SYNTHESIS AND BIOLOGICAL EVALUATIONS OF SOME NOVEL MANNICH BASES OF BENZIMIDAZOLE AND THEIR DERIVATIVES

Kawade D. P., Bhadke M.Y., Asnani A. J., Chaple D. R.

Department of Pharmaceutical Chemistry, Priyadarshini J. L. College of Pharmacy,

Electronic zone, MIDC, Hingna road, Nagpur-440016, Maharashtra, India.

Corresponding Author

Dr. Dinesh Prakashrao Kawade
Associate Professor
Department of Pharmaceutical Chemistry,
Priyadarshini J. L. College of Pharmacy,
Electronic zone, MIDC, Hingna road, Nagpur-440016, Maharashtra, India.
Mobile No: 9545383111
Email Id: drdinesh03@gmail.com

ABSTRACT

The present research work was carried out with a series of Mannich bases of substituted benzimidazole and their derivatives were prepared by both conventional heating and microwave assisted techniques. Benzimidazole and their derivatives were prepared through condensation between *o*-phenylenediamine and six different substituted aliphatic acids in presence of hydrochloric acid, then the product undergone Mannich reaction in presence of formaldehyde, ethanol, primary amines and ethanol to obtained six different substituted Mannich bases of benzimidazole derivatives. All the synthesized compounds were subjected to TLC to find out the purity. The synthesized derivatives were characterized by spectroscopic data and were evaluated for antimicrobial activity and antioxidant activity by using Cup plate method and DPPH free radical scavenging assay respectively. The results of antimicrobial activity (*in-vitro*) revealed that the compounds (2a, 2b, 2c & 2f) possessed significant antimicrobial activity. Among the compound tested, the compound (2d) compound (2d) had shown the highest antibacterial & antifungal activity which was comparable to that of ciprofloxacin and fluconazole respectively. The substituent of amino group on benzimidazole ring contributed significantly towards amino acid. This study suggested that microwave assisted method can be appropriate for the synthesis of benzimidazole and their derivatives with better purity, yield and ecofriendly method.

KEY WORDS

Benzimidazole, Mannich bases, Conventional synthesis and Microwave assisted method, Antimicrobial activity and Anti-oxidant activity.

INTRODUCTION

Benzimidazole ring are the most important nitrogen containing heterocycles, which are widely explored and utilized by the pharmaceutical industry for drug discovery[1]. Numerous benzimidazole based drugs have been extensively used in the clinic to treat various types of diseases with high therapeutic potential [2]. Benzimidazole derivatives play a vital role in biological fields such as anti-inflammatory, anti-cancer[3],anti-convulsant[4], anti-viral, anti-oxidant[5], anti-microbial[6] and anti-tubercular[7] activities.

Mannich reaction is a condensation reaction between a compound containing atl east one active hydrogen atom, formaldehyde and ammonia, a primary or secondary amine (preferably as the hydrochloride). So the net change, during this reaction, is the replacement of the active hydrogen atom by an amino methyl group or substituted amino methyl group. These bases are called Mannich bases, usually isolated as hydrochloride. The Mannich reaction is an example of nucleophilic addition of an amine to a carbonyl group. The Mannich reaction is also considered a condensation reaction [8].

Benzimidazoles are remarkably effective compounds, extensive biochemical and pharmacological studies have confirmed that these molecules are effective against various strains of microorganisms[9][10]. Antimicrobial agents / Antibiotics are antibacterial substances produced by various species of micro-organism (bacteria, fungi, and actionomycetes) 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 [11,12,13]. The prepared compounds were subjected to physiochemical studies like melting point determination, TLC and percentage yield. The structures of synthesized compounds were characterized by IR and NMR spectroscopy. [14],[15] 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.

Benzimidazole is a heterocyclic aromatic organic compound. This bicyclic compound consists of the fusion of benzene and imidazole.

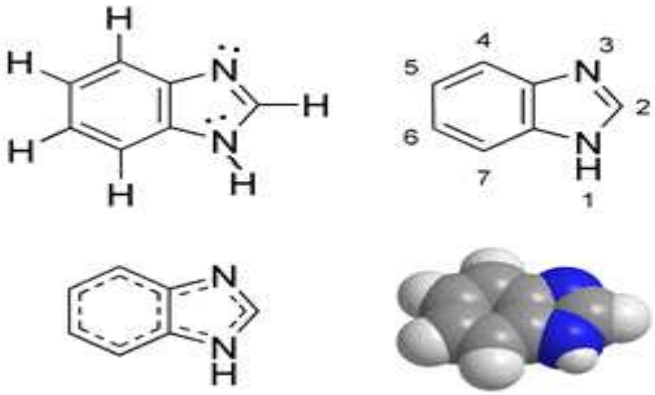


Figure 1: Structure of Benzimidazole

2. General procedure for the synthesis of Mannich bases by substituted benzimidazole derivatives[25,26]

General procedure for preparation of benzimidazole

In a 250ml round bottom flask fitted with a condenser, a mixture of 15gm of O-Phenylene diamine and 9.72ml of 90% formic acid was refluxed thermally at 100°C for 3 hours. The reaction mixture was cooled and 10% sodium hydroxide solution was added slowly, then the crude product was washed with ice cold water, dissolved in 400ml of boiling water add 2gm of decolorizing carbon and digest for 15 min. Filter rapidly at the pump through a preheated Buchner funnel and cool the filtrate to about 10° C, filter of the benzimidazole. Wash with 25ml of cold water and dry 100° C.

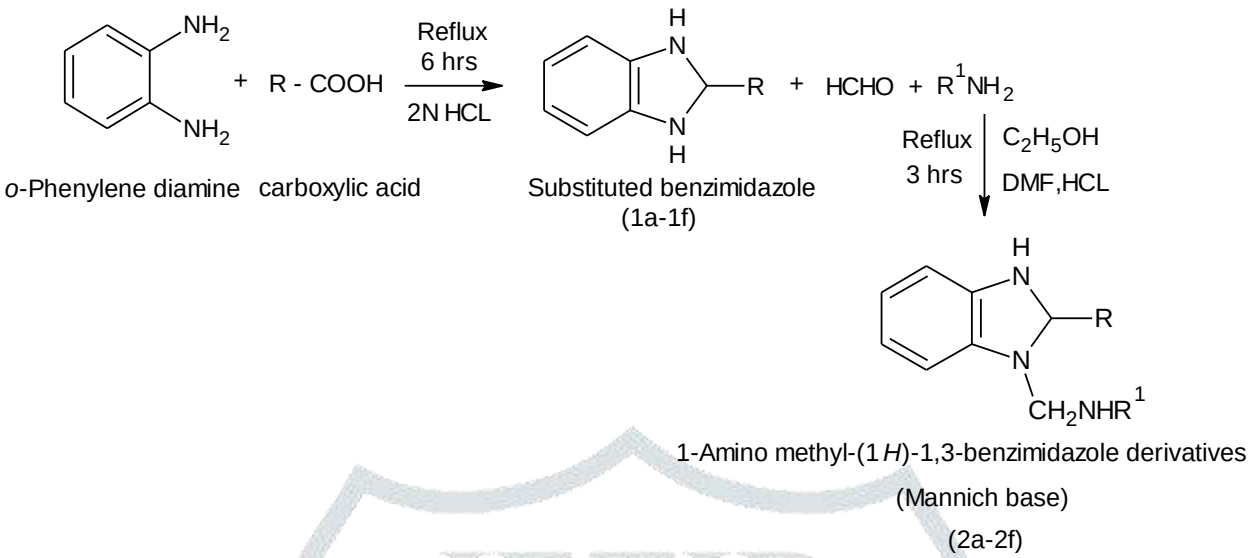
Preparation of substituted benzimidazole [1a-1f]

In a 250ml round bottom flask fitted with a condenser, a mixture of 20mmol (2.162gm) O-Phenylene diamine and acid 36mmol (acetic acid 3.6ml/benzoic acid 7.35gm/ oxalic acid 5.4ml/phthalic acid 9.97gm/salicylic acid 8.16gm) were stirred in 4N HCl (40ml) and refluxed for 6hrs then cooled at room temperature. The completion of this reaction was monitored by TLC. The pH was adjusted to 7.2 using NaOH pellets. The resulting solution was filtered and washed with water dried in vacuum and recrystallised from methanol.

Preparation of Mannich bases [2a-2f]

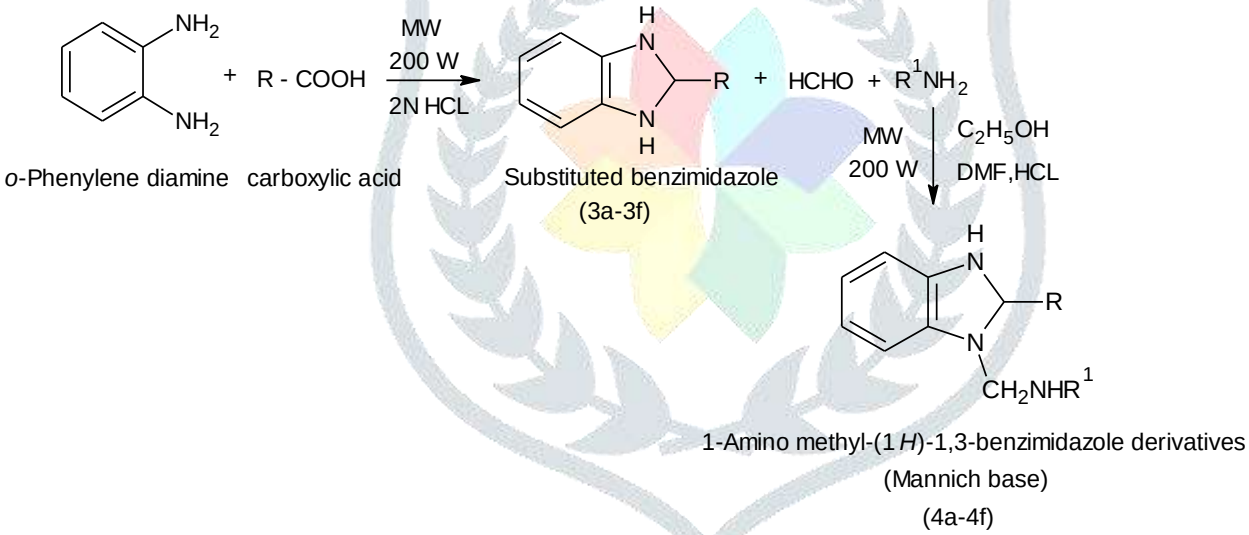
A mixture of Compound **1a/1b/1c** (0.08mol), formaldehyde (0.08mol, 2.66ml) and amines such as aniline (0.08mol, 0.85ml)/ p-Chloro aniline (0.08mol, 0.62ml)/ p-Nitro aniline (0.08mol, 0.57ml)/ methylaniline (0.08mol,0.74ml) and N-ethylaniline (0.08mol, 0.66ml) is dissolved in ethanol; add few drops of hydrochloric acid in a 250ml RBF. Then the mixture was heated under reflux for 3hrs subsequently ethanol was distilled off.The mixture was allowed to cool over night in a refrigerator. The solid thus obtained was recrystallised from ethanol.

Conventional heating method



Scheme 1:Conventional synthesis of Mannich bases

Microwave assisted method



Scheme 2: Microwave assisted synthesis of Mannich bases

Table 1 : Various substitution of conventional heating synthesized Mannich base

Sr. No.	Compounds	R	R^1
1	2a	CH_3COOH	$C_6H_5NH_2$
2	2b	C_6H_5COOH	C_6H_6ClN
3	2c	$C_2H_2O_4$	$C_6H_6N_2O_2$
4	2d	$C_2H_2O_4$	C_7H_9N
5	2e	$C_8H_6O_4$	$C_8H_{11}N$
6	2f	$C_7H_6O_3$	$C_8H_{11}N$

(A) Solvent for recrystallization: Methanol

(B) R_f value = Distance travelled by solute / Distance travelled by solvent

Solvent used = Chloroform: Methanol (9.5:0.5); detecting agent: Iodine vapour

3.2 SPECTRAL STUDIES [27-29]

The IR spectra of all the synthesized compounds were recorded on Shimadzu FT-IR IRA-Affinity1 and absorbance peaks were recorded using KBr pellets.

The actual IR spectra of the synthesized compounds are given in following figures. The interpretation was carried out by observing the graph.

The title compound were further characterized by physicochemical method and spectral analysis. Melting Point was recorded by two different method capillary tube method and visible melting point apparatus methods and was uncorrected. TLC was done to determine purity by using solvent system Chloroform : Methanol (9.5:0.5) and R_f values were reported.

FTIR spectra of all compound showed aromatic C-H stretching vibration 3029 cm^{-1} this is indicates that the C-H bond present in aromatic ring. All derivatives showed a broad absorbance band at about 3000-3500 cm^{-1} associated with stretching vibrations of bonded N-H, indicating present of nitrogen. Each compound showed a strong absorbance band due to NH_2 stretching vibration 3452 cm^{-1}. All derivatives showed broad absorbance at about 2210-2260 cm^{-1} associated with stretching vibrations of bonded –N=C-, indicating present of nitrogen in the ring. Compounds 1a, 1c, 1e, 1h and 1i showed a strong absorbance at 1550-1800 cm^{-1} stretching vibration indicating present of C=N group.

4. BIOLOGICAL EVALUATION

4.1 Antimicrobial activity [27-30]

4.1.1 Principle

The principle of micro-biological assay is an elaborated comparison of the inhibition of growth of the microbes by a measured concentration of the antibiotics under investigation against that produced by the known concentration of a standard preparation of antibiotics with a known activity.

The cylinder plate or cup plate method solely depends upon the diffusion of the antibiotics from a cylinder via a solidified agar layer in a petri-plate to an extent such that the observed growth of the incorporated micro-organism is prevented totally in a zone just around the plate containing a solution of antibiotics.

4.1.2 Requirements

4.1.2.1 Material

a) Micro-organism (*B*.subtilis, *E*.coli, *C*.albians,)

b) Methanol

c) Standard drug Ciprofloxacin for antibacterial and Flucanazole for antifungal study.

d) Synthesized Derivative

e) Petriplate, Nutrient agar Media (Hi-media)

4.1.2.2 Stock Solution

The test compounds (10 mg) were dissolved in methanol (10 ml), to produce 1000µg/ml. Further dilution were made with methanol to produce 100 µg/ml. similarly, the dilution were prepared for standard drug i.e. Ciprofloxacin and Flucanazole in a concentration of 10mg/ml, further 10ml of solution dilute up to 10 ml to produce 100 µg/ml.

4.1.2.3 Media Control

Sterilized medium was kept for growth (approx.48 hours) to assure the sterility of the medium.

4.1.3 Procedure

1. Take a petri- plate and sterilized it.
2. The nutrient agar (Hi-media) was prepared dissolving 28 g of nutrient agar in 1000 ml of distilled water. The medium was sterilized by autoclaving at 15ɹb pressure for 30 minutes and cooled to 40-50°C.
3. The nutrient agar medium was inoculated aseptically with 0.5ml of strains of *B.subtilis* and *E. coli* at room temperature and transfer it in petri plate.
4. All the operation was carried out under aseptic condition. Sterile media was melted on water bath and kept in 45°C in constant temperature in water bath.
5. In each sterile petri dish molten medium was added so that thickness was approximetly 4-5 mm and subculture organism under study was inoculated. The inoculated dish was allowed to set for 30 minute at room temperature. Cup of 6mm diameter were then made with the help of sterile stainless steel bore, a stock solution was added to bore in concentration of 100 µg/ml of each of drug in each of petri-plates.
5. Petri dish was kept in refrigerator for 30 minute so as to allow diffusion of the solution in medium, incubated at 37°C for 24 hours.
6. Zone of inhibition produced by test compounds was measured in mm and minimum concentration of test drug required for inhibition and compound selected on the basis for their concentration.

4.2 Anti-oxidant activity[32]

4.2.1 Requirements

4.2.1.1 Material

a. Test tubes

b. Methanol

c. DPPH

d. Pipptes,volumetric flask

e. Ascorbic acid

4.2.1.2 DPPH ASSAY

Free radical scavenging ability of the Ascorbic acid was tested by DPPH radical scavenging assay. The hydrogen atom donating ability of the drug was determined by the decolorization of methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH produces violet/ purple colour in methanol solution and fades to shades of yellow colour in the presence of antioxidants. Various concentrations of synthesized compound (10µg/ml, 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml) were prepared by dissolving in methanol. To this solution, 1ml of freshly prepared 0.1mM methanolic solution ofDPPH was added. It was then kept in dark for 30 mins. The absorbance was measured at 517nm. 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 no. 11)

$$\% \text{ DPPH radical scavenging activity} = (A0 - A1)/A0 \times 100$$

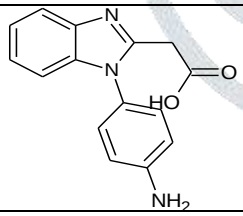
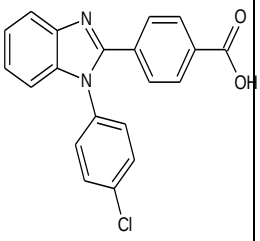
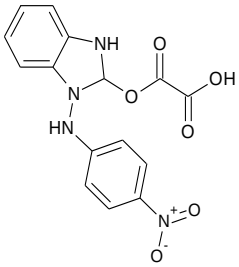
Where, A0 is the absorbance of the control and A1 is the absorbance of the sample.

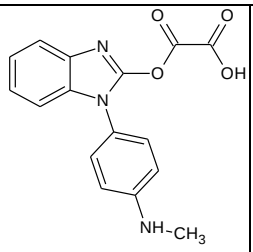
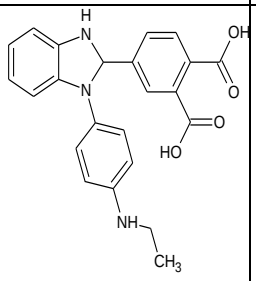
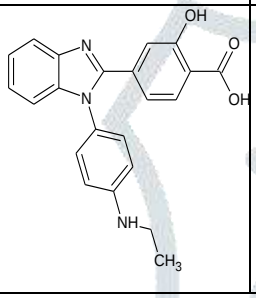
Then % of inhibition was plotted against concentration, and from the graph IC_{50} was calculated. The experiment was repeated three times at each concentration.

6. RESULS AND DISCUSSIONS

5.1 Synthetic studies

Table No. 2 : Physicochemical properties of synthesized compounds (2a-2f)

Sr. No	Compound Name	Structure	Molecular formula	M.W.	M.P. (°C)	% yield		R_f value	Wave length
						Conventional method	Microwave Assisted Method		
2a	[1-(4-amino phenyl)2-acetic acid] 1*H*-benzimidazole		$C_{15}H_{13}N_3O_2$	223.22	212-215	55.62%	87.87%	0.91	445nm
2b	1-(4-chloro phenyl)-2-benzoic acid-1*H*-benzimidazole		$C_{20}H_{13}ClN_2O_2$	304.72	185-187	64.50%	90.90%	0.85	456nm
2c	1-[(4-nitrophenyl)amino] -2-oxalic acid-1*H*-benzimidazole		$C_{15}H_{10}N_4O_6$	342.26	172-175	69.60%	84.75%	0.71	422nm

2d	1-[4-(methylamino)phenyl]-2-oxalic acid-1*H*-benz imidazole		$C_{16}H_{13}N_3O_4$	311.29	170-174	51.45%	66.50%	0.76	436nm
2e	1-[4-(methyl amino)phenyl] -2-phthalic acid -1*H*-benzimidazole		$C_{23}H_{19}N_3O_4$	401.41	163-165	66.02%	89.02%	0.90	426nm
2f	1-[4-(ethylamino)phenyl]- 2-hydroxybenzoic acid-1*H*-benzimidazole		$C_{22}H_{19}N_3O_3$	373.40	186-190	68.05%	84.05%	0.83	447nm

4.2 BIOLOGICAL EVALUATION

4.2.1 Antimicrobial activity

Table No. 3 : Antimicrobial activity of synthesized compound (zone of inhibition)

Compounds	**Zone of inhibition**		
	(B.subtilis)	**(E. coli)**	**C.albicans**
2a	14±0.6	12±0.6	15± 0.5
2b	15±1.0	14±0.5	12± 0.6
2c	13±1.0	17±0.5	14± 1.1
2d	**21±0.7**	**20±1.1**	**18± 0.5**
2e	17±0.5	17±0.5	12± 1.0
2f	17±0.5	13±0.5	13± 1.0
Ciprofloxacin	29±0.5	27±0.5	-
Fluconazole			25± 0.5

The results of antimicrobial activity (*in-vitro*) revealed that the compounds (2a, 2b, 2c & 2f) possessed significant antimicrobial activity. Among the compound tested, the compound (2d) compound (2d) had shown the highest antibacterial & antifungal activity which was comparable to that of ciprofloxacin and fluconazole respectively (Table No.9). The substituent of amino group on benzimidazole ring contributed significantly towards amino acid.

Literature survey revealed that the lipophilicity of the compounds has significant influence on the antimicrobial activity. In general electron withdrawing or donating groups amended the lipophilicityof the test compound (2a-2f) which might have enhanced their permeability across the bacterial cell membrane. In general, the compound (2d) was found to be more active against gram +ve as compared gram -ve bacteria.

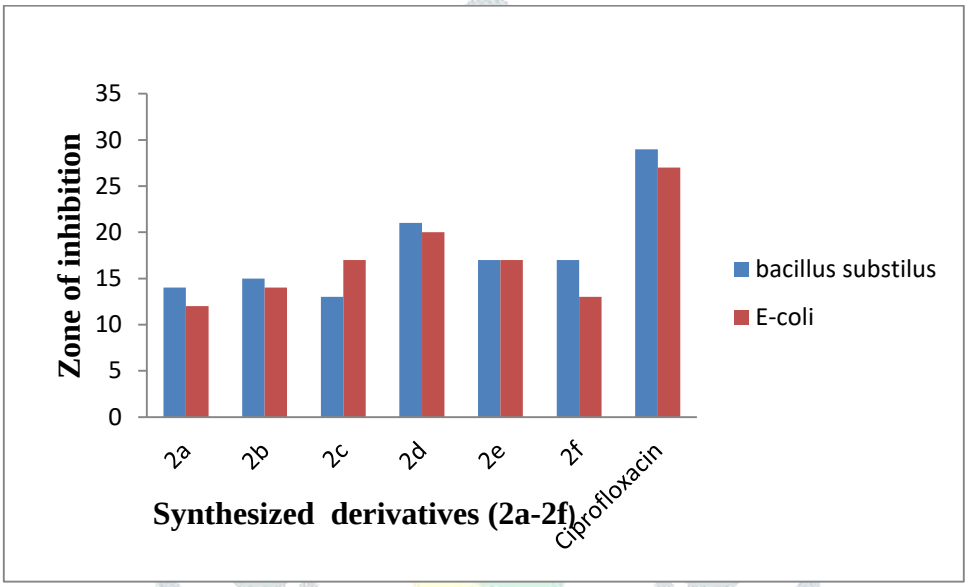


Figure 2 : Antibacterial activity of synthesized derivatives (2a-2f)

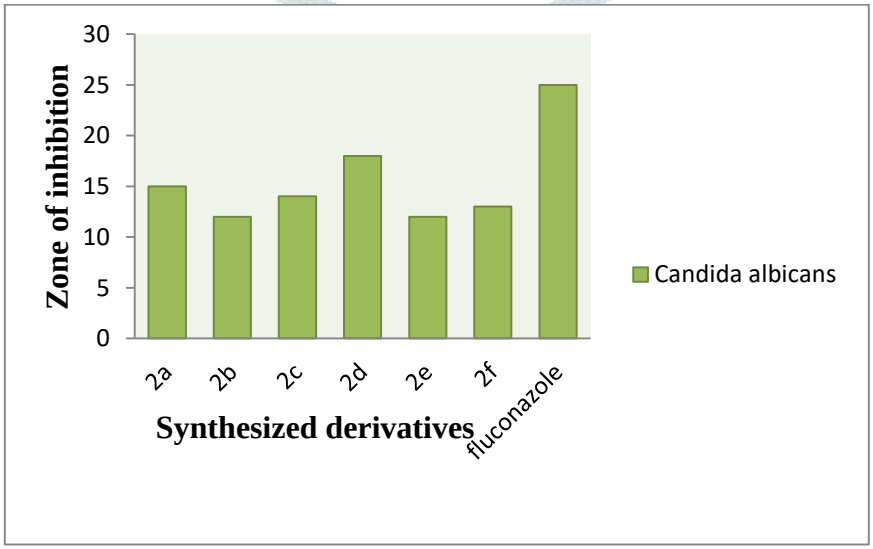


Figure 3 : Antifungal activity of synthesized derivatives (2a-2f)

4.2.2 Antioxidant activity

Table No. 4: DPPH assay of synthesized compounds

Sr. no	compounds	% inhibition						IC_{50}
		10µg/ml	20µg/ml	40µg/ml	60µg/ml	80µg/ml	100µg/ml	
1	2a	35.49±0.5	42.59±0.7	48.60±1.0	56.45±1.0	62.70 ± 0.8	72.46 ± 0.7	17.11
2	2b	39.40±0.6	43.44±0.7	39.05±0.9	54.64±0.8	61.66 ± 0.8	65.78 ± 0.9	44.01
3	2c	40.56±1.0	46.84±1.7	59.56±0.7	68.18±0.7	76.47 ±1.02	84.07 ± 0.8	25.51
4	**2d**	**44.60±1.0**	**55.16±0.9**	**62.15±0.7**	**69.08±0.6**	**76.42 ± 0.6**	**86.80 ± 0.8**	**15.04**
5	2e	41.5±0.6	46.51±0.9	51.32±1.2	58.69±0.8	62.07 ± 0.9	66.35 ± 0.8	35.50
6	2f	44.58±0.5	54.58±0.5	62.15±0.8	69.35±0.8	74.45 ± 0.7	79.18 ± 0.7	31.12
7	Ascorbic Acid	46.50±0.5	56.86±0.9	63.26±0.9	70.45±0.8	79.29 ± 0.8	88.81 ± 1.0	12.19

Antioxidant activity was measured by DPPH assay method and compound (2d) showed best Scavenging activity at concentration of 10, 20, 40, 60, 80 and 100µg/ml, when compared with ascorbic acid as standard. Other compounds with good antioxidant activity are (2a, 2b, 2c, 2e and 2f). Although, all compounds show appreciable amount of antioxidant activity and the IC_{50} value of compound (2d) almost similar as compared to standard drug value.

9. SUMMARY

In the present work, total six compounds (Mannich bases) were synthesized by conventional and microwave assisted methods and a comparative study was also performed.

In the conventional method, the initial step involves the synthesis of benzimidazole and their six derivatives (2a - 2f) by refluxing *o*-phenylenediamine and respective acids such as acetic acid, benzoic acid, oxalic acid, pthalic acid and salicylic acid. Then the step one products are undergone Mannich reaction in presence of formaldehyde, ethanol and five different amines such as aniline, p-chloroaniline, p-nitroaniline, methylaniline and p-ethylaniline respectively to 1a, 1b, 1c, 1d, 1e &1f under reflux were given 2a, 2b,2c, 2d, 2e & 2f. The above six Mannich bases were prepared by microwave assisted method contains two steps. Step one involves the preparation of 3a- 3f by using *o*-phenylenediamine and respective acids such as acids acetic acid, benzoic acid, oxalic acid, pthalic acid, salicylic acid in presence of Hydrochloric acid under microwave oven at 400W.Then these products are treated with formaldehyde, ethanol and five different amines such as aniline, p-chloroaniline, p-nitroaniline, methylaniline and p-ethylaniline respectively to 3a, 3b, 3c, 3d, 3e & 3f under

microwave irradiation to produced 4a, 4b, 4c, 4d, 4e & 4f. All the synthesized compounds were tested for their purity by TLC. The characterization of the synthesized Mannich bases were done by IR and Mass spectroscopic analyzed. All the synthesized compounds were evaluated for their *in vitro* antibacterial activity and results are depicted in (Table no.3) and it was found that compound (2d) was shown better antibacterial activity as compared to ciprofloxacin using gram positive strain of bacteria (*B.substilis*) and gram negative strain of bacteria (*E. coli).*

All the synthesized compounds were evaluated for their (*in vitro)* antifungal activity and results are depicted in (Table no.3) It is found that compound 2d, are comparatively more active than all other compounds against *Candida albicans.*

Antioxidant activity was measured by DPPH assay method and compound (2d) showed best Scavenging activity at concentration of 10, 20, 40, 60, 80 and 100µg/ml, when compared with ascorbic acid as standard. Other compounds with good antioxidant activity are (2a and 2c). Although, all compounds show appreciable amount of antioxidant activity and results are depicted in (Table no. 4)

10. CONCLUSION

In the present study, an attempt was made to synthesized some new Mannich bases of benzimidazole and derivatives by conventional and microwave assisted method and comparative study was done in respect of higher yield, purity, easy one and ecofriendly.

Synthesis were carried out of six various substituted benzimidazole by the condensation reaction of *o*-phenylenediamine and various aliphatic acids in presence of hydrochloric acid in both conventional and microwave assisted methods. Then six different Mannich bases of benzimidazole and their derivatives were formed by action of benzimidazole and their derivatives with formaldehyde, ethanol and various primary and secondary amines in presence of hydrochloric acid by both conventional and microwave methods.

The Mannich bases (4a-4f) synthesized by microwave method gives better yield, purity, and are synthesized within 1-3 minutes. At the same time Mannich bases (2a-2f) synthesized by the conventional method produced lesser yield, less purity compare to microwave method and took 4-6 hrs for synthesizing.(Table no. 2) The melting points of all the synthesized compounds were checked. All the synthesized compounds of the present study were characterized and confirmed by IR and Mass spectra. FT-IR spectra of all compound showed aromatic C-H stretching vibration 3029 cm^{-1} this is indicates that the C-H bond present in aromatic ring. All derivatives showed a broad absorbance band at about 3000-3600 cm^{-1} associated with stretching vibrations of bonded N-H, indicating present of nitrogen. Each compound showed a strong absorbance band due to NH_2 stretching vibration 3452 cm^{-1} associated with stretching vibrations of bonded –N=C= indicating presence of nitrogen in the ring. A carboxylic ester would exhibit a pair of intense absorptions near 2500 and 3100 cm^{-1} due to C-0 stretching modes.

Compounds 2a, 2b, 2c, 2d and 2f showed a strong absorbance at 3300-3600 cm^{-1} stretching vibration indicating present of N-H group and also show the absorbance at 1340-1320 cm^{-1} stretching vibration indicating the

present of C-N group. Then the carbonyl compound C=O showed a strong absorbance band at 1730.-1680 cm^{-1}.

In MS, an isotope peak [m^{+}+z] which appeared at different intensities along with molecular ion peaks, confirmed the molecular weight of the compound (2a,2b and 2c) an isotope peak was obtained due to fairly large abundance of the molecular ion peaks usually occurs in aromatic compounds.

Compound (2d) showed promising Antibacterial, Antifungal and Antioxidant activity when compared with standard drugs. Remaining compounds shown moderate activities.

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.

From the above result, it was concluded that the microwave assisted method is simple, efficient and fast for the synthesis of Mannich bases of Benzimidazole and their derivatives. Among the six derivatives, compound (2d) shown highest antibacterial, antifungal and anti-oxidant activities as compared to standard drugs. All the peaks in FTIR shows the characteristic stretching and bending within the standard limits.

REFERENCES

1. Abraham D and Burger A: Burger's medicinal chemistry and drug discovery, 6th ed. Hoboken NJ: Wiley 2003; 113-120.

2. Lemke T, Williams D, Roche V and Zito S: Foye's Principles of Medicinal Chemistry. 7th ed. Lippincott Williams and Wilkins 2008.

3. Manisha S Kedar, Nachiket S Dighe, Shashikant R Pattan, Deepak S Musmade, Dipak Thakur, Mayur Bhosale and Vinayak M GawareScholars Research Library-Der pharma chemical 2010; 2(2): 249-256.

4. Devender Pathak, Nadeem Siddiquib, Bhanupriya Bhrigu, Waquar Ahsan, M. Shamsher Alam. Benzimidazoles a new profile of biological activities. Scholars Research Library- Der Pharma chemical Lettre 2010; 2(2): 27-34.

5. Oztezkin Algul, Nizam Duran, and Gulay gulbol. Synthesised and evaluation of antimicrobial activity of some 1, 5(6)-H/or- Methyl-2-substituted benzimidazole derivatives. Asian J of Chemistry2007; 19: 3085-3092.

6. S Jubie, R Rajeshkumar, B Yellareddy, G Siddartha, N Sandeep,K Surendrareddy, H S Dushyanth, and K Elango. Microwave assisted synthesis of some novel benzimidazole substituted Fluoroquinalones and their antimicrobial evaluation.J of Pharm Sciences and Res 2010; 2: 69-76.

7. M Shahar Yar, Mustaqeem Abdullah, Jaseela Majeed. Invitro antitubercular screening of newly synthesized benzimidazole derivatives. World Academy of Sciences. Engand Tech 2009; 55: 593-599.

8. Bansal R: Heterocyclic chemistry. 4th ed. Tunbridge Wells: Anshan Ltd., 2007; 1-2.

9. Santosh P, Pandeya S and Pathak A: Benzimidazole: A Versatile Chemical Entity. IJRAP 2011; 2(6): 1726-1737.

10. Rang H and Dale M: Rang and Dale's pharmacology. 7th ed. Edinburgh: Elsevier / Churchill Livingstone 2012.:

11. Rehman M: Mannich base derivatives of Benzimidazole: synthesis and antimicrobial properties. WAP 2013; 3: 558-564.

12. Kalidhar U, Kumar R and Kaur A: Synthesis, Spectral Studies and Biological Evaluation of Some Benzimidazole Derivatives containing Sulfonamide. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2012; 3(2): 1074.

13. Divya S, Ramesh K and Poornima N: Synthesis, Characterization of novel Benzimidazole derivatives. IJPBS 2012; 2(2): 143-149.

14. Kumar S, Subramanian M and Chinnaiyan S: Synthesis, Characterization and evaluation of N-mannich bases of 2-substituted Benzimidazole derivatives. J Young pharm, 2013; 5(4): 154-159.

15. Singh GS, Luntha P. Synthesis and antimicrobial activity of new 1-alkyl/cyclohexyl-3, 3-diaryl-1'-methylspiro [azetidine-2, 3'-indoline]-2', 4-diones. Eur. J. Med. Chem. 2009; 44: 2265-2269.

16. Pardasani RT, Pardasani P, Muktawat S, Chaturvedi V. Reaction of 2-thiozoline-2-thiol with isatin derivatives. Phosp. Silic. Sulp.1998; 142: 221-227.

17. Olomola TO, Bada DA. Synthesis and antibacterial activity of two spiro [indole] thiadiazole derivatives. Toxi. Environ. Chem.2009; 91(5):941-946.

18. Vine KL, Locke JM, Ronson M. In vitro cytotoxicity evaluation of some substituted isatin derivatives. Bioorg. Med. Chem.2007; 15: 931-938.

19. Solomon VR, Lee C, Hu H. Hybrid pharmacophore design and synthesis of isatinbenzothiazole analogs for their anti-breast cancer activity. Bioorg. Med. Chem. 2009; 17: 7585-7592.

20. Wee XK, Yeo WK, Zhang B, Tan VBC. Synthesis and evaluation of functionalized isoindigo as antiproliferative agents. Bioorg. Med. Chem. 2009; 17: 7562-7561.

21. Andrieu BM, Merour JY. Reaction of 3-([(triflouromethyl) sulfonyl] oxy)-1*H* –indole derivatives with diamines and carbon nucleophiles, synthesis of 6*H*-indolo [2, 3-b] quinoxaline derivatives. Tetrahedron. 1998; 54: 11095-11110.

22. Ragavendran JV, Sriram D, Patel SK, Reddy IV, Bhathwajan N. Design and synthesis and anticonvulsant activity from a combined phthalamide-GABA-anilide and hydrazone pharmacophore. Eur. J. Med. Chem.2007; 42: 146-151.

23. Sridhar SK, Pandeya SN, Stables JP, Ramesh A. Anticonvulsant activity of hydrazones, Schiff and mannich bases of isatin derivatives. Eur. J. Pharm. Sci. 2002; 16: 129-132.

24. Kamil A, Akhtar S, Jahan S, Karim A, Rafiq K and Hassan S: Benzimidazole Derivatives: Active Class of Antioxidants. International Journal of Scientific and Engineering Research 2013; 4(8): 1674-1685.

25. Sumayya K: Synthesis, characterization and antioxidation studies of 2-benzylthiobenzimidazole derivatives. Indian journal of chemistry and pharmaceutical research 2013; 1(3): 193-198.

26. Rajendra K, Subban R, Kaveri S, Senniappam V, and Sulaiman AM: Conventional and Microwave Assisted synthesis of 2-Aminothiazoles and Oxazoles and their anticancer activity. Indo American Journal of Pharmaceutical Research.

27. Silverstein R: Spectrometric identification of organic compound. John Wiley & Sons Inc, Sixth edition, 2000-98.

28. Furniss B, Hannford A, Smith W, Tatchell: Vogels textbook of practical organic chemistry. Fifth edition, London 1996, 1032-1034.

29. Bansal RK, Heterocyclic chemistry, New Age International (P) limited, publishers, fourth edition, 2014, 443-514.

30. Fatmah *et al*.: Synthesis and Evaluation of Selected Benzimidazole Derivatives as Potential Antimicrobial Agents, Molecules 2015; 20: 15206-15223. Doi: 10.3390/ molecules200815206.

31. Leonard JT, Jeyaseeli L, Kumar M and Sivakumar R: 'Synthesis, anti- inflammatory and antibacterial activities of 4-substituted phenyl benzimidazoles', Asian J. Chem., 2006; 18: 1104-1106.

32. Marinova, G: Evaluation of the methods for determination of the free radical scavenging activity by DPPH. Bulgarian journal of Agricultural Sciences 2011; 17(1): 11-24.