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## SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF BENZIMIDAZOLE DERIVATIVES

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**ABSTRACT:** Benzimidazoles are an important class of compounds with a wide spectrum of biological activity ranging from anti-hypertensive, anti-viral, anti-fungal, antitumor and anthelmintic activity. Benzimidazole rings are the most important nitrogen-containing heterocycles, which are widely explored and utilized by the pharmaceutical industry for drug discovery. 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. Numerous benzimidazole based drugs have been extensively used in the clinic to treat various types of diseases with high therapeutic potential. A series of benzimidazole derivatives were synthesized by a single step process by reacting o-phenylenediamine and benzoic acid. The purity and structure confirmation of the synthesized compounds were done by TLC and <sup>1</sup>H-NMR. The compounds were evaluated for anti-microbial, anti-fungal and antioxidant activity.

**INTRODUCTION:** Benzimidazole rings are the most important nitrogen-containing heterocycles, which are widely explored and utilized by the pharmaceutical industry for drug discovery<sup>1</sup>. Due to their special structural features and electron-rich environment, Benzimidazole containing drugs bind to a variety of therapeutic targets<sup>2</sup>, thereby exhibiting a broad spectrum of bioactivities. Numerous benzimidazole based drugs have been extensively used in the clinic to treat various types of diseases with high therapeutic potential<sup>3</sup>. Benzimidazole derivatives play important role in medical field with so many Pharmacological activities such as antimicrobial, antiviral, anti-diabetic and anticancer activity<sup>3,4</sup>.

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<sup>5-7</sup>. Due to their enormous medicinal value, the research and development of benzimidazole-containing drugs is an increasingly active and attractive topic of medicinal chemistry. This review enlightens about the chemistry of different derivatives of substituted benzimidazoles along with their pharmacological activities<sup>8-11</sup>. Antimicrobial agents / Antibiotics are antibacterial substances produced by various species of micro-organism (bacteria, fungi, and actinomycetes) that suppress the growth of other micro-organisms. They have been designed to inhibit or kill the infecting organism without having measurable effect on the recipient<sup>4,7,11</sup>.

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Antioxidants are nutrients that help to protect cells from oxidative stress which is a natural damaging physiological process<sup>12</sup>. These nutrients are either present naturally in various types of food or taken as dietary supplements. They play defensive role against oxygen free radical toxicity in our body. Free radicals such as superoxide, hydroxyl and peroxide radicals are capable of damaging all types of biomolecules. They play vital role in causation and progress of oxidative stress related diseases such as Carcinogenesis, Alzheimer, Parkinson, Inflammatory diseases and Cataract. Thus, antioxidants may be considered as scavengers of free radicals. Reactive oxygen species (ROS) are formed when oxygen is present in excess and its reduction is insufficient. Natural occurring antioxidants are flavonoids, phenolic acid and alkaloids<sup>13</sup>.

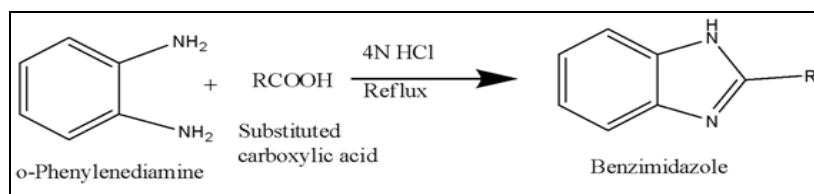
The prepared compounds were subjected to physicochemical studies like melting point determination, TLC and percentage yield. The structures of synthesized compounds were characterized by IR and NMR spectroscopy<sup>14, 15</sup>. The biological evaluation of newly synthesized compounds was carried out against *E. coli* and *Staphylococcus aureus* for antibacterial screening. The other *in vitro* activities carried out were anti-inflammatory and antioxidant activity. Benzimidazoles mainly possess various types of

biological activities like anti-inflammatory, anti-cancer, anti-fungal, antiviral and anthelmintic activity *etc.* The Benzimidazole derivatives have been the center of the attention of researchers over many years due to high practical value of these compounds<sup>16-26</sup>.

#### MATERIALS AND METHODS:

**Chemistry:** Nuclear Magnetic Resonance (<sup>1</sup>H-NMR) spectra were recorded on a Bruker using CDCl<sub>3</sub>. The Chemical shift values are reported in parts per million (ppm) relative to Tetra methyl silane as internal reference. Infra-red (IR) spectra were recorded with a Bruker spectrophotometer. The melting point ranges of newly synthesized compounds were determined by open glass capillary tube using Lab India's visual melting point apparatus and were uncorrected. All the commercially available reagent grade chemicals were used as received. Purity of the compound and progress of the reaction were monitored by thin layer chromatography (TLC), with detection by Ultra-violet (UV) light and / or spots were visualized by exposure to iodine vapors<sup>27</sup>.

**Synthesis:** The title compounds were synthesized using synthetic strategy described in **Fig. 1**<sup>28</sup>. Benzimidazole compounds were synthesized starting from o-phenylenediamine and benzoic acid.

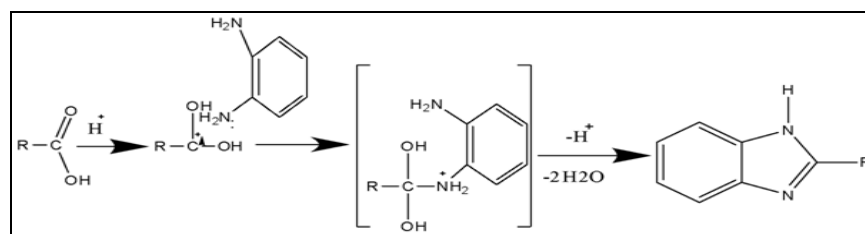


**FIG. 1: SYNTHESIS OF BENZIMIDAZOLE DERIVATIVES**

Substituted carboxylic acid: Formic acid, Benzoic acid, 2-amino benzoic acid, 3, 4-dimethoxybenzoic acid, 3, 4, 5-trimethoxybenzoic acid, 2-chloro-4-nitro benzoic acid, 2-chloro-5-nitro benzoic acid, 2-Iodo benzoic acid, 4-methoxyphenyl acetic acid, 4-ethyl benzoic acid, 2, 4, 5-trifluoro benzoic acid, 4-chloro-3, 5-dinitro benzoic acid

**Mechanism of Reaction:** The role of hydrochloric acid is to activate carboxyl group by addition of a proton to oxygen, forming carbonium ion.

The reaction mechanism involves carbonium ion intermediate.



**General Procedure for Synthesis of Title Compound (1a-l):** O-phenylenediamine (0.01 mole) was refluxed with formic acid, benzoic acid and its derivatives (0.01 mole) in the presence of 4N hydrochloric acid (4N HCl) for 4 - 5 hrs at temperature 80 - 120 °C, rpm 320 on reflux condenser. The completion of the reaction was checked by TLC (chloroform: methanol 9:1). On completion, 10% NaOH (w/v) was gradually added until the reaction mixture turns alkaline. Reaction mixture was cooled in ice bath, allowed to stand for 5 min to obtain precipitate. The product was is filtered, dried and recrystallized from ethanol<sup>14,27,28</sup>.

**CSK - 0:1 H- Benzimidazole (1.a):** Yield 64%, M.P.170-173 °C; ATR: N-H (3512.89 cm<sup>-1</sup>), C = C (1510.45, 1454 cm<sup>-1</sup>), C = N (1665.55 cm<sup>-1</sup>).

<sup>1</sup>HNMR (DMSO, 300MHz): δ 5.00 (s, 2H NH), 7.26 (dd, 2H, *Ar-H*), 7.70 (dd, 2H, *Ar-H*), 8.08 (s, 1H, *Ar-H*).

**CSK-00:2-Phenyl-1H-Benzimidazole (1.b):** Yield 66%, M.P. 293 - 296 °C; ATR: N-H (3529.43cm<sup>-1</sup>), C = C (1443.83, 1409.39 cm<sup>-1</sup>), C = N (1569.57 cm<sup>-1</sup>).

<sup>1</sup>HNMR (DMSO, 300MHz): δ 5.00 (s, 1H NH), 7.24 (dd, 2H, *Ar-H*), 7.54-7.46 (m, 3H, *Ar-H*), 7.66 (dd, 2H), 8.39-8.31 (m, 2H, *Ar-H*).

**CSK- 1: 2- (1H- 1, 3- zbenzodiazol- 2- yl) aniline (1.c):** Yield 77%, M.P. 224 - 226 °C; ATR: N-H (3354.70 cm<sup>-1</sup>), C = C (1289.25, 1351.07 cm<sup>-1</sup>), C = N (1701.08 cm<sup>-1</sup>).

<sup>1</sup>HNMR (DMSO, 300MHz): δ 6.72 (1H, ddd, *Ar-H*), 7.15-7.25 (3H, 7.15 (ddd, *Ar-H Benz*), 7.23 (ddd, *Ar-H Benz*), 7.39 (ddd, *Ar-H*)), 7.49 (1H, ddd, *Ar-H*), 7.57-7.58 (2H, 7.57 (ddd, *Ar-H*), 7.58 (ddd, *Ar-H Benz*)).

**CSK- 2: 2- (3, 4- dimethoxyphenyl)- 1H- 1, 3- benzodiazole (1.d):** Yield 70%, M.P. 234 - 237 °C; ATR: N-H (3547.86 cm<sup>-1</sup>), C = C (1514.94, 1465.30 cm<sup>-1</sup>), C = N (1675.17 cm<sup>-1</sup>), Asymmetric C-O-C (1265.64 cm<sup>-1</sup>), Symmetric C-O-C (1134.19 cm<sup>-1</sup>), Aromatic C-H stretch (3360.64 cm<sup>-1</sup>), Out of plane ring C-O bend (627.52 cm<sup>-1</sup>), Out of plane C-H bend (723.04, 761.76 cm<sup>-1</sup>).

<sup>1</sup>HNMR (DMSO, 300MHz): δ 3.83 (3H, 3.83 (s), 3.83 (s), 3.83 (s)), 3.83 (3H, 3.83 (s), 3.83 (s), 3.83

(s)), 6.99 (1H, dd, *Ar-H*), 7.22 (1H, ddd, *Ar-H*), 7.25-7.28 (2H, 7.25 (dd, *Ar-H*), 7.28 (ddd, *Ar-H*)), 7.54 (1H, dd, *Ar-H*), 7.59 (1H, ddd, *Ar-H*), 7.60 (1H, ddd, *Ar-H*).

**CSK- 3: 2- (3, 4, 5- trimethoxyphenyl)- 1H- 1, 3- benzodiazole (1.e):** Yield 83%, M.P. 206 - 208 °C; ATR: N-H (3381.28 cm<sup>-1</sup>), C = C (1415.25 cm<sup>-1</sup>), C = N (1678.94 cm<sup>-1</sup>), Asymmetric C-O-C (1227.43 cm<sup>-1</sup>), Symmetric C-O-C (1183.41 cm<sup>-1</sup>), Out of plane ring C-O bend (710.92 cm<sup>-1</sup>), Out of plane C-H bend (752.85 cm<sup>-1</sup>).

<sup>1</sup>HNMR (DMSO, 300MHz): δ 3.83 (9H, 3.83 (s), 3.83 (s), 3.83 (s), 3.83 (s), 3.83 (s), 3.83 (s), 3.83 (s), 3.83 (s), O-CH<sub>3</sub>), 6.94 - 7.24 (3H, 6.94 (s), 6.94 (s), 7.24 (ddd, *Ar-H*)), 7.26 (1H, ddd, *Ar-H*), 7.57 (1H, ddd, *Ar-H*), 7.58 (1H, ddd, *Ar-H*).

**CSK- 4: 2- (2- chloro- 4- nitrophenyl)- 1H- 1, 3- benzodiazole (1.f):** Yield 74%, M.P. 185 - 188 °C; ATR: NH (3405.85 cm<sup>-1</sup>), Aromatic C-H stretch (3466.92 cm<sup>-1</sup>), Out of plane C-H bend (851.48, 897.55 cm<sup>-1</sup>), Aromatic C = C (1594.67 cm<sup>-1</sup>), Ortho substitution (738.05 cm<sup>-1</sup>), C - Cl (802.63 cm<sup>-1</sup>) NO<sub>2</sub> (1520.29, 1475.77 cm<sup>-1</sup>).

<sup>1</sup>HNMR (300MHz, DMSO): <sup>1</sup>H NMR: δ 7.26 (1H, td, *Ar-H Benz*), 7.60 (1H, ddd, *Ar-H Benz*), 7.61 (1H, ddd, *Ar-H Benz*), 7.62 (1H, ddd, *Ar-H Benz*), 7.81-8.21 (2H, 7.81 (dd, *Ar-H*), 8.21 (dd, *Ar-H*)), 8.39 (1H, dd, *Ar-H*).

**CSK- 5: 2- (2- chloro- 5- nitrophenyl)- 1H- 1, 3- benzodiazole (1.g):** Yield 60%, M.P. 211 - 214 °C; ATR: NH (3446.35 cm<sup>-1</sup>), Aromatic C = C (1612.28 cm<sup>-1</sup>), C-N, C - C bend (1575.25, 1418.77 cm<sup>-1</sup>), Ortho substitution (734.82 cm<sup>-1</sup>), C-Cl (846.12 cm<sup>-1</sup>), *Ar*-NO<sub>2</sub> Asymmetric and Symmetric (1534.00, 1352.62 cm<sup>-1</sup>).

<sup>1</sup>H NMR (300MHz, DMSO): δ 7.23-7.30 (2H, 7.23 (ddd, *Ar-H Benz*), 7.30 (ddd, *Ar-H Benz*)), 7.57 (1H, ddd, *Ar-H Benz*), 7.71 (1H, dd, *Ar-H*), 8.16 (1H, ddd, *Ar-H Benz*), 8.42 (1H, dd, *Ar-H*), 8.50 (1H, dd, *Ar-H*).

**CSK- 6: 2- (2-iodophenyl)-1H-1, 3-benzodiazole (1.h):** Yield 68%, M.P. 235 - 237 °C; ATR: NH (3344.11 cm<sup>-1</sup>), CH<sub>2</sub> (2892.74cm<sup>-1</sup>), *Ar* C = C (1579.43, cm<sup>-1</sup>), CH<sub>2</sub>I bend (1263.17 cm<sup>-1</sup>), ortho substitution (735.82 cm<sup>-1</sup>), R - I (<600.00 cm<sup>-1</sup>).

$^1\text{H}$  NMR (300 MHz, DMSO):  $\delta$  7.10-7.30 (2H, 7.10 (ddd, *Ar-H Benz*), 7.25 (ddd, *Ar-H Benz*)), 7.38 (1H, ddd, *Ar-H*), 7.50-7.58 (2H, 7.50 (ddd, *Ar-H*), 7.58 (ddd, *Ar-H*)), 7.63 (1H, ddd, *Ar-H*), 7.81-7.84 (2H, 7.81 (ddd, *Ar-H Benz*), 7.84 (ddd, *Ar-H Benz*)).

**CSK- 7: 2- benzyl-1H-1, 3- benzodiazole (1.i):** Yield 75%, M.P. 275 - 278 °C; ATR:  $\text{NH}_2$  (3443.09  $\text{cm}^{-1}$ ),  $\text{CH}_2$  (2978.21, 2884.09  $\text{cm}^{-1}$ ),  $\text{Ar C} = \text{C}$  (1610.06,  $\text{cm}^{-1}$ ),  $\text{CH}_2$  bend (1441.28  $\text{cm}^{-1}$ ).  $^1\text{HNMR}$  (300MHz, DMSO):  $\delta$  4.08 (2H, 4.08 (s), 4.08 (s),  $-\text{CH}_2$ ), 6.62 (1H, ddd, *Ar-H Benz*), 7.19 (1H, ddd, *Ar-H Benz*), 7.21-7.26 (5H, 7.25 (tt, *Ar-H*), 7.21 (ddd, *Ar-H*), 7.21 (ddd, *Ar-H*), 7.26 (td, *Ar-H*), 7.26 (td, *Ar-H*)), 7.53-7.57 (2H, 7.53 (ddd, *Ar-H Benz*), 7.57 (ddd, *Ar-H Benz*)).

**CSK- 8: 2-(4-ethylphenyl)-1H-1, 3-benzodiazole (1.j):** Yield 55%, M.P. 192 - 195 °C; ATR:  $\text{NH}_2$  (3380.33  $\text{cm}^{-1}$ ),  $\text{CH}_2$  (2991.06, 2718.76  $\text{cm}^{-1}$ ),  $\text{Ar C} = \text{C}$  (1603.00  $\text{cm}^{-1}$ ),  $\text{CH}_2$  bend (1475.96  $\text{cm}^{-1}$ ), para substitution (753.89  $\text{cm}^{-1}$ ).

$^1\text{HNMR}$  (300MHz, DMSO):  $\delta$  1.31 (3H, 1.31 (t,  $-\text{CH}_3$ ), 1.31 (t,  $-\text{CH}_3$ ), 1.31 (t,  $-\text{CH}_3$ )), 2.64 (2H, 2.64 (q,  $-\text{CH}_2$ ), 2.64 (q,  $-\text{CH}_2$ )), 7.24-7.27 (2H, 7.24 (dd, *Ar-H Benz*), 7.27 (dd, *Ar-H Benz*)), 7.33 (2H, 7.33 (dd, *Ar-H*), 7.33 (dd, *Ar-H*)), 7.58 (2H, 7.58 (dd, *Ar-H*), 7.58 (dd, *Ar-H*)), 7.62 (1H, ddd, *Ar-H Benz*), 7.62 (1H, ddd, *Ar-H Benz*)

**CSK- 9: 2- (2, 4, 5-trifluorophenyl)-1H-1, 3-benzodiazole (1.k):** Yield 60%, M.P. 182 - 184 °C; ATR:  $\text{NH}$  (3398.54  $\text{cm}^{-1}$ ),  $\text{CH}_2$  (2918.94, 2851.71  $\text{cm}^{-1}$ ),  $\text{Ar C} = \text{C}$  (1475.22  $\text{cm}^{-1}$ ),  $\text{CH}_2$  bend (1462.98  $\text{cm}^{-1}$ ), para substitution (827.88  $\text{cm}^{-1}$ ).

$^1\text{HNMR}$ : (300MHz, DMSO):  $\delta$  6.96-7.25 (2H, 7.24 (d, *Ar-H*), 7.25 (td, *Ar-H Benz*)), 7.35 (1H, ddd, *Ar-H Benz*), 7.56-7.58 (2H, 7.56 (ddd, *Ar-H Benz*), 7.58 (ddd, *Ar-H Benz*)), 7.58 (1H, d, *Ar-H*).

**CSK- 10: 2- (4-chloro-3, 5-dinitrophenyl)-1H-1, 3-benzodiazole (1.l):** Yield 62%, M.P. 161-163 °C; ATR:  $\text{NH}$  (3460.10, 3350.45  $\text{cm}^{-1}$ ),  $\text{CH}_2$  (2924.67, 2853.51  $\text{cm}^{-1}$ ),  $\text{Ar C} = \text{C}$  (1475.11  $\text{cm}^{-1}$ ),  $\text{CH}_2$  bend (1463.55  $\text{cm}^{-1}$ ), para substitution (799.85  $\text{cm}^{-1}$ ).

$^1\text{HNMR}$  (300MHz, DMSO):  $\delta$  5.80(s, 1H,  $\text{NH}$ ), 7.27 (1H, ddd, *Ar-H Benz*), 7.58 (1H, ddd, *Ar-H Benz*), 7.61-7.63 (2H, 7.61 (ddd, *Ar-H Benz*), 7.63

(ddd, *Ar-H Benz*)), 8.80 (2H, 8.80 (s), 8.80 (s) *Ar-H*).

### Pharmacological Evaluation: <sup>16-25, 29-34</sup>

**Antimicrobial Activity:** All synthesised compounds were screened for *in vitro* antibacterial activity against one gram positive strain of bacteria (*S. aureus*) and one gram negative strain of bacteria (*E. coli*) by cup plate method. Cefodoxamine was used as reference antibacterial drug <sup>19, 20, 33</sup>.

**Cup Plate Method:** The nutrient agar medium was prepared by dissolving commercially available agar in distilled water. Immediately it was then autoclaved and cooled to 45 - 50 °C. The nutrient agar medium was inoculated aseptically with 0.5ml of strains of *S. aureus* and *E. coli* at room temperature. Into each sterile Petri dish about 15ml of inoculated molten agar medium was poured. The plates were left at room temperature for solidification. After solidification, the cups of 6mm diameter Petri dish and were made by scooping out the medium with the sterilized corn borer from Petri dish and were labeled. All the synthesized compounds and reference were dissolved in DMSO to prepare appropriate dilution to get required concentration of 25 $\mu\text{g/ml}$ , 50 $\mu\text{g/ml}$  and 100 $\mu\text{g/ml}$ . The solutions of each compound, reference and a control (DMSO) were added separately into each cups. The plates were kept undisturbed for about 24 hours at room temperature. After incubation period of 24 hours the diameter of zone of inhibition was measured with the help of antibiotic zone reader. The results are presented in **Table 1** and **Table 2**.

**TABLE 1: ANTIBACTERIAL ACTIVITY OF COMPOUNDS AGAINST S. AUREUS GRAM POSITIVE BACTERIA (S. AUREUS)**

Conc.	Zone of Inhibition (mm)		
	25	50	100
CSK-0	6	9	13
CSK-00	8	11	14
CSK-1	6	10	13
CSK-2	9	17	19
CSK-3	7	15	20
CSK-4	10	13	15
CSK-5	10	14	17
CSK-6	6	11	15
CSK-7	13	16	19
CSK-8	10	14	18
CSK-9	13	18	21
CSK-10	12	16	20
Standard	16	20	23



**TABLE 2: ANTIBACTERIAL ACTIVITY OF COMPOUNDS AGAINST *E. COLI*, GRAM NEGATIVE BACTERIA (*E. COLI*)**

Concentration	Zone of Inhibition (mm)		
	25	50	100
CSK-0	20	23	24
CSK-00	19	22	24
CSK-1	21	24	26
CSK-2	18	21	23
CSK-3	11	16	21
CSK-4	25	27	33
CSK-5	12	18	20
CSK-6	19	21	25
CSK-7	13	19	23
CSK-8	12	17	19
CSK-9	23	25	30
CSK-10	22	25	29
Standard	28	30	35

**Antioxidant Activity:** <sup>17-19, 34</sup>

**DPPH (2-diphenyl-1-picryl-hydrazyl) Radical-Scavenging Assay:** Various concentrations of synthesized compound (20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml) were prepared by dissolving in DMSO. To this solution, 1ml of freshly prepared 0.1mM methanolic solution of DPPH was added. It was then kept in dark for 30 min. The absorbance was measured at 517nm. DMSO was used as blank and Ascorbic acid was

used as standard. The capability to scavenge the DPPH radical was calculated using the following equation and results of DPPH activity are presented in **Table 3**.

$$\text{DPPH scavenged (\%)} = (A_{\text{control}} - A_{\text{test}}) \times 100 / A_{\text{control}}$$

Where,  $A_{\text{control}}$  was the absorbance of DPPH + methanol, and  $A_{\text{test}}$  was the absorbance of DPPH + sample / standard.

**TABLE 3: DPPH ASSAY RESULTS**

S. no.	Compound	Percentage Inhibition (%)		
		20ug/ml	60ug/ml	100ug/ml
1.	CSK-0	1.92	3.58	7.77
2.	CSK-00	4.97	12.88	23.13
3.	CSK-1	20.15	44.23	66.15
4.	CSK-2	7.15	21.43	40.07
5.	CSK-3	6.22	8.39	12.43
6.	CSK-4	4.32	9.94	17.11
7.	CSK-5	27.02	44.73	55.28
8.	CSK-6	33.86	52.18	72.05
9.	CSK-7	27.64	31.7	36.03
10.	CSK-8	3.97	7.18	11.63
11.	CSK-9	30.05	56.78	78.09
12.	CSK-10	20.91	39.94	68.4
13.	Ascorbic Acid	30.75	57.4	80.84

**ABTS (2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) Radical Scavenging Assay:** This assay is based on the ability of different compounds to scavenge 2, 2'-azino-bis (ethylbenzthiazoline-6-sulfonic acid) radical cation. ABTS radicals have characteristic absorbance at 734nm. This absorbance decreases when radical is reduced by any antiradical compound. The decrease in the absorbance can be measured using UV-VIS spectrophotometer at 734nm. For ABTS assay, the stock solutions of

7mM ABTS and 2.4mM potassium persulfate were prepared. The working solution was then prepared by mixing the two stock solutions in equal quantities (1:1) and allowing them to react for 12h at room temperature in the dark.

The solution (1ml) was then diluted with 60ml methanol to obtain an absorbance of  $0.706 \pm 0.001$  units at 734nm using the UV spectrophotometer. Methanolic solutions of all compounds as well as

ascorbic acid were prepared in the concentration of 20ug/ml, 40ug/ml, 60ug/ml, 80ug/ml and 100ug/ml. Compounds / Ascorbic acid (1ml) of different concentration was allowed to react with 1ml of the ABTS<sup>+</sup> solution and the absorbance was taken at 734nm using UV spectrophotometer. The ABTS<sup>+</sup> scavenging capacity of the extract was compared with that of Ascorbic acid and percentage inhibition calculated using the following equation

and results of ABTS activity are presented in **Table 4**.

$$\text{ABTS radical scavenging activity (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Where, Abs<sub>control</sub> was the absorbance of ABTS radical + methanol, and Abs<sub>sample</sub> was the absorbance of ABTS radical + sample / standard.

**TABLE 4: ABTS ASSAY RESULTS**

S. no.	Compound	Percentage Inhibition (%)		
		20ug/ml	60ug/ml	100ug/ml
1.	CSK-0	1.04	2.3	6.03
2.	CSK-00	16.97	34.03	44.96
3.	CSK-1	23.3	39.75	69.15
4.	CSK-2	7.4	22.44	31.74
5.	CSK-3	6.98	19.57	32.84
6.	CSK-4	0.9	2.88	6.98
7.	CSK-5	22.44	48.6	51.44
8.	CSK-6	30.64	46.65	70.05
9.	CSK-7	24.22	30.37	39.25
10.	CSK-8	5.64	18.63	25.43
11.	CSK-9	19.6	40.32	67.05
12.	CSK-10	13.3	24.08	31.2
13.	Ascorbic Acid	30.88	57.6	80.86

### Antifungal Activity<sup>21, 27, 28, 31</sup>

**Introduction:** Antifungal is also the most widely studied group of antimicrobials. Antifungal is the agent that either kills or inhibits the growth of fungi. It is used in the treatment and prevention of fungal infections. All synthesized compounds were screened for *in vitro* antifungal activity against *Candida albicans* strain of fungi by disc diffusion method. Fluconazole was used as reference antifungal drug.

**Paper Disc Diffusion Method:** The Sabouroud Dextrose agar was prepared by dissolving in distilled water. Its pH was adjusted and immediately it was then autoclaved and cooled to 45-50 °C. Into each sterile petridish about 15ml of molten Sabouroud medium was poured. The Sabouroud agar medium was inoculated aseptically by streaking with strain of *Candida albicans* at

room temperature. The plates were left at room temperature for solidification.

All the synthesized compounds and reference were dissolved in DMSO to prepare appropriate dilution to get required concentration of 10µg/ml, 25µg/ml, 50µg/ml and 100µg/ml and were coated on separate sterile filter paper discs (What man No. 1) measuring 6mm in size. The discs were then placed on the surface of solidified Sabouroud medium of each inoculated Petri dishes. DMSO used as negative control, while fluconazole were used as positive control for obtaining comparative results.

All treated and untreated plates were incubated for 48 h at 37 °C. After incubation period of 48 hours the diameter of zone of inhibition was measured with the help of antibiotic zone reader or simple scale. The results are presented in **Table 5**.

**TABLE 5: ANTIFUNGAL ACTIVITY OF COMPOUNDS AGAINST CANDIDA ALBICANS**

S. No.	Compounds	<i>Candida albicans</i>			
		Zone of Inhibition ( in mm)			
		10ug/ml	25ug/ml	50ug/ml	100ug/ml
1.	CSK-0	-	-	8	16
2.	CSK-00	-	12	16	20
3.	CSK-1	6	14	18	22
4.	CSK-2	-	6	10	18
5.	CSK-3	4	8	14	20

6.	CSK-4	10	16	20	26
7.	CSK-5	8	14	18	22
8.	CSK-6	8	16	18	24
9.	CSK-7	-	-	6	16
10.	CSK-8	10	18	22	28
11.	CSK-9	-	15	19	24
12.	CSK-10	-	9	15	22
13.	Fluconazole	15	20	26	30

**RESULTS AND DISCUSSION:** A series of Benzimidazole derivatives were synthesized, characterized and evaluated for antibacterial, antifungal and anti-oxidant activities. The compounds were synthesized in moderate to good yield. Purity of compounds was determined by TLC on silica gel G plates. The spots were detected by exposure to iodine vapors. Synthesized compounds were characterized by spectral analysis (Fourier Transform Infra-red and <sup>1</sup>HNMR).

**Antibacterial Activity:** All the synthesized compounds were evaluated for their *in vitro* antibacterial activity and results are depicted in **Table 1** and **2**. It was found that compound CSK-3, CSK-4, CSK-9 and CSK-10 are comparatively more active than all other compounds against gram positive strain of bacteria (*S. aureus*) and gram negative strain of bacteria (*E. coli*).

**Antioxidant Activity:** Antioxidant activity was measured by DPPH and ABTS assay method and compound CSK-6 and CSK-9 showed best Scavenging activity at concentration of 40, 60, 80 and 100µg/ml, when compared with ascorbic acid as standard. Other compounds with good antioxidant activity are CSK-1, CSK-5 and CSK-10. Although, all compounds show appreciable amount of antioxidant activity and results are depicted in **Table 3** and **4**.

**Antifungal Activity:** All the synthesized compounds were evaluated for their *in vitro* antifungal activity and results are depicted in **Table 5**. It is found that compound CSK-8, CSK-4, CSK-6 and CSK-9 are comparatively more active than all other compounds against *Candida albicans*.

**CONCLUSION:** A series of Benzimidazole derivatives were synthesised and screened for biological activity. In summary, all the derivatives showed promising Antibacterial, Antioxidant and Antifungal activity when compared with that of standard.

The diverse aspects clearly show the high potential of Benzimidazole derivatives and the relevance and importance of research done with these compounds. This work will hopefully be used for further development of potential inhibitor drugs.

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