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# Synthesis of 1,2 Disubstituted Benzo 1,3-Diazole Derivatives and Evaluation of Their *In-vitro* Antifungal Activities

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## ABSTRACT

The present research work was meant to blend some Mannich bases of benzo1,3-diazole dervivatives and was explored for their organic exercises. The recently incorporated mixes have been portrayed by their explanatory and unearthly (IR, 1HNMR, Mass spectra) properties. Every one of the mixes have been screened for their antifungal exercises by standard strategies. $BZ_2$, $BZ_3$ and $BZ_6$ displayed Promising antifungal activity.

**Keywords:** Benzo1,3-diazole, o-phenylenediamine, urea, benzamide, Hydroxylamine, Antifungal activity.

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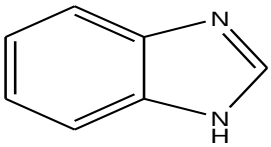
## 1. INTRODUCTION



Benzo1,3-diazole is a bicyclic heterocyclic system consisting of two nitrogen atoms and fused phenyl ring. Benzo1,3-diazole derivatives dervatives take part in a vital role in biological fields such as antitubercular, antineoplastic, antiviral, antioxidant, anticonvulsant, antiinflammatory, antibacterial and antifungal activities. (Kedar M.S et al 2010). The synthesis of newer benzimidazole derivatives remains a main center of attention of medicinal research (Hubschwerlen.C et al 1992).

latest observations imply that substituted benzo1,3-diazole and heterocycles, which are the structural isoster's of nucleotides owing fused heterocyclic nuclei in their structures that allow them to interact easily with the biopolymers, posses potential activity with lower toxicities in the chemotherapeutic approach in man. (Furniss et al 1996). As an outgrowth of our investigation to discover novel antitubercular and antifungal agents a new series of 1, 2 disubstituted benzimidazole analogs were synthesized and both anti tubercular and anti fungal activity were evaluated.

## 2. MATERIALS AND METHOD

### 2.1 General

Melting points (mp) were determined by open capillary tube method. The purity of the compounds was checked on precoated TLC plate made of silica Gel-G and spots were visualized by iodine Vapour. The IR Spectra of the compounds were recorded in the range of 4000-500 $cm^{-1}$ on shimadzu FT-IR, Affinity I, FT - IR spectrometer using KBr disc $^1$HNMR was scanned and chemical shifts are expressed in δ (ppm) relative to TMS as an internal standard using ethanol as solvent Mass Spectra were recorded by EI-MS method.

### 2.2 Chemistry

#### 2.2.1 Synthesis of benzo1,3-diazole derivatives

The different 2-(2 or 5 – substituted) phenyl benzo1,3-diazole derivatives were prepared as reported in the literature. ( Linga S et al 2011).

**2.2.2 Synthesis of 1-{[2-4-methoxyphenyl)- 1-H benzimidazol-1-y1]methyl} urea ($BZ_1$)** ( Messmary M.A.et al 2010.)

Formaldehyde (0.05 mol) was added slowly to 0,05 mol of 2-( 4-methoxy phenyl ) 1-H Benzimidazole and 0.05 mol of urea in 15 ml of ethanol, with continuous stirring for 1 hour

and refrigerated overnight. The product was filtered, recrystallized using absolute alcohol.

**2.2.3 Synthesis of [2-(4-methoxyphenyl)-1H benzimidazol-1-y1] methyl}benzamide( $BZ_2$ )**

Formaldehyde (0.05mol) was added slowly to 0.05 mol of 2-(4-methoxyphenyl) 1-H benzimidazole and 0.05 mol of benzamide in 15 ml of ethanol, with continuous stirring for 1 hour, and refrigerated overnight the product was filtered, recrystallized using absolute alcohol.

**2.2.4 Synthesis of N-hydroxy[2-(4-methoxyphenyl) 1-H benzimidazole 1-y1] methyl} methanamine ( $BZ_3$ )**

Formaldehyde (0.05 mol) was added slowly to 0.05 mol of 2-(4-methoxy phenyl) 1-H benzimidzole and 0.05 mol of hydroxyl amine in 15 mol of ethanol with continuous stirring for 1 hour, and refrigerated overnight the product was filtered, recrystallized using absolute alcohol.

**2.2.5 Synthesis of 1-{[2-(2-hydroxyphenyl)-1H-benzimidazol 1-y1]methyl}urea ($BZ_4$)**

Formaldehyde (0.05 mol) was added slowly to 0.05 mol of 2-(1H-benzimidazol-2-y1) Phenol and 0.05 mol of urea in 15 ml of ethanol, with continuous stirring for 1 hour and refrigerated overnight. The product was filtered, refrigerated overnight. The product was filtered, recrystallized using absolute alcohol.

**2.2.6 Synthesis of N-{(2-hydroxy phenyl ) -1H-benzomidazol – 1y1] methyl} benzamide ($BZ_5$)**

Formaldehyde (0.05 mol) was added slowly to 0.05 mol of 2-(1-H benzimidazol-2-y1) phenol and 0.05 mol of benzamide in 15 ml of ethanol, with continuous stirring for 1hour, and refrigerated overnight. The product was filtered, recrysralized using absolute alcohol.

**2.2.7 Synthesis of N-[(1-hydroxy amino) ($BZ_6$) methyl] 1H - benzimidazol -2-y1] } Phenol**

Formaldehyde (0.05 mol) was added to 0.05 mol of 2-(1H-benzimidazol-2-y1) Phenol and 5 m mol of hydroxylamine in 15 ml of ethanol, with continuous stirring for 1 hour and refrigerated overnight. The product was filtered, recrystallized using absolute alcohol.

**2.2.8 Synthesis Synthesis of 4-({[2-(2-hydroxy phenyl)-1H-benzimidazol-1-y1]l} methyl} amino)benzoic acid ($BZ_7$)**

Formaldehyde (0.05mol) was added slowly to 0.05 mol of 2-(1H-benzimidazol-2-y1) Phenol and 0.05 mol of para-amino benzoic acid in 15ml of ethanol, with continuous stirring for 1 hour, and refrigerated overnight. The product was filtered, recrystallized using absolute alcohol.

**2.2.9 Synthesis of 1-{[2-(4-chloro phenyl) 1H-benzimidazol1-y1] methyl} urea ($BZ_8$)**

Formaldehyde (0.05mol) was added slowly to 0.05 mol of 2-(4-chlorophenyl) – 1H-benzimidazole and 0.05 mol of urea in 15 ml of ethanol, with continuous stirring for 1 hour, and refrigerated overnight the product was filtered, recrystalized using absolute alcohol.

**Scheme : Synthesis of 1,2 – disubstituted benzimidazole derivatives.**

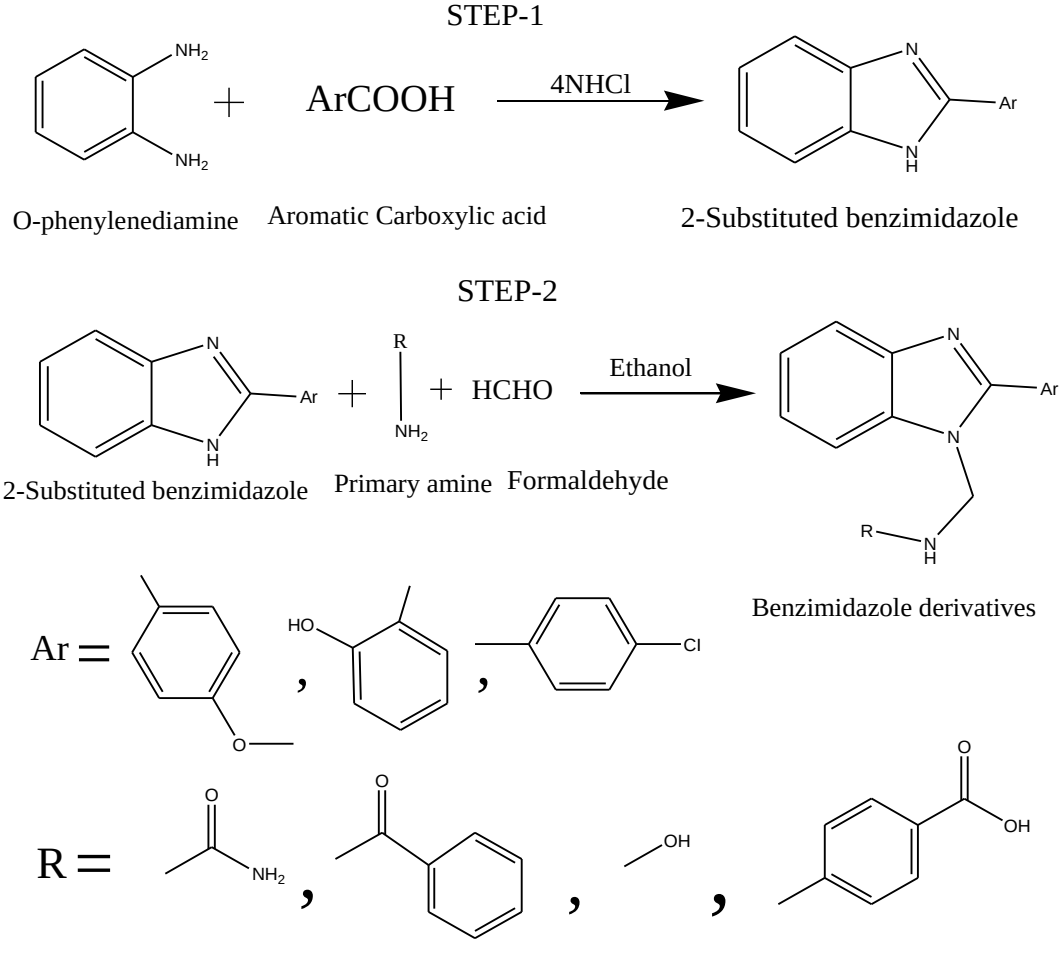
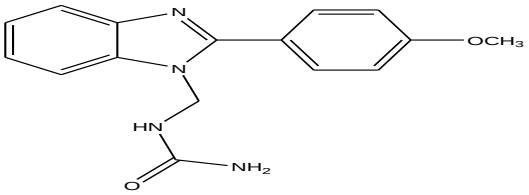
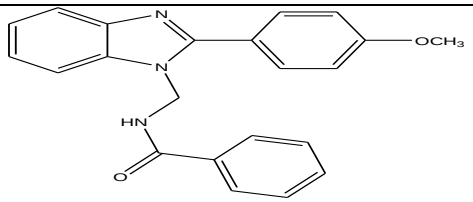
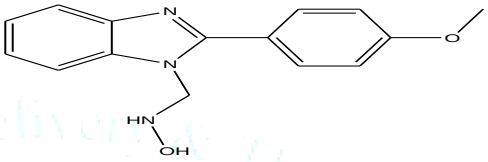
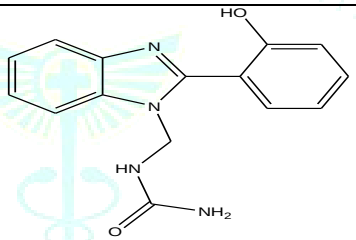
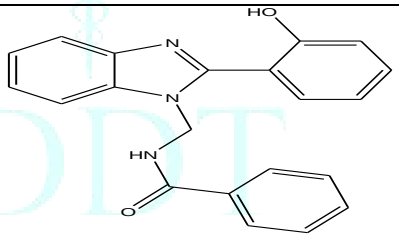
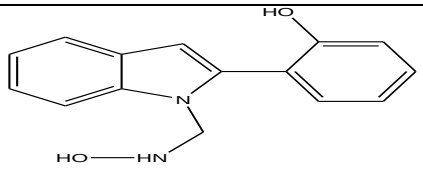
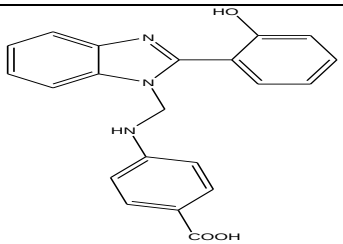
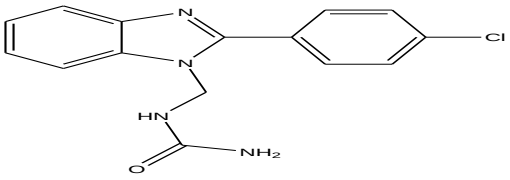


**Table 1: Derivatives of 1,2-disubstituted Benzo1,3-diazole derivatives**

| CODE | STRUCTURES |
|---|---|
| $BZ_1$ |  |
| $BZ_2$ |  |
| $BZ_3$ |  |
| $BZ_4$ |  |
| $BZ_5$ |  |
| $BZ_6$ |  |
| $BZ_7$ |  |
| $BZ_8$ |  |

**2.3 Spectral data of synthesized compounds ( $BZ_1$ -$BZ_8$)**

(Silverstein RM., 2011)

2.3.1 1-{[2-4-methoxyphenyl)- 1-H benzimidazol-1-y1]methyl} urea ($BZ_1$)

**IR υ ( $cm^{-1}$ )** : 1678.13 (C=O amide str) , 3025.34 (aromatic C –H str), 2924.89(aliphatic C-H str) , 1259.38(C-O str), 1323.22(C-N str) 1601.06 (aromatic C=C str)

**$^1$HNMR ( DMSO –$d_6$) δ ppm:**

2.3 .2 [2-(4-methoxyphenyl)-1H benzimidazol-1-y1] methyl} benzamide( $BZ_2$ )

**IR υ ( $cm^{-1}$ )** :1652.26 (C = O amide str) , 3162.31 (aromatic C –H str), 2925.83(aliphatic C-H str) ,1297.06(C-O str), 1622.34 (aromatic C=C str), 1576.54(N-H bend)

**$^1$HNMR ( DMSO –$d_6$) δ ppm:**

2.3.3 N-hydroxy[2-(4-methoxyphenyl) 1-H benzimidazole **1-**y1] methyl} methanamine ( $BZ_3$ )

**IR (υ $cm^{-1}$)** 3402.34 (Secondary amine N.H str),3614.23 (O-H str),3337.74 (aromatic C-H Str), 2924.61 (Aliphatic C-H Str), 1392.46 (C-O Str), 1590.51 (Aromatic C=C Str) 7.155 – 8.004 (m, 8H, Ar-H) , 10.311- 10.451 (t, 1H,NH) 4.066-4.160(d,2H,$CH_2$) , 5.631(s,2H,$NH_2$) 0.977(s,3H,$CH_3$)

**$^1$HNMR ( DMSO –$d_6$) δ ppm:**

2.3 .4 1-{[2-(2-hydroxyphenyl)-1H-benzimidazol 1-y1]methyl}urea ($BZ_4$) **IR (υ $cm^{-1}$) :** 1649.41 (C=O amide Str), 1356.25 (C-N Str), 3371.42 (O-H Str in Phenol), 1649 (C=O amide Str), 3203.49 (Primary aromatic amine N-H Str),3024.91 (Aromatic C-H Str), 2923.76 (Aliphatic C-H Str)
**$^1$HNMR ( DMSO –$d_6$) δ ppm:**

2.3.5 N-{(2-hydroxy phenyl ) -1H-benzomidazol – 1y1] methyl} benzamide ($BZ_5$) **IR (υ $cm^{-1}$) :** 1645.64 (C=O amide Str), 3196.24 (aromatic C=C Str),2924.16 (aliphatic C-H Str),1293.29 (C-OStr), 1588.15 (aromatic C=C Str), 3370.00 (O-H Str in Phenol)**$^1$HNMR ( DMSO –$d_6$) δ ppm:**

2.3.6 N-[(1-hydroxy amino) ($BZ_6$) methyl] 1H - benzimidazol -2-y1] } Phenol **IR (υ $cm^{-1}$):**3371.06 (O-H Str in Phenol), 3401.32 (Secondary amine N-H Str), 3146.32 (Aromatic C-H Str), 2836.92 (Aromatic C-H Str), 1289.06 (C-O Str), 1608.05 (Aromatic C = C Str) 1323.31 (C-N Str) **$^1$HNMR ( DMSO –$d_6$) δ ppm:**

2.3.7 4-({[2-(2-hydroxy phenyl)-1H-benzimidazol-1-y1]l} methyl} amino)benzoic acid ($BZ_7$) **IR (υ $cm^{-1}$):** 3370.06 (Secondary amine N-H Str), 3161.93 (aromatic C-H Str) 2907.10 (Aliphatic C-H Str), 1294.45 (C-O Str), 1668.24 (C=NStr), 1598.79 aromatic C=C Str), 923.91 (aromatic C-C Str), 1583.24 (C=O Str in carboxylic acids) ,2544.68 (-OH) Str in carboxylic acid. **$^1$HNMR ( DMSO –$d_6$) δ ppm:** 2.3.8 1-{[2-(4-chloro phenyl) 1H-benzimidazol1-y1] methyl} urea ($BZ_8$) **IR (υ $cm^{-1}$)**: 1678.34 (C=O amide Str), 3201.06 (Primary aromatic amine N-H Str), 3024.96 (aromatic C-H Str),2921.36 (Aliphatic C-H Str), 1262.08 (C-O Str), 1590.52 (Aromatic C = C Str), 1663.26 (C=N Str ),629.93 ($C_6H_4$) 1057.32(aromatic C-Cl Str)

**$^1$HNMR ( DMSO –$d_6$) δ ppm:**

## 3. BIOLOGICAL ACTIVITY

3.1Evaluation of Antifungal activity against *Aspergillus niger* (Schwalve et al.,2007)

Using a loop or swab, the colonies are transferred to the plates. The turbidity was visually adjusted with broth to equal that of a 0.5 McFarland turbidity standard that had been vortexed. Alternatively, the suspension can be standardized with a photometric device. A sterile cotton swab was dipped into the inoculum, within 15min of adjusting the inoculum to McFarland 0.5 turbidity standard and was rotated against the wall of the tube above the liquid to remove excess inoculum. The entire surface of the agar plate was swabbed three times. To ensure even distribution, the plates were rotated approximately $60^0$ between streaking. To prevent the formation of aerosols, avoid hitting the sides of petriplate. The inoculated plates were allowed to stand for at least 3 min but no longer than 15min before making wells. A hollow tube of 5mm diameter was heated and pressed on above the inoculated agar plate and was removed immediately. Similarly five wells were made on each plate.75ml, 50ml, 25ml, 10ml and 5ml of sample solutions were added into respective wells on each plate. Plates were incubated within 15 min of sample application. Plates were inverted and stacked no more than five high. Incubated for 18-24hrs at $37^0$C in an incubator. The plates were read only if the lawn of growth was confluent or nearly confluent. The diameter of inhibition zone was measured to the nearest whole millimetre by holding the measuring device

## 4. RESULTS AND DISCUSSION

4.1 Chemistry

In the present examination, the mannich bases of benzo1,3-diazole derivatives were planned utilizing CADD, of which eight subordinates fulfilling the necessary criteria were integrated as portrayed in plot. The physical information of all incorporated mixes were given in Table 2. Every one of the mixes were cleansed by recrystallization utilizing absolute alcohol as dissolvable and the response culmination was set up by single spot on TLC plate. The unearthly information of orchestrated mixes were described by IR,1HNMR and mass phantom examination.

**Table 2: Physical data of all synthesized compounds.**

| Sl.No | Compounds | Mol.Formula | Mol.wt | % Yield | m.p ($^0$C) |
|---|---|---|---|---|---|
| 1 | $BZ_1$ | $C_{16}H_{16}N_4O_2$ | 296.33 | 72 | 130-132 |
| 2 | $BZ_2$ | $C_{22}H_{19}N_3O_2$ | 357.413 | 81 | 99-101 |
| 3 | $BZ_3$ | $C_{15}H_{15}N_3O_2$ | 269.304 | 70 | 204-206 |
| 4 | $BZ_4$ | $C_{15}H_{15}N_4O_2$ | 282.303 | 64 | 126-128 |
| 5 | $BZ_5$ | $C_{21}H_{17}N_3O_2$ | 343.386 | 79 | 100-102 |
| 6 | $BZ_6$ | $C_{14}H_{13}N_3O_2$ | 233.277 | 60 | 207-209 |
| 7 | $BZ_7$ | $C_{21}H_{17}N_3O_3$ | 359.385 | 58 | 163-165 |
| 8 | $BZ_8$ | $C_{15}H_{13}N_4OCl$ | 300.749 | 67 | 129-131 |

4.2. Antifungal Activity

4.2.1 Antifungal Screening

The synthesized compounds were evaluated for its antifungal activity against *A.niger* using Fluconazole (50µg/ml) as standard. Compound $BZ_2$,$BZ_3$ and $BZ_6$ showed significant Disc Diffusion of 24mm,25mm, 20 mm at a concentration of 50µg/ml when compared to other compounds. The standard value of Disc Diffusion for Fluconazole at 50µg/ml was 24mm. Therefore compounds $BZ_2$,$BZ_3$ and $BZ_6$ were found to possess better antifungal activity. Result of antifungal screening is depicted in Table 5.

**Table 3. Antifungal activity of compounds at different concentration by disc diffusion method**

| Compound Code | *Aspergillus niger* | | | |
|---|---|---|---|---|
| | 50µg/ml | 100µg/ml | 250µg/ml | 500µg/ml |
| $BZ_1$ | 00mm | 00mm | 00mm | 00mm |
| $BZ_2$ | 24 mm | 26mm | 27mm | 29mm |
| $BZ_3$ | 25mm | 27mm | 29mm | 30mm |
| $BZ_4$ | 00mm | 00mm | 00mm | 00mm |
| $BZ_5$ | 00mm | 00mm | 00mm | 00mm |
| $BZ_6$ | 20mm | 22mm | 24mm | 26mm |
| $BZ_7$ | 00mm | 00mm | 00mm | 00mm |
| $BZ_8$ | 00mm | 00mm | 00mm | 00mm |
| Control | - | - | - | - |
| Fluconazole (50µg/ml) | 24 | | | |

4.2.2 Minimum inhibitory concentration

The compound $BZ_6$ was found to be sensitive at concentration 0.8 µg/ml. Compound $BZ_3$ showed sensitivity at 3.125 µg/ ml. Compound $BZ_2$ showed sensitivity at 1.6 µg/ ml. Standard value of MIC for Fluconazole is 16µg/ml. Result of MIC is given in Table5.

**Table 7. MIC in µg/ml of all synthesized compounds**

| Fungal strain | Code | Concentration (µg/ml) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 100 | 50 | 25 | 12.5 | 6.25 | 3.125 | 1.6 | 0.8 | 0.4 | 0.2 |
| *A.niger* | $BZ_2$ | S | S | S | S | S | S | S | R | R | R |
| | $BZ_3$ | S | S | S | S | S | S | R | R | R | R |
| | $BZ_6$ | S | S | S | S | S | S | S | S | R | R |

S-Sensitive, R- Resistant

## 5. CONCLUSION

A new series of maanic bases of 1, 2-disubstituted benzimidazole derivaties were synthesized from O-phenylene diamine starting material.The compounds were evaluated for its anti fungal activity. , compound $BZ_6$ posses better anti fungal activity

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## REFERENCES

1. Furniss, Hannaford,Smith, and Tatchell (1996), *Text book of practical organic chemistry* , 5th edition Singapur publishers. Lougmain.
2. Hubschwerlen C, Pflieger P, Specklin J.L, Gubermator K, Gmuendertl, Angerhrn P, and Kompis I (1992), Pyrimido (1,6,-a) benzimidazoles: a new class of DNA gyrase inhibitors. *Journal of Medicinal chemistry*, 35; 1385-1392.
3. Kedar M.S, Dighe N.S,Pattan S.R, Musmade D.S, Thakur D, Bhosale M and Gaware V.M (2010). Benzimidazole in medicinal chemistry; An overview . Scholars Research Library-*Derpharma chemical* 2(2):249-256.
4. Lingala.S, Nerella R and Sambasiva Rao KRS (2011). synthesis, Antimicrobial and Anthelkminitic activity of some novel benzimidazole derivatives. *International journal of pharmaceutical sciences,* 10, 100-105.
5. Maria C.S, Lourenco , Marcus V N , Desouza, Pinheiro D, Ferreira, Rasnisb B, Thais Cristina M and Monica A ( 2007). .Evalution of anti-Tubercular activity of nicotinic acid and isonizid analogues .*ARKIVOC*,XV ,181-191
6. Messmary.M.A, Elarfi.M.G and Mohamed.R (2010). Synthesis and Spectral studies of Mannich Bases Derived from 2-substituted Benzimidazoles. *International journal of chem.Tech Research,* 2 ; 1714-1716.
7. Schwalve, Moore and Goodwin (2007) Antimicrobial susceptibility testing protocols, Crc.press.
8. Silverstein R.M and Webster F.X 9(2005). *Spectro photometric Identification of organic compounds,* 6th edition, John Wiley & Sons, New York
9. Vazquez G N, Vilehis M M R, Mulia L Y Melendez V, Gerena L CamposA H, Castillo R and Luis F H (2006). Synthesis and antiprotozoal activity of some 2- (trifluoromethyl)-1H-benzimidazole bioisosters. *Euoropean Journal of Medicinal Chemistry*, 41, 131-141.
10. Vera K, Jan K, Karel and Jarmila K (2002). New benzimidazole derivatives as antimycobacterial agents. *Farmaco*, 57, 259-265.
11. Walia R, Dhamija K, Vandana, Akhtar M J and Laba H S (2012). Synthesis of novel substituted benzimidazole derivatives as potential antimicrobial agents. *International Journal of Pharmaceutical,Chemical and biological Sciences,* 2, 293-298.

12. Yar M S, Abdullah M M and Majeed J (2009). Synthesis and *in vitro* antimycobacterial activity of some novel benzimidazole derivatives. *world academy of science, engineering and technology,* 55, 394-393.

13. Zao G and Lu M (2012). Simple and efficient Synthesis of benzimidazole derivatives using cobalt (11) acetylacetone at room temperature.*Chiang mai Journal of Science*, 40, 618-624.

14. Zhang Z H, WangY M and Yin L (2007). Efficient lewis acid mediated synthesis of benzimidazole derivatives. Catal communication, 8, 1126-1131.

15. Ziolkowska N E, Michejda C J and Bujacz G D (2010). Synthesis and anti HIV-activity of N-benzyl- benzimidazole derivative. *Journal Molecular Structure*, 9, 188-193.

16. Zygmunt K, Jacqueline A V, Peter U, Agata G, Bohdan S sand Agnieszka L (2002). Synthesis, antiprotozoal and antibacterial activity of nitro and halogeno substituted benzimidazole derivatives. *Acta Biochemical Polonica,* 49, 185-195.