DMSO. Brine shrimp eggs were hatched in a shallow rectangular dish filled with artificial sea water which was prepared with a commercial salt mixture and double-distilled water. After 24 hours the phototropic nauplii were collected by pipette from the lighted side, having been separated by the divider from their shells. Ten shrimps were transferred to each sample vial. The nauplii can be counted macroscopically in the stem of pipette against a lighted back-ground.

The vials were maintained under illumination. Survivors were counted, after 24 hours, and the percent deaths at each dose and control (solvent) were determined. $LD_{50}$'s was determined from the 24 hour counts. The $LD_{50}$ was derived from the best fit line obtained by regression analysis, after transforming dose-response data into a straight line by means of logarithmic transformation.

## 3. RESULTS AND DISCUSSION

3.1. Caracterization of synthesized compounds

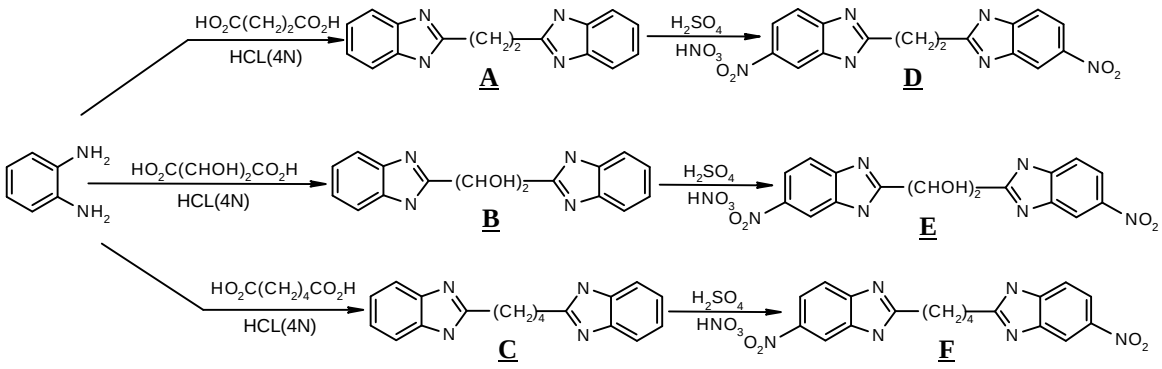


**Figure 1 :** Synthesis of Bis-benzimidazole compounds

2,2'-Ethylenebis-benzimidazole (**A**) : mp >300°C, $^1$HNMR (DMSO-$d_6$) δ 7.2-7.8 (m, 8H, ArH), 2.88 (s, 4H, -$CH_2$-); IFR (KBr) ν 1621 (C=N); 3452 $cm^{-1}$ (NH).

2,2'-Bis(benzimidazolyl)-ethanediol (**B**) : mp 245°C, $^1$HNMR (DMSO-$d_6$) δ 7.18 (m, 4H, ArH), 7.58 (m, 4H, ArH), 5.48 (s, 2H, -CH(OH)-CH(OH)-); IFR (KBr) ν 1630 (C=N); 3570 $cm^{-1}$ (NH).

2,2'-Buthylenebis-benzimidazole (**C**) : mp 265-267°C, $^1$HNMR (DMSO-$d_6$) δ 7.5-8.05 (m, 8H, ArH), 2.55 (s, 4H, -**$CH_2$**-($CH_2$)$_2$-**$CH_2$**-); 1.62 (s, 4H, -$CH_2$-**($CH_2$)$_2$**-$CH_2$-); IFR (KBr) ν 1623 (C=N); 3500 $cm^{-1}$ (NH).

2,2'-Ethylene-bis(5-nitrobenzimidazol) (**D**) **:** mp 286-288°C, $^1$HRMN (DMSO-$d_6$) δ 8.34 (d, 2H, ArH), 7.96 (dd, 2H, ArH) 7.56 (d, 2H, ArH), 3.40 (s, 4H, -$CH_2$-); IR (KBr) ν 1615 (C=N); 1517 (N=O) ; 1342 (N=O); 3250 $cm^{-1}$ (NH).

2,2'-Buthylene-bis(5-nitrobenzimidazol) (**E**) : mp 264-266°C, $^1$HRMN (DMSO-$d_6$) δ 8.36 (d, 2H, ArH), 8.07 (dd, 2H, ArH), 7.63 (d, 2H, ArH), 2.94 (s, 4H, -$CH_2$-), 1.87 (s, 4H, -$CH_2$-); IR (KBr) ν 1620 (C=N); 1516 (N=O) ; 1342 (N=O) ; 3250 $cm^{-1}$ (NH).

2,2'-Bis(5-nitrobenzimidazolyl)-ethanediol (**F**) : mp 285-287°C, $^1$HNMR (DMSO-$d_6$) δ 8.35 (d, 2H, ArH), 8.25 (s, 2H, ArH), 7.85 (d, 2H, ArH), 5.97 (s, 2H, -CH(OH)-CH(OH)-); IFR (KBr) ν 1625 (C=N); 1520 (N=O) ; 1352 (N=O); 3250 $cm^{-1}$ (NH).

3.2. Bioactivities of synthesized compounds

3.2.1. antibacterial and antifungal assays

Antibacterial and antifungal activity of synthesized compounds was measured as radius of zone of inhibition around the disc. Results of these tests are shown in table 1.

3.2.2. Cytotoxicity test

Compounds has been tested at 20, 40, 60 and 100 µg/ml. $LD_{50}$ value are given in table 2.

**Table 1:** The in vitro antibacterial and antifungal activity of synthesised compounds

| | Growth inhibition zone diameter (mm) | | |
|---|---|---|---|
| Comp. | *E. coli* | *S. aureus* | *C. albicans* |
| **A** | NI | NI | 7 |
| **B** | NI | NI | 8 |
| **C** | NI | NI | 10 |
| **D** | NI | NI | 8 |
| **E** | NI | NI | 7 |
| **F** | NI | NI | 9 |
| Fluconazole | - | - | 16.5 |
| Miconazole | - | - | 18 |
| Amplcillin | 22 | 29 | - |

NI, no inhibition

**Table 2:** Cytotoxicity of synthesized compounds

| Comp. | $LD_{50}$ µg/ml |
|---|---|
| **A** | 35.21 |
| **B** | 38.07 |
| **C** | 10.85 |
| **D** | 16.65 |
| **E** | 17.56 |
| **F** | 5.72 |
| Digitalin | 151 |
| Caffeine | 306 |

## 4. CONCLUSION

This study found that these novel bis-benzimidazoles are actively cytotoxic against Artemia salina larva. Their toxicity is more than digitalin ($DL_{50}$ =151 µg/ml) and

caffeine ($DL_{50}$ =306 µg/ml) toxicity. These compounds can present a particular interest for cytotoxicity on KBS and 9PS cells. With regard to the antimicrobial and antifungal tests, none of the synthesised bis-benzimidazoles exhibited activity towards E. coli and S. aureus. However, the test compound have been found to exhibit considerable activity against C. albican but none of them superceeded the activity of standard compounds.

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