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## Synthesis Characterization and Evaluation of Novel Substituted Azole Derivatives

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### ABSTRACT

Benzimidazole is a very stable nucleus and the derivatives of 2-methyl benzimidazole may show different and better biological activity. The presence of the thiazolidinone ring in a benzimidazole derivative enhances its antimicrobial activity. 2-methyl benzimidazole derivative are formed by the condensation of o-phenylenediamine with acetic acid. Combination of two or more active moieties into one is a common procedure, can result in augmenting the activity and removes untoward side effects. Hence, the present work focuses on synthesis of potent antimicrobial benzimidazole derivatives by the condensation of Schiff's bases of 2-methyl benzimidazole moiety with thiomalic acid affording the thiazolidinone ring and to study their antimicrobial activity. In antibacterial activity (*Staphylococcus aureus* and *Escherichia coli*), it shows that compound TE<sub>6</sub> is the most active compound and in antifungal activity (*Candida albicans*), TE<sub>3</sub> & TE<sub>4</sub> are found to be most active.

**Keywords:** 2 methyl benzimidazole, Antifungal, Antibacterial, O-phenylenediamine.

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### INTRODUCTION

The practice of medicinal chemistry is devoted to the discovery and development of new agents for treating diseases. The process of establishing new drugs is exceedingly complex and involves the talents of people from a variety disciplines, including Chemistry, Biochemistry, Physiology, Pharmacology, Pharmaceutics and Medicine. Medicinal chemistry is concerned mainly with the organic, analytical and biologic chemical aspects of this process, but its people must interact productively with those in other disciplines. Thus it occupies a strategic position at the interface of chemistry and biology [1].

Medicinal chemistry is a science whose roots lie in all branches of chemistry and biology. It concerns essentially the understanding and explanation of the mechanism of actions of drugs. On this base it attempts to establish relationship between chemical structure and biological activity and link biodynamic behavior to the chemical reactivity and physical properties of therapeutic agents [1].

The present work deals with the characterization of some new Benzimidazoles (Figure 1) containing Thiazolidinone derivatives. Despite a numerous attempts to develop new structural prototype in the search for more effective antimicrobials, the benzimidazoles still remains as one of the most versatile class of compounds against microbes and therefore are useful substrate for further molecular exploration.

Benzimidazole is a heterocyclic aromatic organic compound. This bicyclic compound consists of the fusion of benzene and imidazole. The most prominent benzimidazole compound in nature is N-ribosyl-dimethylbenzimidazole, which serves as an axial ligand for cobalt in vitamin B<sub>12</sub>. Benzimidazole, in an extension of the well-elaborated imidazole system, has been used as carbon skeletons for N-heterocyclic carbenes. The NHCs are usually used as ligands for transition metal complexes. They are often prepared by deprotonating an N, N'-disubstituted benzimidazolium salt at the 2-position with a base.

Benzimidazole is white coloured, slightly solid, slightly soluble in water and soluble in ethanol. It is a dicyclic compound having imidazole ring (containing two nitrogen atoms at nonadjacent positions) fused to benzene. Benzimidazole and its derivatives are used in organic synthesis and vermicides or fungicides as they inhibit the action of certain microorganisms. Examples of benzimidazole class fungicides include carbendazim, chlorfenazole, cypendazole, debacarb, fuberidazole, furophanate, mecarbinzid, rabenzazole, thiabendazole, thiophanate. Benzimidazole structure is the nucleus in some drugs such as proton pump inhibitors and anthelmintic agents.

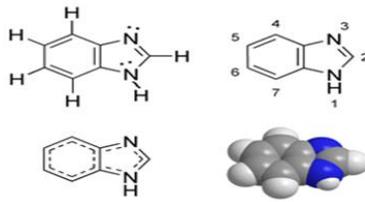
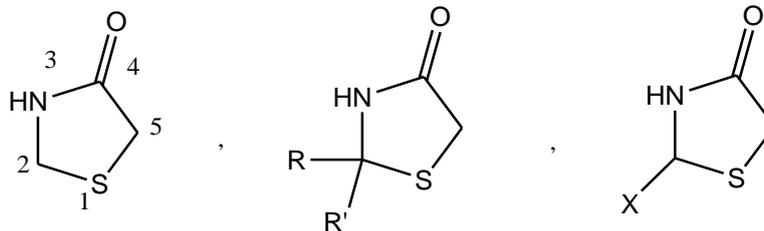


Figure 1: Structure of Benzimidazole

IUPAC name : 1H-benzimidazole  
 Molecular formula : C<sub>7</sub>H<sub>6</sub>N<sub>2</sub>  
 Molar mass : 118.14 g mol<sup>-1</sup>  
 Melting point : 170-172°C  
 Boiling point : 360°C

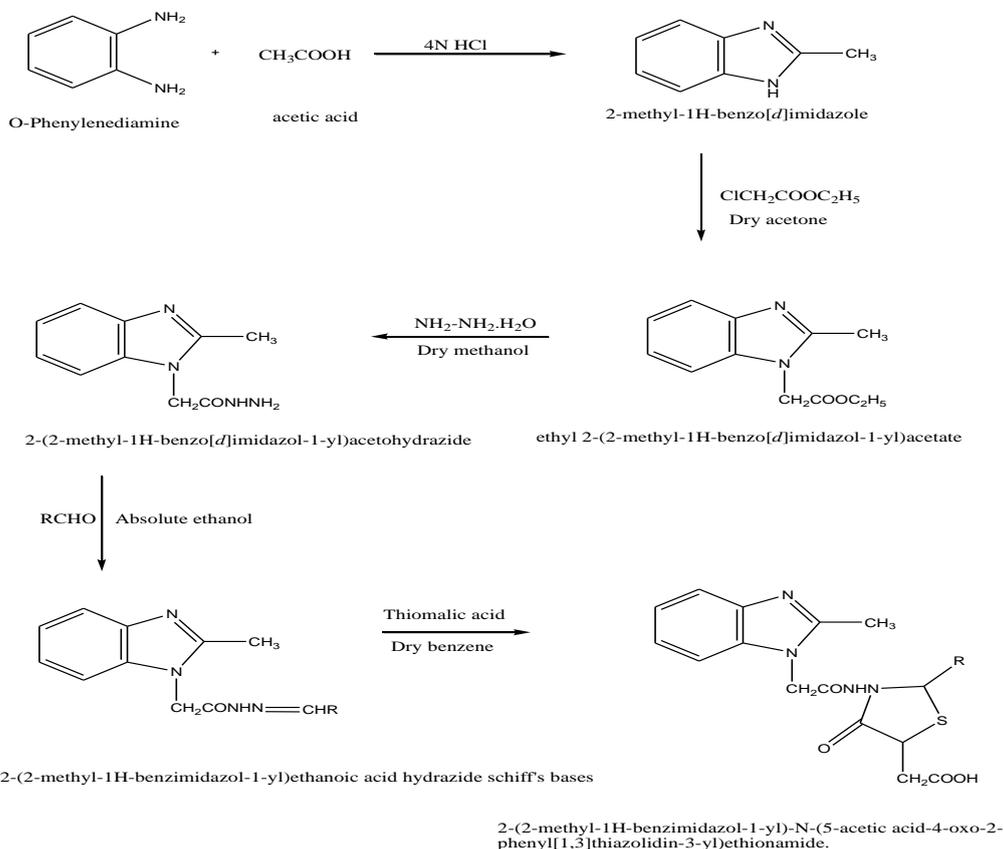
### Thiazolidinone [2]

The broad and potent activity of 4-thiazolidinones has established it as one of the biologically important scaffold. Thiazolidine is an important scaffold known to be associated with several biological activities. 4-Thiazolidinones are derivatives of thiazolidine with a carbonyl group at the 4-position. Substituents in the 2-, 3-, and 5-positions may be varied, but the greatest difference in structure and properties is exerted by the group attached to the carbon atom in the 2-position (R, R' and X).



4-thiazolidinones are well known heterocyclic compounds for their spectrum of biological activities such as antibacterial, antifungal, antitubercular, anthelmintic, antithyroid, local anaesthetic, monoamine oxidase inhibition, Anticonvulsant activity, Hypnotic activity, Anticancer activity, Antihistaminic activity (H<sub>1</sub>-antagonist), Antiviral activity, Anti-inflammatory activity (COX-inhibitors), Follicle stimulating hormone (FSH) receptor agonist activity.

### MATERIALS AND METHODS



The above scheme represents the complete process of the synthesis of the compounds (Table 1).

#### Procedure for synthesis of 2-methyl-1H-benzimidazole [MB] [3]

O-phenylenediamine (4 g, 0.04 mol) was condensed with acetic acid (1.7 ml, 0.03 mol) in 50 ml 4 N HCl. The reaction mixture was stirred for about 4 h. With magnetic stirrer at 80°C. The compound was precipitated by adding concentrated ammonia solution, filtered and washed with Compound was recrystallized from water and ethanol. The percentage yield was 80% and melting point was found to be 192°C-194°C.

#### Procedure for synthesis of ethyl 2-(2-methyl-1H-benzimidazol-1-yl) acetate [BA] [4]

Ethylchloroacetate (1.06 ml, 0.01 mol) was added to a solution of 2-methyl-1H-benzimidazole (1.32 g, 0.01 mol) in dry acetone (20 ml). To that mixture, anhydrous K<sub>2</sub>CO<sub>3</sub> was added and the reaction mixture was refluxed for 1 h. Acetone was removed after completion of reaction and the residue crystallized from ethanol. The percentage yield was 85% and melting point was found to be 186°C-188°C.

#### Procedure for synthesis of 2-(2-methyl-1H-benzimidazol-1-yl) acetohydrazide [4]

To a solution of ethyl 2-(2-methyl-1H-benzimidazole-2-yl) acetate (2.18 g, 0.01 mol) dissolved in dry methanol (50 ml), 99% hydrazine hydrate (1 ml) was added and the mixture was refluxed for 4-5 h. The reaction mixture was cooled and the solid obtained was filtered, washed with small quantity of cold methanol. The percentage yield was 37% and melting point was found to be 248°C-250°C.

#### Procedure for synthesis of 2-(2-methyl-1H-benzimidazol-1-yl) ethanoic acid hydrazide schiff's bases [5]

A mixture of 2-(2-methyl-1H-benzimidazol-1-yl) acetohydrazide (2.04 g, 0.01 mol) and the appropriate aromatic aldehyde, namely o-chlorobenzaldehyde, p-chlorobenzaldehyde, p-nitrobenzaldehyde, p-hydroxybenzaldehyde, anisaldehyde, p-methyl benzaldehyde (0.012 mol), was refluxed in absolute ethanol (20 ml) for 5 h. The reaction mixture was concentrated, cooled and the formed precipitate was filtered off, dried and then recrystallized from ethanol and water. The percentage yield and melting points are given in Table 2.

#### Procedure for synthesis of 2-(2-methyl-1H-benzimidazol-1-yl)-N-(5-acetic acid-4-oxo-2-phenyl [1, 3] thiazolidin-3-yl) ethionamide [TE] [5]

A mixture of 2-(2-methyl-1-H-benzimidazole-1-yl) ethanoic acid hydrazide Schiff's base (0.005 mol) and thiomalic acid (0.9 g, 0.006 mol) was refluxed in dry benzene (50 ml) for 6 h. The solvent was evaporated and the reaction mixture was neutralized with cold dilute sodium bicarbonate solution. The formed product was filtered off and recrystallized from ethanol and water [6-8].

Table 1: IUPAC Names of the synthesized compounds TE<sub>1</sub>-TE<sub>6</sub>.

S. NO.	Code	IUPAC Name
1.	TE <sub>1</sub>	2-(2-methyl-1H-benzimidazol-1-yl)-N-{5-aceticacid-4-oxo-2-(4-methoxyphenyl) [1, 3] thiazolidin-3-yl}ethionamide.
2.	TE <sub>2</sub>	2-(2-methyl-1H-benzimidazol-1-yl)-N-{5-aceticacid-4-oxo-2-(4-chlorophenyl) [1, 3] thiazolidin-3-yl}ethionamide.
3.	TE <sub>3</sub>	2-(2-methyl-1H-benzimidazol-1-yl)-N-{5-aceticacid-4-oxo-2-(4-methylphenyl) [1, 3] thiazolidin-3-yl}ethionamide.
4.	TE <sub>4</sub>	2-(2-methyl-1H-benzimidazol-1-yl)-N-{5-aceticacid-4-oxo-2-(2-chlorophenyl) [1, 3] thiazolidin-3-yl}ethionamide.
5.	TE <sub>5</sub>	2-(2-methyl-1H-benzimidazol-1-yl)-N-{5-aceticacid-4-oxo-2-(4-hydroxyphenyl) [1, 3] thiazolidin-3-yl}ethionamide.
6.	TE <sub>6</sub>	2-(2-methyl-1H-benzimidazol-1-yl)-N-{5-aceticacid-4-oxo-2-(4-nitrophenyl) [1, 3] thiazolidin-3-yl}ethionamide.

Table 2: Yield % and melting points of the synthesized compounds TE<sub>1</sub>-TE<sub>6</sub>

Compound name	R	MP°C	Yield%	Molecular formula	Spectral data		
					IR KBr (g,cm <sup>-1</sup> )	<sup>1</sup> H-NMR (CDCl <sub>3</sub> ,d,ppm)	Mass spectra (m/z)
TE <sub>1</sub>	- OCH <sub>3</sub> C <sub>6</sub> H <sub>4</sub>	160-162	87	C <sub>22</sub> H <sub>22</sub> N <sub>4</sub> O <sub>5</sub>	3423.08 N-H, O-H(stretching) 2926.05, 2850.67 Aliphatic O-H stretching 1739.73 C=O stretching (Thiazolidinone) 1603.14 Bending (Amide) 1460.21 CH <sub>3</sub> Bending	7.520 Aromatic C-H (4H) 6.096 C-2 proton of thiazolidinone N-CH <sub>2</sub> (2H) proton	357
TE <sub>2</sub>	- ClC <sub>6</sub> H <sub>4</sub>	223-225	92	C <sub>21</sub> H <sub>19</sub> N <sub>4</sub> O <sub>4</sub> Cl	3403.46 N-H stretching and O-H stretching Aliphatic O-H stretching 1716 1636.42 Bending (Amide)	7.585 Aromatic C-H (4H) of phenyl group 7.082 Aromatic C-H	358

TE <sub>3</sub>	- CH <sub>3</sub> C <sub>6</sub> H <sub>4</sub>	193-195	76	C <sub>22</sub> H <sub>22</sub> N <sub>4</sub> O <sub>4</sub>	C=O stretching (Thiazolidinone) 2.915 CH <sub>2</sub> of CH <sub>2</sub> COOH	6.045 C-2 proton of thiazolidinone 4.345 N-CH <sub>2</sub> (2H) proton 3.185 C-5 proton of thiazolidinone
TE <sub>4</sub>	- ClC <sub>6</sub> H <sub>4</sub>	172-174	89	C <sub>21</sub> H <sub>19</sub> N <sub>4</sub> O <sub>4</sub> Cl	1636.52 Bending (Amide)	7.532 Aromatic C-H (4H) 7.089 Aromatic C-H 6.039 C-2 proton of thiazolidinone 4.3514.351 N-CH <sub>2</sub> (2H) proton
TE <sub>5</sub>	- OHC <sub>6</sub> H <sub>4</sub>	205-207	67	C <sub>21</sub> H <sub>20</sub> N <sub>4</sub> O <sub>5</sub>	1383.14 CH <sub>3</sub> Bending (alkane)	4.332 C-5 proton of thiazolidinone 2.905 CH <sub>2</sub> of CH <sub>2</sub> COOH 2.2653H (S) CH <sub>3</sub> OF C <sub>2</sub> of benzimidazole N-CH <sub>2</sub> (2H) proton
TE <sub>6</sub>	- NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	212-215	73	C <sub>21</sub> H <sub>19</sub> N <sub>5</sub> O <sub>6</sub>	3403.98 N-H stretching and O-H stretching 2925.89, 2852.16 Aliphatic O-H stretching 1708.42 C=O stretching (Thiazolidinone) 1618.13 Bending (Amide)	6.053 C-2 proton of thiazolidinone 4.352 N-CH <sub>2</sub> (2H) proton

### Microbiological screening

The antimicrobial activity of the various synthesized compounds TE<sub>1</sub>, TE<sub>2</sub>, TE<sub>3</sub>, TE<sub>4</sub>, TE<sub>5</sub>, and TE<sub>6</sub> were studied in the following manner (Table 3).

#### Antibacterial activity [9]

Organism used: *Staphylococcus aureus* (MTCC 96), *Escherchia. Coli* (MTCC 722)

Standard used: Amikacin (30 µg/disc)

Control: Dimethyl sulfoxide (DMSO)

Test Concentration: 50 µg/ml

Incubation temperature: 37°C

Incubation time: 18 h.

Method: Filter paper disc method

Principle: Gel diffusion

#### Composition of media [10]

#### Muller-Hinton agar media

Beef extract: 30 g

Casein hydrolysate: 17.5 g

Soluble starch: 1.5 g

Agar: 20 g

Sodium hydroxide: 5 g

Distilled water: 1000 ml

Final p<sup>H</sup> at 25°C: 7.4 ± 0.2

#### Procedure [9,10]

Muller Hinton agar media of 100 ml was prepared as per the composition and sterilized in the autoclave at 15 Lbs/in<sup>2</sup> for 20 min. When the media was in warm molten state, 15 ml of the medium was transferred (using sterile boiling tube marked already for measuring) into the sterile petri plates in a laminar air flow bench aseptically. The medium was allowed to solidify and incubated at 37°C to check free from contamination.

## Protocol [9,1]

Assay was carried out by Filter paper disc method. The method followed was spread plate technique. The agar plates free from contamination were spreaded with 50  $\mu$ l of 48 h old culture of bacterial test organism using sterile buds. The standard disc of Amikacin (sterile) of 8 mm diameter was placed on the petri plates. Then the discs (sterile) of 8 mm were soaked in 1 ml of test solution and in solvent DMSO. After evaporating the solvent in a sterile atmosphere, the drug impregnated discs were placed in petriplates. After 15 min the plates were incubated at 37°C for 18 h in an inverted position. The clear zones of inhibition were measured using Hi media zone reader scale. The zones of test solutions were compared with standard Amikacin.

**Antibacterial activity****Table 3: Antimicrobial activity of the various synthesized compounds TE<sub>1</sub>- TE<sub>6</sub>**

S. No.	Compound	Zone of inhibition against	
		Staphylococcus aureus	Escherichia coli
1	TE <sub>1</sub>	13 mm	15 mm
2	TE <sub>2</sub>	15 mm	14 mm
3	TE <sub>3</sub>	17 mm	19 mm
4	TE <sub>4</sub>	13 mm	18 mm
5	TE <sub>5</sub>	14 mm	14 mm
6	TE <sub>6</sub>	16 mm	11 mm
7	Amikacin (std)	16 mm	17 mm
8	DMSO (control)	Resistant	Resistant

*Antifungal activity [11,12]*

Organism Used: *Candida albicans* (MTCC 227)

Standard Used: Ketoconazole (30  $\mu$ g/disc)

Control used: DMSO

Test concentration: 50  $\mu$ g/ml

Incubation temperature: 28°C

Incubation time: 24 h

Method: Filter paper disc method

Principle: Gel diffusion

*Composition of media***Saboured dextrose agar media**

Glucose: 40 g

Peptone: 10 g

Agar: 15 g

Distilled water: 1000 ml

Final P<sup>H</sup>: 5.4

*Protocol*

Assay was carried out by diffusion plate method. The method followed was spread plate technique. The agar plates free from contamination were spread with 50  $\mu$ l of 48 h old culture of fungal test organism using sterile buds. The standard disc of Ketoconazole (sterile) of 8 mm diameter was in the petriplates. Then the discs (sterile) of 5 mm were soaked in 1 ml (1 mg/ml) of the test solution and in solvent control DMSO. After evaporating the solvent in a sterile atmosphere, the drug impregnated discs were placed in petriplates. After 15 min the plates were incubated for 24 h at 28°C in an inverted position. The clear zones of inhibition were measured using Hi media zone reader scale. The zones of test solutions were compared with standard Ketoconazole.

*Antifungal activity***Table 4: Antifungal activity of the various synthesized compounds TE<sub>1</sub>- TE<sub>6</sub>**

S. No.	Compound	Zone of inhibition against <i>Candida albicans</i>
1	TE <sub>1</sub>	14 mm
2	TE <sub>2</sub>	12 mm
3	TE <sub>3</sub>	11 mm
4	TE <sub>4</sub>	11 mm
5	TE <sub>5</sub>	14 mm
6	TE <sub>6</sub>	17 mm
7	Ketoconazole (std)	Resistant
8	DMSO (control)	Resistant

## RESULTS AND DISCUSSION

**The KBr Spectrum of all synthesized compounds**

The synthesized compounds show a band at 3400-3425  $\text{cm}^{-1}$  indicates the presence of N-H stretching and O-H stretching. The band at 2926  $\text{cm}^{-1}$  and 2850  $\text{cm}^{-1}$  confirms the presence of aliphatic C-H stretching in the synthesized compounds. The band at 1739-1625  $\text{cm}^{-1}$  is due to the C=O stretching of thiazolidinone ring. The presence of amide group in the synthesized compounds is confirmed by the band at 1603  $\text{cm}^{-1}$ . The band at 1460-1382  $\text{cm}^{-1}$  is due to the presence of alkane  $\text{CH}_3$  bending in the synthesized compounds. From this data we confirmed the functional groups of the synthesized compounds and extra bond at 777  $\text{cm}^{-1}$  in the compound  $\text{TE}_6$  and  $\text{TE}_4$  confirms the C-Cl stretching. The compounds  $\text{TE}_6$  contain a band at the 1522  $\text{cm}^{-1}$  which indicates the presence of aromatic C- $\text{NO}_2$  stretching.

**NMR Spectrum of all synthesized compounds**

The 300  $\text{MHz}$   $^1\text{H}$ -NMR spectrum shows a signal at  $\delta$  7.5 ppm assigned for the aromatic protons of the phenyl substitution. A signal at  $\delta$  7 ppm (m) indicates the aromatic C-H protons of the benzimidazole ring. A signal at the  $\delta$  6 ppm (s) assigned for the 1H proton at C-2 position of thiazolidine ring. A singlet signal at  $\delta$  4.3 ppm assigned to the 2H proton of N- $\text{CH}_2\text{A}$  signal at 3.7 ppm is assigned for the 1H proton of the C-5 position of thiazolidinone ring. A signal at 2.9 ppm is assigned for the 2H proton of the  $\text{CH}_2\text{COOH}$  of the compounds. A signal at 2.55 assigned for the 3H protons of the methyl substitution C-2 position of benzimidazole.

**Antimicrobial activity**

All the synthesized compounds were screened for the antimicrobial activity. In antibacterial activity (*S. aureus* and *E. coli*), it shows that compound  $\text{TE}_3$  is the most active compound and in antifungal activity (*C. albicans*),  $\text{TE}_6$  is found to be most active. In the antibacterial activity of the synthesized compounds  $\text{TE}_1$ ,  $\text{TE}_2$ ,  $\text{TE}_3$ ,  $\text{TE}_4$ ,  $\text{TE}_5$ , and  $\text{TE}_6$ , *E. coli* shows comparatively good sensitivity to the compound  $\text{TE}_6$  as that of standard used. *S. aureus* shows comparatively good sensitivity to the compound  $\text{TE}_1$  and  $\text{TE}_5$  as that of standard used. In the antifungal activity of the synthesized compounds  $\text{TE}_1$ ,  $\text{TE}_2$ ,  $\text{TE}_3$ ,  $\text{TE}_4$ ,  $\text{TE}_5$ , and  $\text{TE}_6$ , *C. albicans* shows comparatively good sensitivity to the compound  $\text{TE}_3$  and  $\text{TE}_4$  as that of standard used. The compound shows significant activity than the standard used.

**CONCLUSION**

The approach of the present study was to synthesize 2-(2-methyl-1H-benzimidazol-1-yl)-N-(5-acetic acid-4-oxo-2-phenyl (substituted) [1, 3] thiazolidin-3-yl) ethionamide and hence forth evaluate the antibacterial and antifungal activity. It was established that introduction of thiazolidinone nucleus in the 2-methyl benzimidazole enhanced the antimicrobial activity. We conclude that there is vast scope for 2-methyl benzimidazole as an effective antimicrobial agent. From the  $R_f$ , melting point, solubility, IR, NMR, and mass pattern were given the evidence for the proposed structure of the synthesized novel derivatives of benzimidazole. In antibacterial activity (*S. aureus* and *E. coli*), it shows that compound  $\text{TE}_6$  is the most active compound and in antifungal activity (*C. albicans*),  $\text{TE}_3$  &  $\text{TE}_4$  are found to be most active. In the antibacterial activity of the synthesized compounds  $\text{TE}_1$ ,  $\text{TE}_2$ ,  $\text{TE}_3$ ,  $\text{TE}_4$ ,  $\text{TE}_5$ , and  $\text{TE}_6$ , *E. coli* shows comparatively good sensitivity to the compound  $\text{TE}_6$  as that of standard used. *S. aureus* shows comparatively good sensitivity to the compound  $\text{TE}_1$  and  $\text{TE}_5$  as that of standard used. In the antifungal activity of the synthesized compounds  $\text{TE}_1$ ,  $\text{TE}_2$ ,  $\text{TE}_3$ ,  $\text{TE}_4$ ,  $\text{TE}_5$ , and  $\text{TE}_6$ , *C. albicans* shows comparatively good sensitivity to the compound  $\text{TE}_3$  and  $\text{TE}_4$  as that of standard used.

**REFERENCES**

- [1] D.J. Abraham, Chemotherapeutic agents as sulfonamide drug-Burger Medicinal Chemistry Drug Discovery (chemotherapeutic agents), A John Wiley and sons, Inc 6<sup>th</sup> publication, **2001**, 5, 557-566.
- [2] V. Amit, K.S. Shailendra, *Eur. J. Med. Chem.*, **2008**, 43, 897-905.
- [3] T.A. Kumar, M. Anil, *Ind. J. Chem.*, **2006**, 45B, 489-493.
- [4] K.F. Ansari, C. Lal, *Eur. J. Med. Chem.*, **2009**, 44, 4028-4033.
- [5] H.El-masry Afaf, H.H. Fahmy, S.H. Abdelwahed Ali, *Molecules.*, **2000**, 5, 1429-1438.
- [6] R.M. Silverstein, B. Morrill, C. Terence, Interpretation of Infrared spectra, Nuclear Magnetic Resonance Spectroscopy, Spectromagnetic identification of organic compounds, John Willey and sons, Inc, 5<sup>th</sup> (Edn.), **1991**, 100-131, 202-225.
- [7] Y.R. Sharma, Application of infrared spectroscopy, Elementary organic spectroscopy, principle and chemical application, S. Chand and company Ltd., **2005**, 90-135.
- [8] D.A. Skoog, F.J. Holker, A. Timothy, Nieman, Interpretation of Proton Nuclear Magnetic Resonance Spectroscopy, Principle of Instrumental Analysis, Thomson Brooks, 5<sup>th</sup> (Edn.), **2005**, 457-476.
- [9] A. Mori, C. Nishino, *Phytochemistry.*, **1987**, 26, 2231-2234.
- [10] R.C. Dubey, Culture media-A text book of biotechnology, S. Chand, Publication, 1<sup>st</sup> (Edn.), 1993, 224-228.
- [11] A. Chakravarti, A. Ghosh, A. Kanta, P. Kumar, *Ind. J. Med. Res.*, **1995**, 102, 13-19.
- [12] S. Saisivam, V.B. Kishan, *Ind. J. Microbiol.*, **2006**, 46, 197.