WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 13, Issue 23, 847-862. Research Article ISSN 2277–7105

SYNTHESIS, CHARACTERIZATION & BIOLOGICAL EVALUATION OF SOME NEWER BENZIMIDAZOLE DERIVATIVES

Ankit* and Nitin Mittal

Department of Pharmaceutical Chemistry, Lords University, Alwa r, Alwar-bhiwadi
Highway, Chikani, Alwar Rajasthan, India-30102.

Article Received on
10 October 2024,
Revised on 30 October 2024,
Accepted on 20 Nov. 2024
DOI: 10.20959/wjpr202423-32667

***Corresponding Author**
Ankit
Department of
Pharmaceutical Chemistry,
Lords University, Alwa r,
Alwar-bhiwadi Highway,
Chikani, Alwar Rajasthan,
India-30102.

ABSTRACT

Benzimidazole derivatives play important role in medical field with so many Pharmacological activities such as antimicrobial, antiviral, antidiabetic and anticancer activity. The potency of these clinically useful drugs in treatment of microbial infections and other activities encouraged the development of some more potent and significant compounds. Benzimidazoles are remarkably effective compounds, extensive biochemical and pharmacological studies have confirmed that these molecules are effective against various strains of microorganisms. Firstly *o*-phenylenediamine was condensed with 3-hydroxy benzoic acid in the presence of 4N HCl to give 3-(1*H*-benzo[*d*]imidazole-2-yl) phenol. In the second step, 3-(1H-benzo[d]imidazole-2-yl) phenol was reacted with HBr in the presence of $ZnCl_2$ to give 2-(3-bromophenyl)-1*H*-benzo[*d*]imidazole. In the third step, 2-(3-bromophenyl)-1*H*-benzo[*d*]imidazole was reacted hydrazine hydrate and finally reacted with substituted benzaldehyde to give benzimidazole derivatives **(4a-4i).** The resultant products **(4a-4i)** have consistent values of C, H and N contents with predicted structure. The structures of newly synthesized benzimidazole derivatives were accomplished through IR, ^{1}HNMR and mass spectral data. All the newer Benzimidazole derivatives were evaluated for antibacterial action with respect to the mentioned species: **the strain of Gram +ve bacteria includes *B. subtilis, S.aureus* and the strains of Gram –ve incorporates *P.aeruginosa, E.coli*** and antifungal action with respect to ***Aspergillus niger* and *Candida albicans*** species of fungi. The antibacterial information shows that compounds containing nitro and methoxy substitution were more potent and the antifungal information recommend that compounds having methoxy were most active.

KEYWORDS: Benzimidazole, Antifungal, antimicrobial, Synthesis.

1. INTRODUCTION

Benzimidazole has a heterocyclic pharmacophore, which is an essential scaffold in drug and pharmaceutical development (Lee et.al, 2023).

Benzimidazole is a significant heterocyclic compound which is naturally dynamic. The benzimidazole core is of huge significance in restorative science. The most unmistakable benzimidazole compound in nature is N – ribosyl-dimethyl benzimidazole, which fills in as a pivotal ligand for cobalt in nutrient B 12 (Wikipedia.org, Walia et.al, 2011).

Benzimidazole derivatives play important role in medical field with so many Pharmacological activities such as antimicrobial, antiviral, antidiabetic and anticancer activity. The potency of these clinically useful drugs in treatment of microbial infections and other activities encouraged the development of some more potent and significant compounds. Benzimidazoles are remarkably effective compounds, extensive biochemical and pharmacological studies have confirmed that these molecules are effective against various strains of microorganisms (Pardeshi et. Al, 2021).

The discovery and development of medications with Benzimidazole moiety is currently a significant and appealing subject of intrigue given their tremendous remedial qualities (Tahlan et al, 2019).

In current years, Benzimidazole amalgams have developed as an eminent examination theme because of their different biological actions (Vasava et al, 2020).

Rings of benzimidazole moiety are importantly significant nitrogen containing heterocycles, which are generally investigated and used by the pharmaceutical business for medicate disclosure (Anand et.al, 2018).

Recent findings suggest that substituted benzimidazole derivatives possess potential chemotherapeutic activity with reduced toxic effects (Vashist et. Al, 2018).

2. MATERIAL AND METHOD

Chemistry

All the required chemicals were purchased from SDFCL and Sigma Aldrich. The melting points of the target compounds were determined in open capillary method using melting point apparatus and were used without correction. The purity of synthesized compounds were checked by a single spot in TLC on precoated silica gel aluminum sheets (Type 60 GF254, Merck) and the spots were visualized by iodine vapors and UV-lamp (254) nm. NMR spectra were determined on a Bruker Avance NMR Spectrometer. DMSO-d6 was used as a solvent at 300 MHz (1H NMR) and tetramethylsilane (TMS) as an internal standard. Splitting patterns are designated as: s, singlet; bs, broad singlet; d, doublet; t, triplet; m, multiplet. The infrared (FT-IR) spectra were recorded on Perkin elmer infrared spectrophotometer by using KBr discs.

Synthetic procedures

STEP 1 - Procedure for the preparation of 3-(1*H*-Benzo[*d*]imidazole-2-yl) phenol (1)

Equivalent molar amount of *o*-phenylenediamine (0.04mol) and 3-methoxy benzoic acid (0.04mol) were dissolved in 80ml of 4N HCl in a 500ml RBF. The mixture was refluxed for 17 hrs. On cooling, needle shaped crystals were obtained which were washed with ice-cold water. Crude product was recrystallized by hot water to get pure white crystalline compound (1). (Yield 78.90%, M.P. 138- 139^{0}C).

STEP 2- Procedure for the preparation of 2-(3-Bromophenyl)-1*H*-Benzo[*d*] imidazole (2)

In a RBF, attached with a reflux condenser (the top of which is associated to a guard tube for absorbing hydrogen chloride gas), Zinc chloride (2.09g,0.02 mol) was dissolved in 30 ml of hydrobromic acid by shaking and 3-(1*H*-Benzo[*d*]imidazole-2-yl) phenol (1) (2.1g,0.01 mol) was add on to this blend. The blend so formed was heated on water bath by means of reflux condenser for 15 hours. The Finishing point of chemical response was checked by Thin Layer Chromatography. At the time when the finishing of chemical reaction was observed, then reaction mixture was maintained in fridge for one night, product obtained was strained, recrystallized repeatedly by means of water to acquire the brown colored product (Yield 59.45%, m.p.152-153^{0}C).

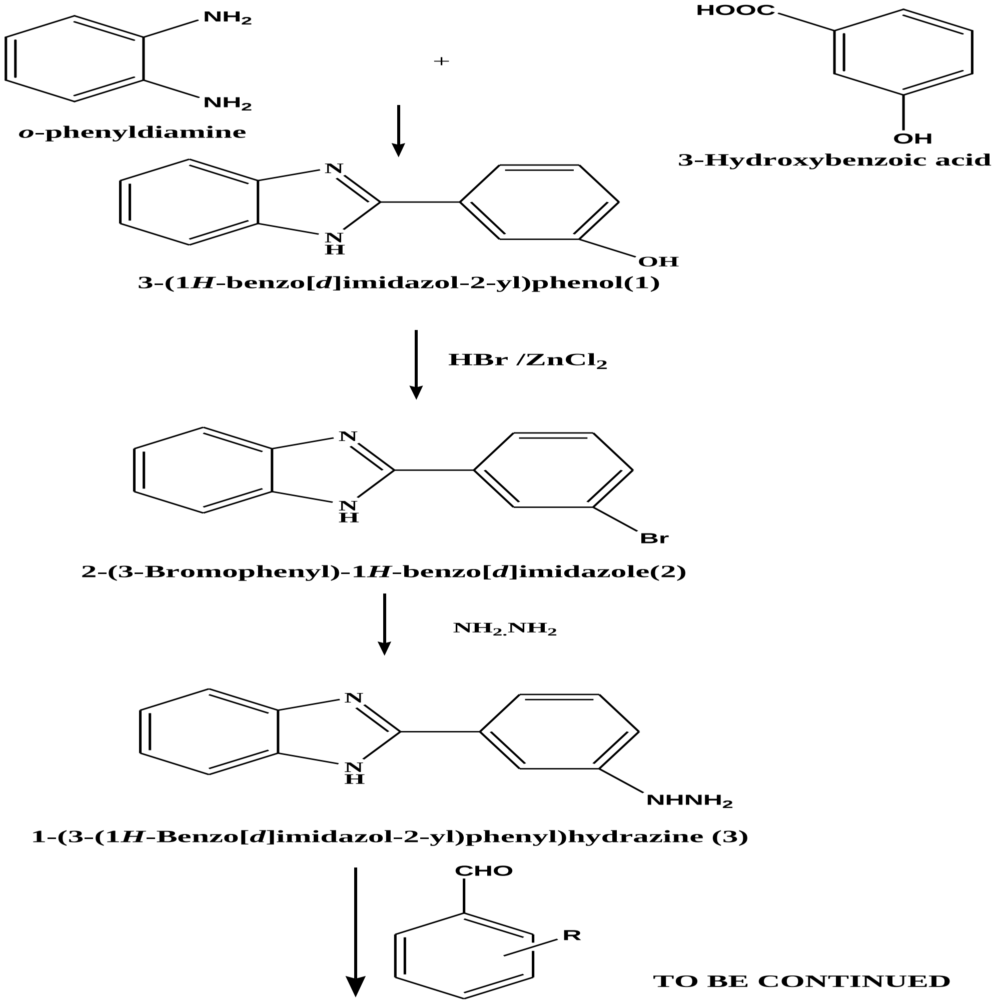
STEP 3- Procedure for the preparation of 1-(3-(1*H*-Benzo[*d*]imidazole-2-yl)phenyl) hydrazine (3)

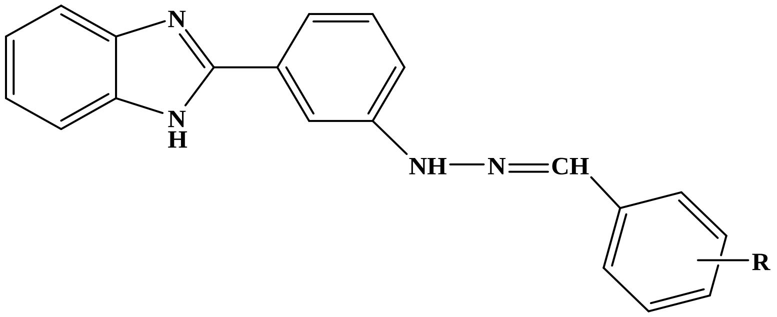
The intermediate obtained in previous reaction 2-(3-Bromophenyl)-1*H*-benzo[*d*] imidazole (2) (0.05 mole) was refluxed with hydrazine hydrate (0.05 mole) in 1,4- dioxane (35 ml)on water bath for around 5 hrs. Excessive amount of dissolving agent was withdrawn and the outcome obtained was recrystallized repeatedly by methanol to get the desired product.

STEP 4 - Procedure for the preparation of 1-(3-(1*H*-Benzimidazole-2-yl)phenyl)-2-(substitutedbenzylidene)hydrazine (4a-4i)

Various benzaldehyde derivatives (0.01 mol) was add on to a blend of 1-(3-(1*H*-Benzo[*d*]imidazole-2-yl)phenyl) hydrazine (3) (0.01 mol) in methanol (20 ml). The blend was boiled on heating mantle through reflux condenser at 65^{0}C for around 8 hrs, the reaction mixture so formed was cooled and crystallized by ethanol to get compounds **(4a-4i)**

Scheme of work





1-(3-(1*H*-Benzimidazole-2-yl)phenyl)-2-(substitutedbenzylidene)hydrazine (4a-4i)

Compound Code	R
4a	2-NO_2
4b	2-Cl
4c	2- OCH_3
4d	3-Cl
4e	3- OCH_3
4f	3-NO_2
4g	4-OCH_3
4h	4-Cl
4i	4-NO_2

1-(3-(1*H*-Benzo[*d*]imidazole-2-yl)phenyl)-2-(2-nitrobenzylidene)hydrazine (4a)

FTIR (KBr, ν, cm-1): 3334(Ar N-H stretch (2^oamine)), 3083(Ar C-H str.), 2954(Al C-H stretch), 1647(C=N stretch), 1547 (Ar C=C str.), 1521 (asymmetric N=O streching), 1420(C-N stretch), 1351 (symmetric N=O str.).

^{1}**H NMR** (300 MHz, DMSO, δ ppm): 6.370-7.863 ppm (m, 12H, Aromatic-H), 8.460 (s, 1H, Aromatic-H), 8.489 (s, 1H, N-H, D_2O exchangeable), 8.967(s, 1H, Ar. N-H, D_2O exchangeable).

MS (ESI) m/z [% rel. abundance]: 357.15 $[M]^+$, 358.13 $[M+1]^+$

Fragments- 193.07, 164.46, 118.53, 105.12, 90.34, 78.11

Elemental analysis

Calcd. For $C_{20}H_{15}N_5O_2$: C, 67.62; H, 4.48; N, 19.75; O, 8.95%.

Obtained: C, 67.69; H, 4.44; N, 19.51%

1-(3-(1*H*-Benzo[*d*]imidazole-2-yl)phenyl)-2-(2-chlorobenzylidene)hydrazine (4b)

FTIR (KBr) ν (cm-1): 3310(Ar N-H str. (2^oamine)), 3071(Ar C-H str.), 2907(Aliphatic C-H str.), 1672(Aromatic C-C str.), 1656(C=N stretching), 1489(C-N str.), 1049(C-Cl stretching).

^{1}**H NMR** (300 MHz, DMSO, δ ppm): 6.487-7.787 ppm (m, 12H, Ar-H), 7.321 (s, 1H, CH), 8.564 (s, 1H, N-H, D_2O exchangeable), 9.864 (s, 1H, Ar. N-H, D_2O exchangeable).

MS (ESI) m/z [% rel. abundance]: 346.10 $[M]^+$, 347.52 $[M+1]^+$, 348.47 $[M+2]^+$
Fragments- 193.77, 153.02, 117.63, 105.23, 90.45, 78.44

Elemental analysis
Calcd. For $C_{20}H_{15}ClN_4$: C, 69.67; H, 4.61; N, 16.21%,
Obtained: C, 69.47; H, 4.68; %; N,16.23%

1-(3-(1*H*-Benzo[*d*]imidazole-2-yl)phenyl)-2-(2-methoxybenzylidene)hydrazine (4c)
FTIR (KBr) ν (cm-1): 3283 (Ar N-H str. (2^{o}amine)), 3044 (Ar C-H stretch), 2875 (Al C-H str.), 1654(C=N stretching), 1585 (Ar C-C stretching), 1513 (C-N str.), 1342 (C-O-C, asymmetric).

^{1}**H NMR** (300 MHz, DMSO, δ ppm): 3.71(s, 3H, CH_3), 6.973-7.794 ppm (m, 12H, Aromatic H), 8.232 (s, 1H,-CH), 8.921 (s, 1H, N-H, D_2O exchangeable), 9.182 (s, 1H, Ar. N-H, D_2O exchangeable).

MS (ESI) m/z [% rel. abundance]: 342.52 $[M]^+$, 343.46 $[M+1]^+$, 344.89 $[M+2]^+$
Fragments- 342.52, 193.73, 149. 63, 118.56, 105.32, 90.57, 78.66

Elemental examined
Calcd. for $C_{21}H_{18}N_4O$: C, 73.64; H, 5.51; N, 16.17; O, 4.70%,
Obtained: C, 73.62; H, 5.53; N, 16.13%,O,4.72%

1-(3-(1*H*-Benzo[*d*]imidazole-2-yl)phenyl)-2-(3-chlorobenzylidene)hydrazine (4d)
FTIR (KBr) ν (cm-1): 3321(Ar N-H stretch (2^{o}amine)), 3092(Ar C-H stretching), 2910(Al C-H str.), 1661(C=N stretching), 1617(Ar C-C stretching), 1481(C-N str.), 1041(C-Cl str.).

^{1}**H NMR** (300 MHz, DMSO, δ ppm): 6.484(s, 1H, Ar-H), 7.101-7.852 ppm (m, 11H, Ar-H), 7.982 (s, 1H,-CH), 8.232 (s, 1H, N-H, D_2O exchangeable), 9.543 (s, 1H, Ar. N-H, D_2O exchangeable).

MS (ESI) m/z [% rel. abundance]: 346.25 $[M]^+$, 347.68 $[M+1]^+$, 348.56 $[M+2]^+$
Fragments- 193.67, 153.12, 117.54, 105.24, 90.42, 78.46

Elemental analysis

Calcd. For $C_{20}H_{15}ClN_4$: C, 69.67; H, 4.31; N, 16.21; Cl, 10.34%,

Found: C, 69.64; H, 4.32; N,16.23 Cl, 10.36%

1-(3-(1*H*-Benzo[*d*]imidazole-2-yl)phenyl)-2-(3-methoxybenzylidene)hydrazine (4e)

FTIR (KBr) ν (cm-1): 3291 (Aromatic N-H str. (2^{o}amine)), 3078 (Ar C-H str.), 2891 (Aliphatic C-H str.), 1644(C=N stretching), 1562 (Ar C-C str.), 1512 (C-N str.), 1304 (C-O-C, asymmetric).

^{1}**H NMR** (300 MHz, DMSO, δ ppm): 3.654 (s, 3H, CH_3), 6.526-7.827 ppm (m, 11H, Ar-H), 7.1453(s, 1H, Aromatic-H), 8.234 (s, 1H,-CH), 8.584 (s, 1H, Ar. N-H, D_2O exchangeable), 8.484 (s, 1H, N-H, D_2O exchangeable).

MS (ESI) m/z [% rel. abundance]: 342.67 $[M]^+$, 343.50 $[M+1]^+$, 344.94 $[M+2]^+$

Fragments- 193.67, 149. 59, 118.56, 105.34, 90.43, 78.65

Elemental analysis

Calcd. For $C_{21}H_{18}N_4O_2$: C, 73.04; H, 4.51; N, 16.47; O: 4.78%,

Found: C, 73.07; H, 4.53; N, 16.45; O: 4.79%

1-(3s-(1*H*-Benzo[*d*]imidazole-2-yl)phenyl)-2-(3-nitrobenzylidene)hydrazine (4f)

FTIR (KBr) ν (cm-1): 3318(Aromatic N-H stretching (2^{o}amine)), 3083(Ar C-H str.), 2934(Al C-H str.), 1687(C=N stretching), 1525 (Ar C-C stretch), 1512 (asymmetric N=O stretch), 1353 (symmetric N=O stretching), 1421(C-N stretching),

^{1}**H NMR** (300 MHz, DMSO, δ ppm): 6.546 -8.694 ppm (m, 11H, Ar-H), 8.547 (s, 1H,-CH), 8.683 (s, 1H, Ar-CH), 8.690 (s, 1H, N-H, D_2O exchangeable), 9.545 (s, 1H, Ar. N-H, D_2O exchangeable).

MS (ESI) m/z [% rel. abundance]: 357.48 $[M]^+$, 358.62$[M+1]^+$

Fragments-193.16, 164.39, 118.43, 105.23, 90.65, 78.43

Elemental analysis

Calcd. For $C_{20}H_{15}N_4O_2$: C, 67.66; H, 4.28; N, 19.55; O, 8.85%

Found: C, 67.63; H, 4.26; N, 19.54; O, 8.87%

1-(3-(1*H*-Benzo[*d*]imidazole-2-yl)phenyl)-2-(4-methoxybenzylidene)hydrazine (4g)

FTIR (KBr) ν (cm-1): 3285 (Ar N-H str. (2^{o}amine)), 3072 (Ar C-H str.), 2882 (Aliphatic C-H str.), 1672(C=N stretching), 1546 (Ar C-C str.), 1531 (C-N str.), 1305 (C-O-C, asymmetric).

^{1}**H NMR** (300 MHz, DMSO, δ ppm): 3.463 (s, 3H,-CH_3), 6.822- 7.543(dd, 4H, Ar-CH), 6.983 -7.794 ppm (m, 8H, Ar-H), 8.393 (s, 1H, -CH), 8.744 (s, 1H, Ar. N-H, D_2O exchangeable), 9.643 (s, 1H, N-H, D_2O exchangeable).

MS (ESI) m/z [% rel. abundance]: 342.74 $[M]^+$, 343.58 $[M+1]^+$, 344.62 $[M+2]^+$

Fragments- 193.61, 149. 43, 118.64, 105.32, 90.17, 78.56

Elemental analysis

Calcd. For $C_{21}H_{18}N_4O$: C, 73.64; H, 5.31; N, 16.47; O, 4.85%,

Found: C, 73.62; H, 5.32; N, 16.45%, O, 4.83%,

1-(3-(1*H*-Benzo[*d*]imidazole-2-yl)phenyl)-2-(4-chlorobenzylidene)hydrazine (4h)

FTIR (KBr) ν (cm-1): 3316(Aromatic N-H stretching (2^{o}amine)), 3082(Ar C-H stretch), 2906(Al C-H str.), 1683(C=N stretching), 1613(Ar C-C stretching), 1486(C-N str.), 1047(C-Cl str.).

^{1}**H NMR** (300 MHz, DMSO, δ ppm): 6.462- 7.896ppm (m, 8H, Ar-H), 7.454 – 7.643 (dd, 4H, Ar-CH), 8.562 (s, 1H, -CH), 8.683 (s, 1H, Ar. N-H, D_2O exchangeable), 9.432 (s, 1H, N-H, D_2O exchangeable).

MS (ESI) m/z [% rel. abundance]: 346.38 $[M]^+$, 347.70 $[M+1]^+$, 348.69 $[M+2]^+$

Fragments-193.61, 153.16, 117.59, 105.33, 90.39, 78.56

Elemental analysis

Calcd. For $C_{20}H_{15}ClN_4$: C, 69.35; H, 4.31; N, 16.27; Cl, 10.34%,

Found: C, 69.37; H, 4.33; N, 16.29% ; Cl, 10.32%,

1-(3-(1*H*-Benzo[*d*]imidazole-2-yl)phenyl)-2-(4-nitrobenzylidene)hydrazine (4i)

FTIR (KBr) ν (cm-1): 3315(Ar N-H str (2^{o}amine)), 3092(Ar C-H stretch), 2929(Al C-H stretching), 1676(C=N stretch), 1534 (Ar C-C stretching), 1511 (asymmetric N=O str.), 1443 (symmetric N=O str.), 1353 (C-N stretching).

^{1}H NMR (300 MHz, DMSO, δ ppm): 6.544- 7.701 ppm (m, 8H, Ar-H), 7.944 – 8.294 (dd, 4H, Ar-CH), 8.425 (s, 1H, -CH), 8.624 (s, 1H, Ar. N-H, D_2O exchangeable), 9.645 (s, 1H, N-H, D_2O exchangeable).

MS (ESI) m/z [% rel. abundance]: 357.65 $[M]^+$, 358.86$[M+1]^+$
Fragments-193.65, 164.32, 118.21, 105.89, 90.75, 78.91

Elemental examination
Obtained For $C_{20}H_{15}N_5O_2$: C, 67.66; H, 4.48; N, 19.55; O, 8.72%
Found: C, 67.68; H, 4.46; N, 19.54; O, 8.74%

Biological evaluation

2.3.1 Antibacterial activity

The antibacterial activity of newly synthesized compounds was performed by paper disc diffusion method using nutrient agar medium against following microorganisms: ***Staphylococcus aureus, Bacillus subtilis*** (Gram positive) and ***E. Coli, Pseudomonas aeureginosa*** (Gram negative).

In the paper disc-diffusion method, paper disc impregnated with compounds dissolved in DMSO at concentration 25, 50 and 100μg/ml were used. Disc impregnated with DMSO were used as solvent control for antibacterial activity because of free solubility of test compounds. Ciprofloxacin at concentration 50 μg/ml was used as standard drug for antibacterial activity. A series of compounds **4a-4i** were tested for antibacterial activity. The microorganism culture was spread over nutrient agar media in Petri dishes, and then the disc impregnated with the solution was placed on the surface of the media inoculated with bacterial strain.

The plates were incubated at 35^{o}C for 24 hours for bacterial culture. After incubation, the zones of inhibition around the disc were observed. The zones of inhibition indicates that the compounds inhibit growth of microorganism. Each testing is done in triplicate. Results were interpreted in terms of diameter (mm) of zone inhibition.
The results of antibacterial activity are shown in table: **2.4 & 2.5.**

Table 2.4: Antibacterial activity of the synthesised compounds.

No. of compound	**E.coli**			**B. subtilis**		
	25 μg ml^{-1}	**50 μg ml^{-1}**	**100 μg ml^{-1}**	**25 μg ml^{-1}**	**50 μg ml^{-1}**	**100 μg ml^{-1}**
4a	8.22±0.38	11.16±0.19	15.87±0.16	8.42± 0.21	11.28±0.64	15.28±0.17
4b	9.54±0.29	14.07±0.41	16.67±0.24	9.94± 0.34	10.57±0.17	16.65±0.15
4c	7.54 ±0.23	10.97±0.40	14.82±0.37	8.46± 0.19	11.69±0.91	15.53±0.34
4d	10.24±0.28	14.21±0.27	15.18±0.43	7.43± 0.16	11.51±0.26	14.72±0.27
4e	9.54± 0.19	11.57±0.64	15.02± 0.42	10.28±0.23	14.64±0.46	17.22±0.41
4f	9.21 ± 0.37	13.94±0.19	15.94± 0.84	8.87± 0.12	11.35±0.94	15.57±0.47
4g	7.56 ±0.51	10.14±0.72	14.54± 0.74	9.29± 0.57	11.69±0.16	16.32±0.51
4h	8.11±0.14	11.56±0.72	14.57± 0.41	10.95±0.78	14.39±0.42	16.97±0.24
4i	7.79±0.64	11.34±0.43	15.24± 0.87	9.42± 0.57	10.64±0.72	15.27±0.43
Ciprofloxacin		14.38±0.13			14.82±0.21	
Control						

Table 2.5: Antibacterial activity of the synthesised compounds.

No. of compound	**P. Aeruginosa**			**S.aureus**		
	25 μg ml^{-1}	**50 μg ml^{-1}**	**100 μg ml^{-1}**	**25 μg ml^{-1}**	**50 μg ml^{-1}**	**100 μg ml^{-1}**
4a	10.57±0.35	11.36±0.54	14.54±0.17	10.52± 0.11	14.08±0.49	17.06±0.35
4b	9.54± 0.12	12.04±0.31	15.28±0.35	9.14± 0.17	11.61±0.35	14.35±0.29
4c	10.24±0.18	14.38±0.46	17.5±1 0.17	8.87± 0.42	12.26±0.14	15.37±0.64
4d	8.18± 0.21	11.64±0.54	15.21± 0.13	7.12 ±0.11	10.84±0.49	15.87±0.27
4e	10.64±0.17	14.24±0.12	17.69± 0.37	8.57± 0.53	11.64±0.35	15.49±0.17
4f	9.42± 0.72	11.03±0.31	15.68± 0.68	7.28± 0.33	12.54±0.15	16.65±0.24
4g	8.27± 0.34	11.26±0.19	14.34± 0.54	10.84± 0.29	13.97±0.46	17.28±0.15
4h	8.86± 0.47	12.35±0.42	15.69±0.36	7.39± 0.74	11.31±0.79	14.10±0.48
4i	10.86±0.24	14.38±0.68	16.51 ±0.74	10.27± 0.31	14.38±0.42	15.84±0.28
Ciprofloxacin		14.49±0.16			14.79±0.12	
Control						

Antifungal activity

The newly synthesized compounds were tested for their antifungal activity using disc diffusion method on nutrient agar medium. The *in vitro* study was done for all the synthesized compounds for the comparison of antifungal activity. The following strains were used: ***Aspergillus niger*** and ***Candida albicans.***

A spore suspension in normal saline was prepared from the culture of the test fungi on sabouraud's broth media. Each Petri plate was divided into 5 equal portions along the diameter to place one disc in each portion. Three discs of test sample were placed on three portions together with one disc of reference drug Fluconazole and the last one impregnated with the solvent (DMF) as negative control. Test samples were tested at 50, 100 and

200µg/ml concentration in DMF. Fluconazole in the concentration of 50µg/ml was used as a standard drug for antifungal activity. A series of compounds **4a-4i** were tested for antifungal activity.

The Petri plates inoculated with fungal cultures were incubated at 25^{o}C for 48 hours. Antifungal activity was determined by measuring the diameter of the inhibition zone for triplicate sets. The diameters obtained for the test samples were compared with that produced by the standard drug Fluconazole.

The results of antifungal activity are shown in table: **2.6**

Table 2.6: Antifungal activity of the synthesised compounds.

No. of compound	**C. albicana**			**A. niger**		
	25μg ml^{-1}	**50μg ml^{-1}**	**100μg ml^{-1}**	**25μg ml^{-1}**	**50μg ml^{-1}**	**100μg ml^{-1}**
4a	6.39 ±0.5	8.55± 0.3	11.6± 0.42	6.47±0.31	8.65±0.59	10.9 ±0.6
4b	7.48±0.2	8 ± 0.9	10.8 ± 0.8	8.37±0.31	9.8± 0.5	10.42±0.21
4c	8.19±0.17	11.47±0.5	13.56±0.40	8.75±0.37	11.15±0.9	13.26± 0.7
4d	7.60±0.4	10.3±0.9	12.55±0.5	8.04±0.71	10.4±0.8	12.5±0.31
4e	6.3±0.9	9.9±0.2	11.3 ±0.15	6.3 ±0.6	9.63±0.7	12.70±0.29
4f	7.77±0.3	10.9±0.7	13.20±0.6	8.6±0.3	10.6±0.5	12.7±0.12
4g	7.89±0.60	11.6 ±0.9	13.3±0.41	7.52±0.7	11.10±0.4	13.51±0.4
4h	8.13±0.11	11.09±0.97	13.02±0.11	7.65 ±0.9	11±0.2	13.6±0.46
4i	7.9 ±0.14	10.16±0.3	12.53±0.46	6.63 ±0.5	9.86 ±0.1	12.4±0.8
Fluconazole		12.4 ±0.5			11.2 ±0.8	
Control						

3. BIOLOGICAL RESULT AND DISCUSSION

Antibacterial activity

The newly synthesized compounds were tested in vitro for their antibacterial activity using disc diffusion method. The antibacterial activity of all the synthesized compounds were conducted against two gram positive bacteria ***B.subtilis, S.aureus*** and two gram negative bacteria ***E.coli, P.aureginosa*** at concentrations 25, 50 and 100µg/ml, compared to standard drug Ciprofloxacin (50µg/ml). Zone of inhibition was measured by antibiotic zone reader.

The result revealed that the newly synthesized compound **4b, 4d, 4f** showed good antibacterial activity with 14.07, 14.21 and 13.94 mm zone of inhibition respectively against *E.coli* when given at concentration 50 μg ml^{-1} whereas under identical conditions standard drug ciprofloxacin showed 14.38mm zone of inhibition. Compounds 4a, 4c, 4e, 4g, 4h, 4i showed moderate antibacterial action having values 11.16, 10.97, 11.57, 10.14, 11.56, 11.34 mm zone of inhibition respectively against *E.coli*. Compounds **4e, 4h** showed good antibacterial activity with 14.64, 14.39 mm zone of inhibition respectively against *B. subtilis* when given at concentration 50 μg ml^{-1} whereas under identical conditions standard drug ciprofloxacin showed 14.82 mm zone of inhibition. Compounds 4a, 4b, 4c, 4d, 4f, 4g, 4i showed moderate antibacterial activity11.28, 10.57,11.69,11.51,11.35,11.69,10.64 mm zone of inhibition respectively against *B. subtilis.* Compounds **4c, 4e, 4i** showed good antibacterial activity with 14.38, 14.24, 14.38 mm zone of inhibition respectively against *P.aeruginosa* when given at concentration 50 μg ml^{-1} whereas under identical conditions standard drug ciprofloxacin showed 14.49 mm zone of inhibition. Compounds 4a, 4b, 4d, 4f, 4g, and 4h showed moderate antibacterial activity 11.36, 12.04, 11.64, 11.03, 11.26, 12.35 mm zone of inhibition respectively with respect to *P.aeruginosa*. Compounds **4a, 4g, 4i** indicated good antibacterial activity with 14.08, 13.97, 14.38 mm zone of inhibition respectively against *S.aureus* when given at concentration 50 μg ml^{-1} whereas under identical conditions standard drug ciprofloxacin showed 14.79 mm zone of inhibition. Compounds 4b, 4c, 4d, 4e, 4f, and 4h indicated moderate antibacterial action 11.61, 12.26, 10.84, 11.64, 12.54, 11.31 mm zone of inhibition respectively against *S.aureus.*

The result of antibacterial studies indicated the significant activity of the newly synthesized benzimidazole derivatives when compared to standard drug Ciprofloxacin.

Antifungal activity

The newly synthesized compounds were screened for *in-vitro* antifungal activity using disc diffusion method. The following fungal strains were used: ***C. albicans*** and ***A. niger.***

The results showed that the newly synthesized compounds **4c, 4g, 4h** indicates good antifungal activity with11.47, 11.6, 11.09 mm zone of inhibition respectively against *Candida albicans* when given at concentration 50 μg ml^{-1} whereas under identical conditions standard drug fluconazole showed 14.40 mm zone of inhibition. Compounds 4a, 4b, 4d, 4e, 4f, 4i indicates moderate antifungal action with 8.55, 8, 10.3, 9.9, 10.9, 10.16 mm zone of inhibition respectively against *Candida albicans.* Compounds **4c, 4g, 4h** showed good antifungal activity with 11.15, 11.10, 11 mm zone of inhibition respectively against *Aspergillus niger* when given at concentration 50 μg ml^{-1} whereas under identical conditions standard drug fluconazole showed 11.2 mm zone of inhibition. Compounds 4a, 4b, 4d, 4e, 4f, 4i indicates moderate antifungal action with 8.65, 9.8, 10.4, 9.63, 10.6, 9.86 mm zone of inhibition respectively against *Aspergillus niger.*

4. CONCLUSION

Firstly *o*-phenylenediamine was condensed with 3-hydroxy benzoic acid in the presence of 4N HCl to give 3-(1*H*-benzo[*d*]imidazole-2-yl) phenol. In the second step, 3-(1H-benzo[d]imidazole-2-yl) phenol was reacted with HBr in the presence of $ZnCl_2$ to give 2-(3-bromophenyl)-1*H*-benzo[*d*]imidazole. In the third step, 2-(3-bromophenyl)-1*H*-benzo[*d*]imidazole was reacted hydrazine hydrate and finally reacted with substituted benzaldehyde to give benzimidazole derivatives **(4a-4i).** The resultant products **(4a-4i)** have consistent values of C, H and N contents with predicted structure. The structures of newly synthesized benzimidazole derivatives were accomplished through IR, ^{1}HNMR and mass spectral data.

All the newer Benzimidazole derivatives were evaluated for antibacterial action with respect to the mentioned species: **the strain of Gram +ve bacteria includes *B. subtilis, S.aureus* and the strains of Gram –ve incorporates *P.aeruginosa, E.coli*** and antifungal action with respect to ***Aspergillus niger* and *Candida albicans*** species of fungi. The antibacterial information shows that compounds containing nitro and methoxy substitution were more potent and the antifungal information recommend that compounds having methoxy were most active.

ACKNOWLEDGEMENT

Authors are grateful to the management of Lords University, Alwar, Alwar-Bhiwadi Highway, Chikani, Alwar, Rajasthan, India-301028 for the help in the research.

Conflict of Interest: No conflict of interest between the authors regarding this research work.

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