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SYNTHESIS OF 1,2 DISUBSTITUTED BENZO 1,3-DIAZOLE DERIVATIVES AND EVALUATION OF THEIR INVITRO ANTI-TUBERCULAR ACTIVITIES

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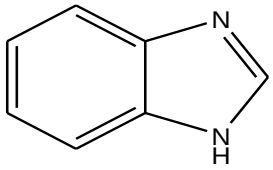
ABSTRACT:
The present research work was aimed to synthesize some Mannich bases of benzo1,3-diazole dervivatives and was investigated for their biological activities. The newly synthesized compounds have been characterized by their analytical and spectral (IR, ^{1}HNMR, Mass spectra) properties. All the compounds have been screened for their antitubercular activities by standard methods. Antitubercular study revealed that Both BZ_2 and BZ_5 have promising activity.
Keywords: Benzo1,3-diazole, o-phenylenediamine, urea, benzamide, Hydroxylamine, Antitubercular activity.

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)

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.

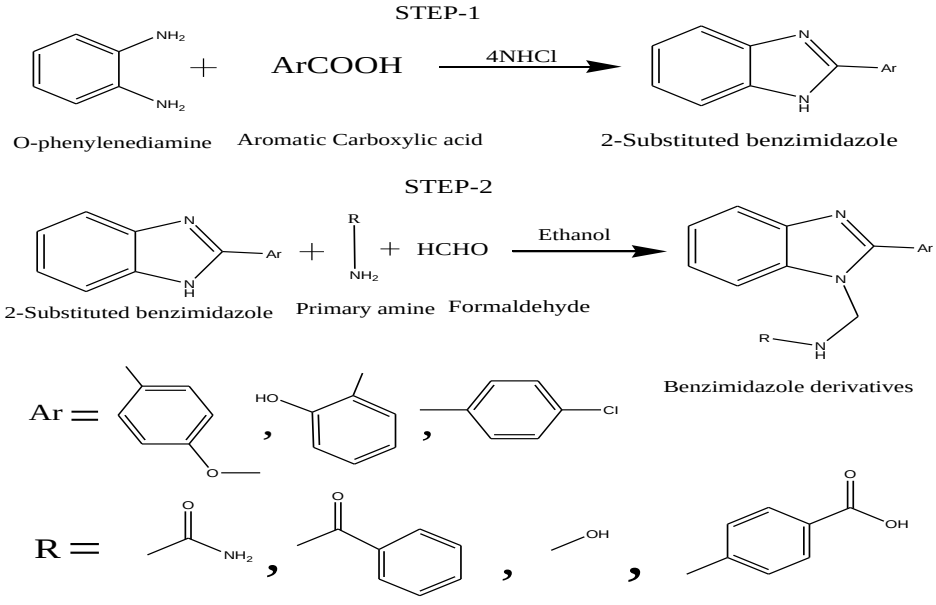


Table 1: Derivatives of 1,2-disubstituted benzimidazole derivatives

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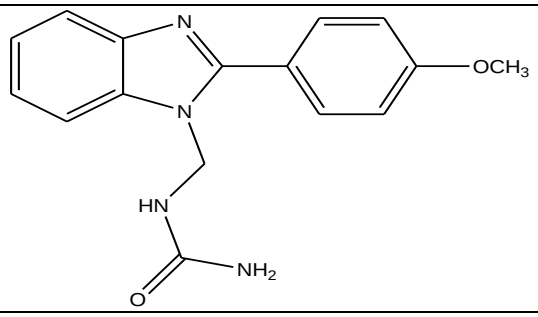
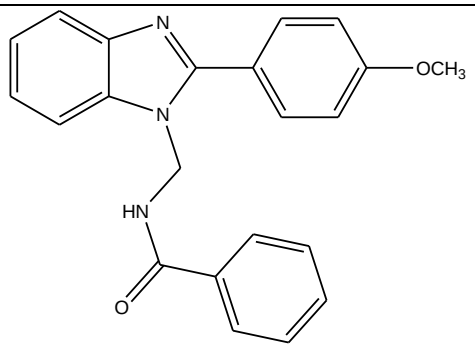
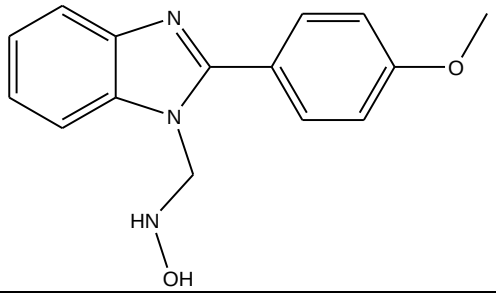
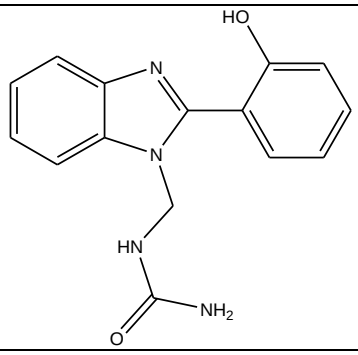
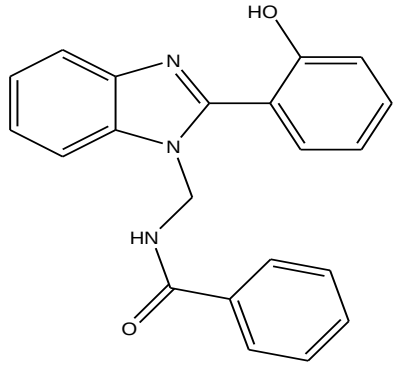
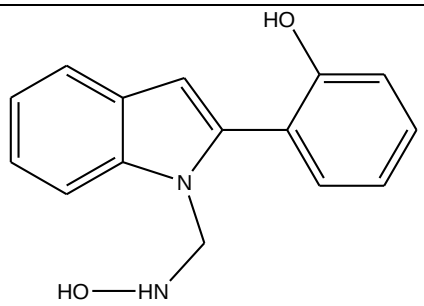
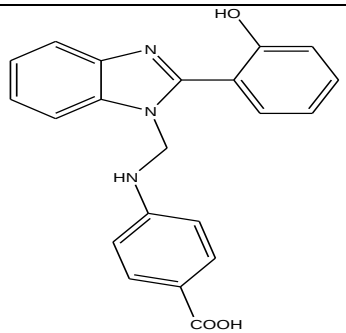
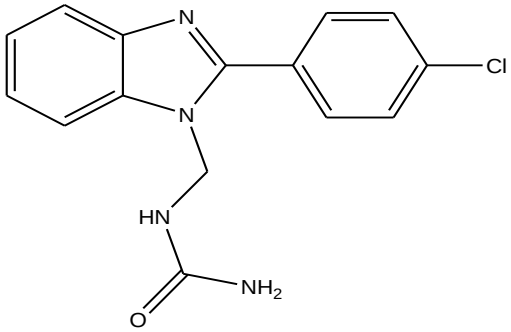
BZ_1	
BZ_2	
BZ_3	
BZ_4	

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)

^{1}HNMR (DMSO –d_6) δ ppm:

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)

2.3.4 1-{[2-(2-hydroxyphenyl)-1H-benzimidazol1-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-benzimidazol –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:

2.3.74-({[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

3.1. Evaluation of Anti tubercular activity (Maria et al., 2007).

The anti mycobacterial activity of compounds were assessed against *M.tuberculosis* using micro plate Alamar Blue assay (MABA) This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and Bacteic radiometric method. Briefly, 200μl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimize evaporation of medium in the test wells during incubation. The 96 wells plate received 100μl of the Middle Brook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 μg/ml.Plates were covered and sealed with parafilm and incubated at 37^{0}C for five days. After this time, 25 μl of freshly prepared 1.1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the colour change from blue to pink.

4. RESULTS AND DISCUSSION

4.1 Chemistry

In the present study, the mannich bases of benzimidazole derivatives were designed using CADD, of which eight derivatives satisfying the required criteria were synthesized as depicted in scheme. The physical data of all synthesized

compounds were given in Table 2. All the compounds were purified by recrystallization using absolute alcohol as solvent and the reaction completeness was established by single spot on TLC plate. The spectral data of synthesized compounds were characterized by IR,^{1}HNMR and mass spectral analysis.

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 Anti tubercular Activity

The synthesized derivatives BZ_2 and BZ_5 were tested for their anti – myco bacterial activity against *mycobacterium tuberculosis*. Both BZ_2 and BZ_5 showed significant activity with MIC of 12.5 μg/ml against *mycobacterium tuberculosis* H37RV strain. The MIC values of screened analogues were shown in Table No.3

Table 3: Anti-myco bacterial screening

No.	CODE	CONCENTRATIONS (μg/ml)							
		100	50	25	12.5	6.25	3.125	1.6	0.8
1.	BZ_2	Blue	Blue	Blue	Blue	Pink	Pink	Pink	Pink
2.	BZ_5	Blue	Blue	Blue	Blue	Pink	Pink	Pink	Pink

Blue denotes sensitive; pink denotes resistant

Standards used.

Pyrazinamide, streptomycin and ciprofloxacin were used as standards.Pyrazinamide was given minimum inhibitory concentration of 3.125 μg/ml. Streptomycin was given minimum inhibitory concentration of 6.25 μg/ml and ciprofloxacin was given minimum inhibitory concentration of 3.125 μg /ml.

Table 4: ANTI-TB Result

No.	CODE	CONCENTRATIONS (μg /ml)							
		100	50	25	12.5	6.25	3.125	1.6	0.8
1.	BZ_2	S	S	S	S	R	R	R	R
2.	BZ_5	S	S	S	S	R	R	R	R

S denotes sensitive ; R denotes resistant

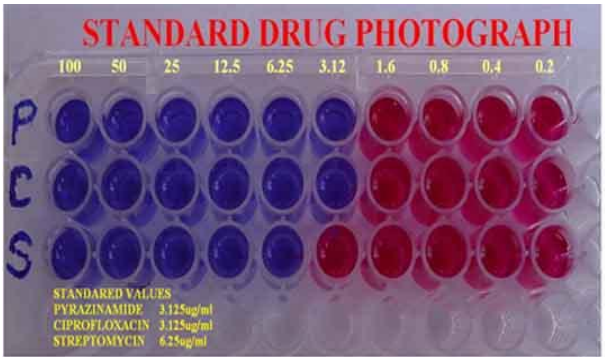


Figure 1:

Microplate showing the results of standard Microplate showing the results compounds of test compounds

Both compound BZ_2 and BZ_5 showed significant anti-tubercular activity which may be due to the formation of hydrogen bonding interaction with in the enzyme active binding site. This result revealed that the computational docking scores are in good agreement with the experimental values.

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 tubercular and anti fungal activity. Compounds BZ_2, BZ_5 posses significant anti-tubercular activity, compound BZ_6 posses better anti fungal activity.

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