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Research Article

SYNTHESIS AND CHARACTERIZATION OF BENZIMIDAZOLE DERIVATIVES FOR ANTI- B ACTERIAL ACTIVITY

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ABSTRACT

The present study was conducted to evaluate 3 synthesised benzimidazole derivatives and the derivatives were prepared by treating with primary amine (methyl amine) and secondary amine (pyridine) and hence they are confirmed by IR, NMR and GC-MS. The synthesized compound shows anti bacterial activity activity against *E.Coli*, *S.Aureus* and they shows more, most and significant activity towards the microorganism hence the compound is beneficial to treat bacterial infection.

Keywords: Benzimidazole Substituents, IR, NMR, GCMS, Anti- bacterial activity.

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INTRODUCTION

Benzimidazole is a heterocyclic compound and it is the combination of benzene and imidazole. Imidazole a five membered heterocyclic ring system which contain imino group in addition to a tertiary nitrogen atom, which is located in the position 1&3 respectively [1].

Benzimidazole show both types of characters i.e acidic as well as basic features [2].

PREPRATION OF BENZIMIDAZOLE

I) Synthesis of benzimidazole from o-phenylenediamine:

Benzimidazole has been formed by the rxn of ortho-phenylenediamine with carboxylic acid using strong acids such as poly phosphoric and conc. hydrochloric acid.

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PHYSICAL PROPERTIES OF BENZIMIDAZOLE:

Benzimidazole with organic compound gas are sometime soluble in polar solvents(such as oxygen and hydrogen) or less soluble in organic solvents.

Benzimidazoles are sufficiently acidic, they are soluble inbinar of compound (aqueous alkali and N-metallic compounds).

CHEMICAL PROPERTIES OF BENZIMIDAZOLE:

Oxidation: Benzimidazoles are a unit stable in oxidation & the oxidation of($KMNO_4$ in hot alkaline solution)in vigorous condition partially oxidize benzimidazoles and provide a small touch of imidazole dicarboxylic acid. They have no effect on benzimidazole ring throughout the oxidation of substituent group.

Reduction: The standard method for benzimidazole reduction involves hydrogenation using platinumin as a catalyst in acetic acid, palladium used [3].

USES OF BENZIMIDAZOLE

I. Benzimidazole and its derivatives have an massive use in the industry of textile as a dyeing agent.

II. Benzimidazole is also used as a bleaching agent as well as it is also employed to improve the brightness of the undyed material.

III. Amino benzimidazoles are widely used for sulphur and azo dyes preparation in textile industry.
IV. Benzimidazole is also used in preparing fluorescent dyes to make the ink dyed clothes clean.
V. 2-mercaptobenzimidazole(3H)-benzimidazolethione and many other Benzimidazoles has also been used in the industry of photography.
VI. 2-mercaptobenzimidazole has been use, as oxidant for rubber and this Compound also been used in detection of various metals.
VII. Many other benzimidazole compounds are broadly used in sunburn preservatives and hence protect the skin [4].

MATERIAL AND METHODOLOGY

The present study is designed to synthesise Benzimidazole Derivative as an anti-bacterial activity. This project is consist of different research methodology.

SYNTHESIS OF BENZIMIDAZOLE DERIVATIVE SYNTHETIC REACTIONS

Synthesis of Benzimidazole Nucleus (Compound-I)

0.1 mol of o-phenylenediamine and 0.1 mol of 2-chloroacetic acid were taken in a round bottom flask and then the round bottom flask was kept in heating mentle and the mixture is heated for around 9-10hrs.After that the mixture was cooled down at room temperature and basified with 4N HCL solution, filtered, washed with ice cold water and compound I obtained is recrystallized with water [5].

Synthesis of schiff bases by using different substituted benzaldehydes with primary amine (methyl amine)

A. Schiff base by using 4-chloro benzaldehyde

IIaEquimolar amount of **4-chloro benzaldehyde**(0.1mol) and Methylamine (0.1mol) in ethanol containing few drops of acetic acid is refluxed in a heating mentle for around 9-10 hrs.Aftercompletion ,the reaction mixture is checked by TLC and than the reaction mixture is cooled at room temperature and poured over ice cold water and the solid product is filtered and recrystallized with water.

B. Schiff base by using 4-nitro benzaldehyde IIb Equimolar amount of **4-nitrobenzaldehyde** (0.1mol) and (0.1mol) Methylamine in ethanol containing few drops of acetic acid is refluxed in a heating mentle for around 9-10 hrs.Aftercompletion ,the reaction mixture is checked by TLC and than the reaction mixture is cooled at room temperature and poured over ice cold water and the solid product is filtered and recrystallized with water.

C. Schiff base by using 4-bromo benzaldehyde

IIcEquimolar amount of **4-bromobenzaldehyde** (0.1mol) and Methylamine (0.1mol) in ethanol containing few drops of acetic acid is refluxed in a heating mentle for around 9-10 hrs. After completion ,the reaction mixture is checked by TLC and than the reaction mixture is cooled at room temperature and poured over ice cold water and the solid product is filtered and recrystallized with water [6].

REDUCTION OF SCHIFF BASE BY SODIUM BOROHYDRIDE

Reduction of schiff base of 4-chlorobenzaldhyde IIIaRxn

The product obtained by the Istrxn**(4-Chlorobenzaldehyde)** is dissolved in ethanol ,and little amount of **sodium borohydride ($NABH_4$)** was added within solution and immediately change in colour in a product is observed .the rxn mixture is stirred at room temperature and allowed to stand for few min. then the excess of solvent is evaporated and residue is washed with cold water and crystallized from water to give compound.

Reduction of schiff base of 4-nitro benzaldehyde IIIb Rxn

The product obtained by the Istrxn (4-nitro benzaldehyde) is dissolved in ethanol ,and little amount of**($NABH_4$)** was added within solution and immediately change in colour in a product is observed .the rxn mixture is stirred at room temperature and allowed to stand for few min. then the excess of solvent is evaporated and residue is washed with cold water and crystallized from water to give compound.

Reduction of schiffbaseof 4-bromo benzaldehyde III cRxn

The product obtained by the Istrxn (4-bromobenzaldehyde) is dissolved in ethanol ,and little amount of **($NABH_4$)** was added within solution and immediately change in colour in a product is observed .the rxn mixture is stirred at room temperature and allowed to stand for few min. then the excess of solvent is evaporated and residue is washed with cold water and crystallized from water to give compound [7].

FUSION OF SUBSTITUTED BENZIMIDAZOLE & SCHIFF BASES

Fusion of substituted benzimidazole & 4-chloro benzaldehyde Iva

Theproduct obtained by the rxn of the ortho-phenyleneddiamine and chloroacetic acid will be added to a secondary amine (4-chlorobenzaldehyde) and**K_2CO_3** in dry acetone .the rxn will stirred for 6-8 hours and ambient temperature and acetone will then evaporate. Distilled water will add to the residue and the formed ppt. The rxn mixture is checked by TLC. And filtered product is wash with water ,dry and recrystallization for appropriate solvent.

Fusion of substituted benzimidazole & 4-nitrobenzaldehyde IVb

The product obtained by the rxn of the ortho-phenylene d diamine and chloroacetic acid will be added to a secondary amine (4-nitrobenzaldehyde) and$\mathbf{K_2CO_3}$dry acetone .the rxn will stirred for 6-8 hours and ambient temperature and acetone will then evaporate. Distilled water will add to the residue and the formed ppt. The rxn mixture is checked by TLC. And filtered product is wash with water ,dry and recrystallization for appropriate solvent.

Fusion of substituted benzimidazole & 4-bromo benzaldehyde IVc

The product obtained by the rxn of the ortho-phenyleneddiamine and chloroacetic acid will be added to a secondary amine (4-bromobenzaldehyde) andin$\mathbf{K_2CO_3}$ dry acetone .the rxn will stirred for 6-8 hours and ambient temperature and acetone will then evaporate. Distilled water will add to the residue and the formed ppt. The rxn mixture is checked by TLC. And filtered product is wash with water ,dry and recrystallization for appropriate solvent[5].

Synthesis of different benzaimidazole derivative with secondary amine(pyridine)

Synthesis of 4-chloro benzaldehyde with pyridine

VI-M4 the mixture of compound 4^{th}rxn (0.004 Mol) and formaldehyde (0.004 Mol), and 2^0amine (0.004Mol) and HCL(2ml) & methanol reflux with 2-3 hours. the mixture will filtered and solution was cooled on ice water &crystal was obtained by filteration&dried& recrystallization from suitable solvent.

Synthesis of 4-nitrobenzaldehyde with pyridine

VI-M5 the mixture of compound 4^{th}rxn (0.004 Mol) and formaldehyde (0.004 Mol), and 2^0amine (0.004Mol) and HCL(2ml) & methanol reflux with 2-3 hours. the mixture will filtered and solution was cooled on ice water &crystal was obtained by filteration& dried & recrystallization from suitable solvent.

Synthesis of 4-bromo benzaldehyde with pyridine

VI-M6 the mixture of compound 4^{th}rxn (0.004 Mol) and formaldehyde (0.004 Mol), and 2^0amine (0.004Mol) and HCL(2ml) & methanol reflux with 2-3 hours. the mixture will filtered and solution was cooled on ice water &crystal was obtained by filteration&dried& recrystallization from suitable solvent [6,7]

ANTI-BACTERIAL ACTIVITY

The serial dilution method will use to evaluate antibacterial activity of synthesised compounds against different bacteria.

PRINCIPLE

The serial dilution method is stepwise dilution of the substance in solution usually the dilution factor at each step is constant, resulting in ageometric progession test to antibiotics.it most commonly used method to perform these test . The lowest concentration (highest dilution)of antibiotic preventing appearance of turbidity is considered to be the MIC.

MATERIAL

HIMEDIA M210-500G

Brain heart infusion broth 500g

Ingredients	Gms/litre
Calf brain, infusion from	200.00
Beef heart, infusion from	250.00
Proteose peptone	10.00
Dextrose	2.00
Sodium chloride	5.00
Disodium phosphate	2.50

Final pH (at 25ºC) 7.4+/-0.2

NOTE: For facultative anaerobes, tubes were incubated at 37°C for 48-72 hrs in Co2 Jar. For strict anaerobes, tubes were incubated in anaerobic jars for 48-72 hrs.

STANDARD VALUESfor the MIC test which was performed.

For anti-bacterial Ciprofloxacin (10µg):

S.aureus - 2µg/ml GRAM +VE

E.coli - 2µg/ml GRAM -VE facultative anaerobe

MIC TEST PROCEDURE

I. 9 dilutions of each drug have to be done with BHI (brain heart infusion) for MIC.

II. In the initial tube 20microliter of drug was added into the 380microliter of BHI broth.

III. For dilutions 200microliter of BHI (brain heart infusion) broth was added into the next 9 tubes separately.

IV. Then from the initial tube 200microliter was transferred to the first tube containing 200microliter of BHI broth. This was considered as 10-1 dilution.

V. From 10-1 diluted tube 200microliter was transferred to second tube to make 10-2 dilution.

VI. The serial dilution was repeated up to 10-9 dilution for each drug.

VII. From the maintained stock cultures of required organisms, 5microliter was taken and added into 2ml of BHI (brain heart infusion) broth.

VIII. In each serially diluted tube 200microliter of above culture suspension was added.

IX. The tubes were incubated for 24 hours and observed for turbidity [8].

^{1}H NMR of compound VI-M_4: δ 1.57 (3H, t, *J* = 7.7 Hz), 1.61 (8H, R-CH2-R),3.97 (4H, dd, *J* = 14.6, 10.3, 2.7 Hz, CH2-N-CH2),4.66 (2H, t, *J* = 2.7 Hz, N-CH2-R), 4.68 (2H, s, N-CH2-AR), 4.82 (2H, s, N-CH2), 5.24 (2H, s, N-CH2-N), 6.49 (1H, AR-H), 6.97 (1H, 0.5 Hz, AR-H), 7.16 (4H, AR-H).

^{1}H NMR of compound VI-M_5: δ 0.91 (3H, t, *J* = 7.7 2.7 Hz, CH2-N-CH2),2.89 (2H, t, *J* = 2.7 Hz, N-CH2-R), 4.00 (2H, s, N-CH2-AR), 4.48 (2H, s, N-CH2), 4.89 (2H, s, N-CH2-N), 6.48 (1H, AR-H), 6.84 (1H, 0.5 Hz, AR-H), 7.49 (4H, AR-H).

^{1}H NMR of compound VI-M_6: δ 0.92 (3H, t, *J* = 7.7 Hz), 1.86 (8H, R-CH2-R),1.94 (4H, dd, *J* = 14.6, 10.3, 2.7 Hz, CH2-N-CH2),3.61 (2H, t, *J* = 2.7 Hz, N-CH2-R), 3.65 (2H, s, N-CH2-AR), 4.98 (2H, s, N-CH2), 4.91 (2H, s, N-CH2-N), 7.12 (1H, AR-H), 7.34 (1H, 0.5 Hz, AR-H), 7.56 (4H, AR-H).

IR SPECTROSCOPY OF SYNTHESISED COMPOUND

IR (KBr) of compound **VI-M_4**: 3341 cm-1 (stretching, N-H), 3023 cm-1 (stretching, Ar-H), 2920 cm-1 (stretching, C-H, aliphatic), 1640 cm-1 (stretching, C=C, unsaturated), 1465 cm-1 (stretching, -CH3) and 742 cm-1 (stretching, C-Cl)

IR (KBr) of compound **VI-M_5**: 3341 cm-1 (stretching, N-H), 3023 cm-1 (stretching, Ar-H), 2920 cm-1 (stretching, C-H, aliphatic), 1421 cm-1 (stretching, N-O), 1640 cm-1 (stretching, C=C, unsaturated) and 1465 cm-1 (stretching, -CH3)

IR (KBr) of compound **VI-M_6**: 3341 cm-1 (stretching, N-H), 3023 cm-1 (stretching, Ar-H), 2920 cm-1 (stretching, C-H, aliphatic), 1640 cm-1 (stretching, C=C, unsaturated), 1465 cm-1 (stretching, -CH3) and 678 cm-1 (stretching, C-Br).

GC-MS SPECTROSCOPY OF SYNTHESISED COMPOUND

GC-MS of compound.VI-M_4

Analyzed or confirmed thatin this compoundwithmolecular formula ($C_{20}H_{32}N_5Cl$), molecular weight(412.1) and molecular positve ion peak$(M+H)^+$ 413.1 (spectra no.-7)

GC-MS of compound.VI-M_5

Analyzed or confirmed thatin this compoundwithmolecular formula ($C_{20}H_{32}N_6O_2$), molecular weight (423.4) and molecular molecularpositve ion peak$(M+H)^+$ 424.4 (spectra no.-8).

GC-MS spectra of compound VI-M_6

Analyzed or confirmed that in this compound with molecular formula ($C_{20}H_{32}N_5$ Br), molecular weight(456.4) and molecular positve ion peak$(M+H)^+$ 457.4show (spectra no.-9).

STANDARD VALUES for the MIC test which was performed.

For anti-bacterial Ciprofloxacin (10µg):

S.Aureus - 2µg/ml **GRAM +VE**

E.coli - 2µg/ml **GRAM -VE** FACULTATIVE ANAEROBE

Table 1. General information of benzimidazole

IUPAC NAME - 1H-BENZIMIDAZOLE	
OTHER NAME-1H-BENZO[d]IMIDAZOLE	
PROPERTIES	
Chemical formula	$C_7H_6N_2$
Melting point	170-172°C(338-342°F
Acidity(pk_a)	12.8 for benzimidazole &5.6 for conjugate acid
Molar mass	118.14gmol^{-1}

Table 2. Antibacterial activity of synthesized compounds

Sr. No.	Sample	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.12 mg/ml	1.6 mg/ml	0.8 mg/ml	0.4 mg/ml	0.2 mg/ml
E.Coli											
01	VI-M4	S	S	S	S	S	S	S	R	R	R
02	VI-M5	S	S	S	S	S	S	R	R	R	R
03	VI-M6	S	S	S	S	S	R	R	R	R	R
S.Aureus											
01	VI-M4	S	S	S	S	R	R	R	R	R	R
02	VI-M5	S	S	S	S	S	S	S	S	R	R
03	VI-M6	S	S	S	S	S	S	R	R	R	R

INDEX: S - Sensitive, R- Resistant.

Table 1. NMR SPECTROSCOPY OF SYNTHESISED COMPOUND

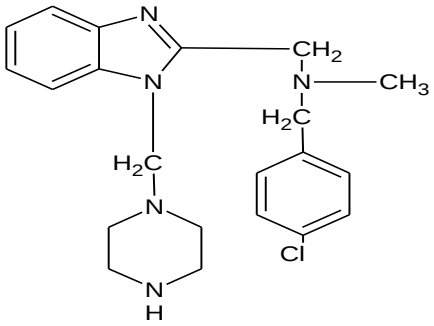
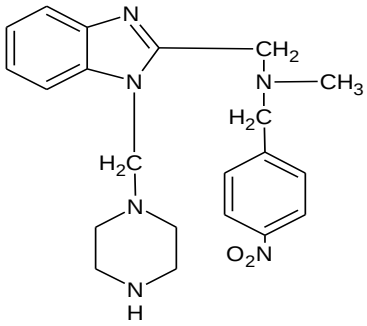
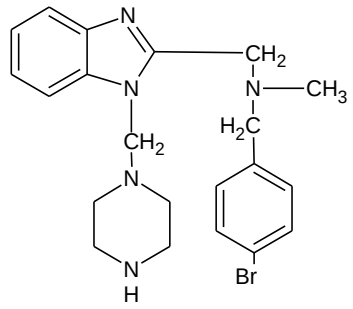
 **VI-M_4**	 **VI-M_5**	 **VI-M_6**

Fig 1. Structure of 1H-Benzimidazole

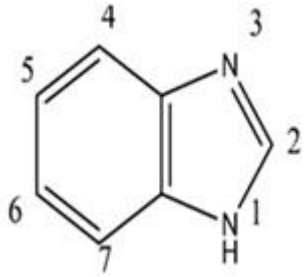


Fig 2. Synthesis of benzimidazole from o-phenylenediamine

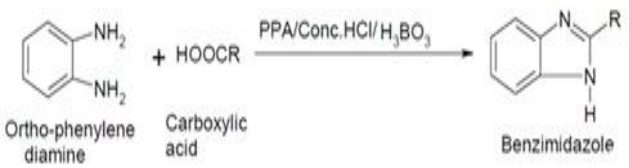
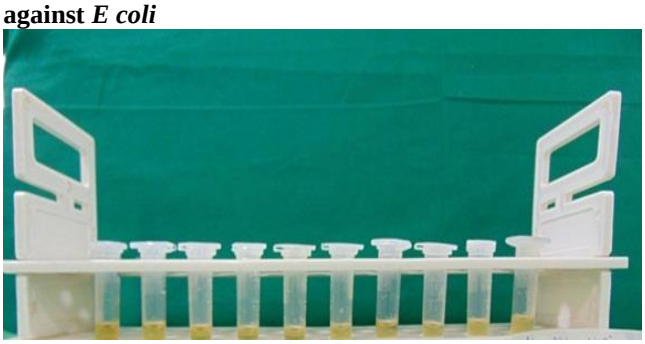


Fig 3. Anti-bacterial activity of synthesised compound against *E coli*



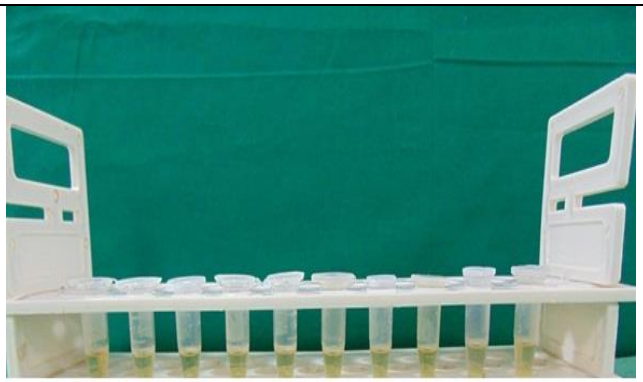
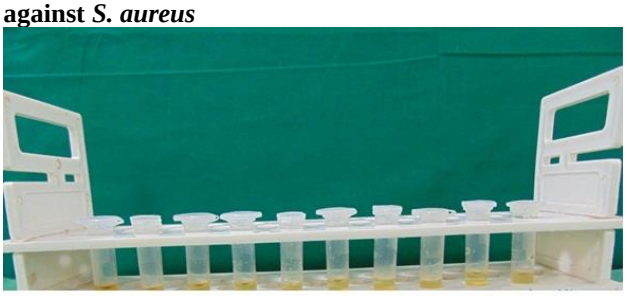
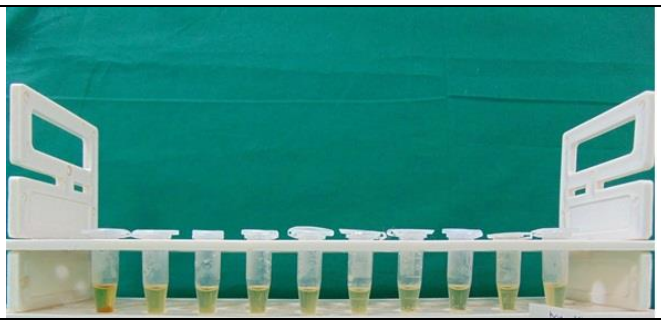
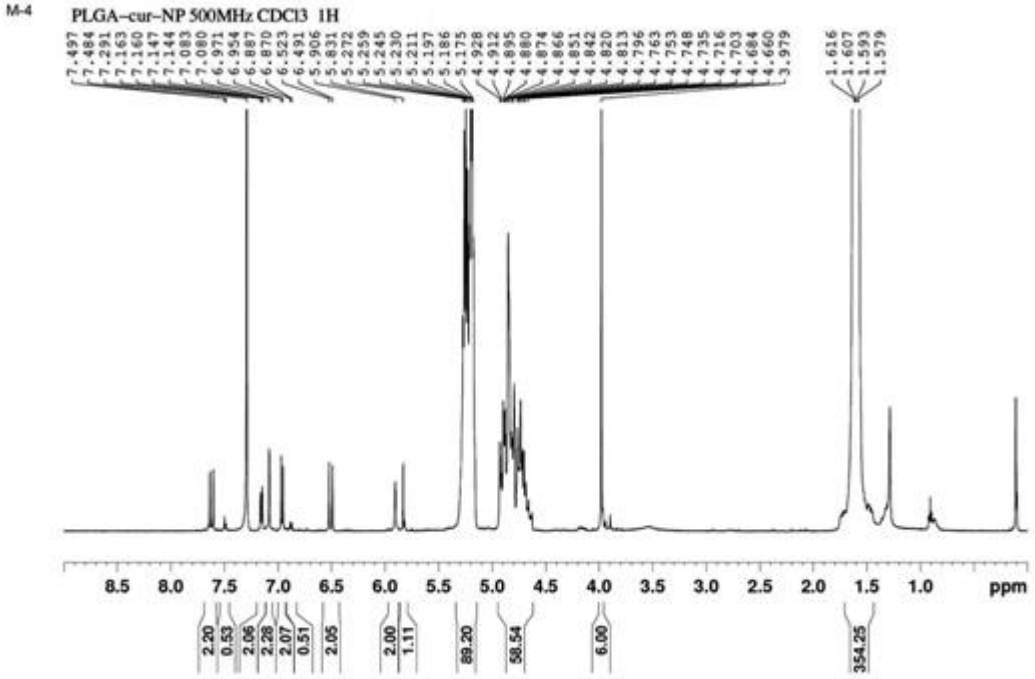


Fig 4. Anti-Bacterial Activity of Synthesised Compound against *S. aureus*

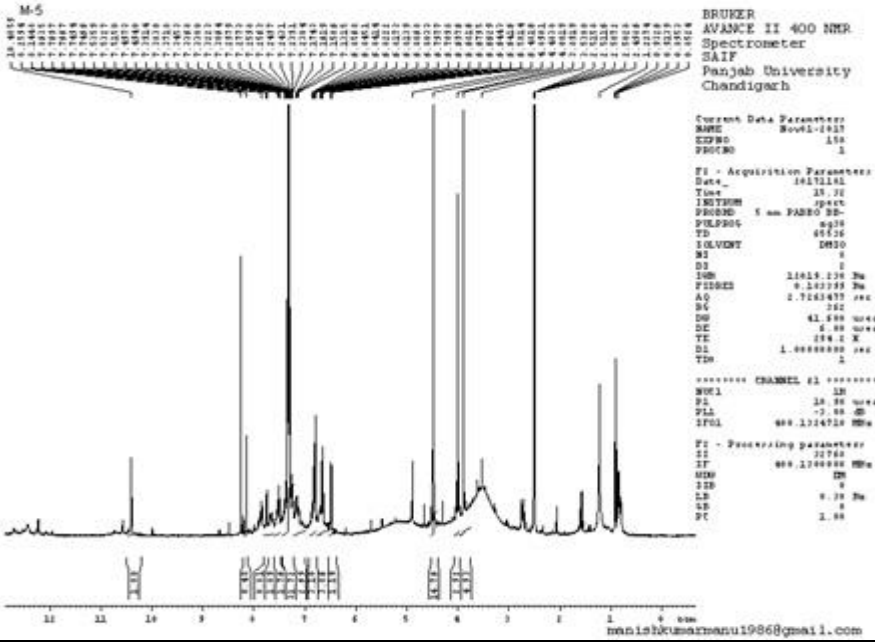




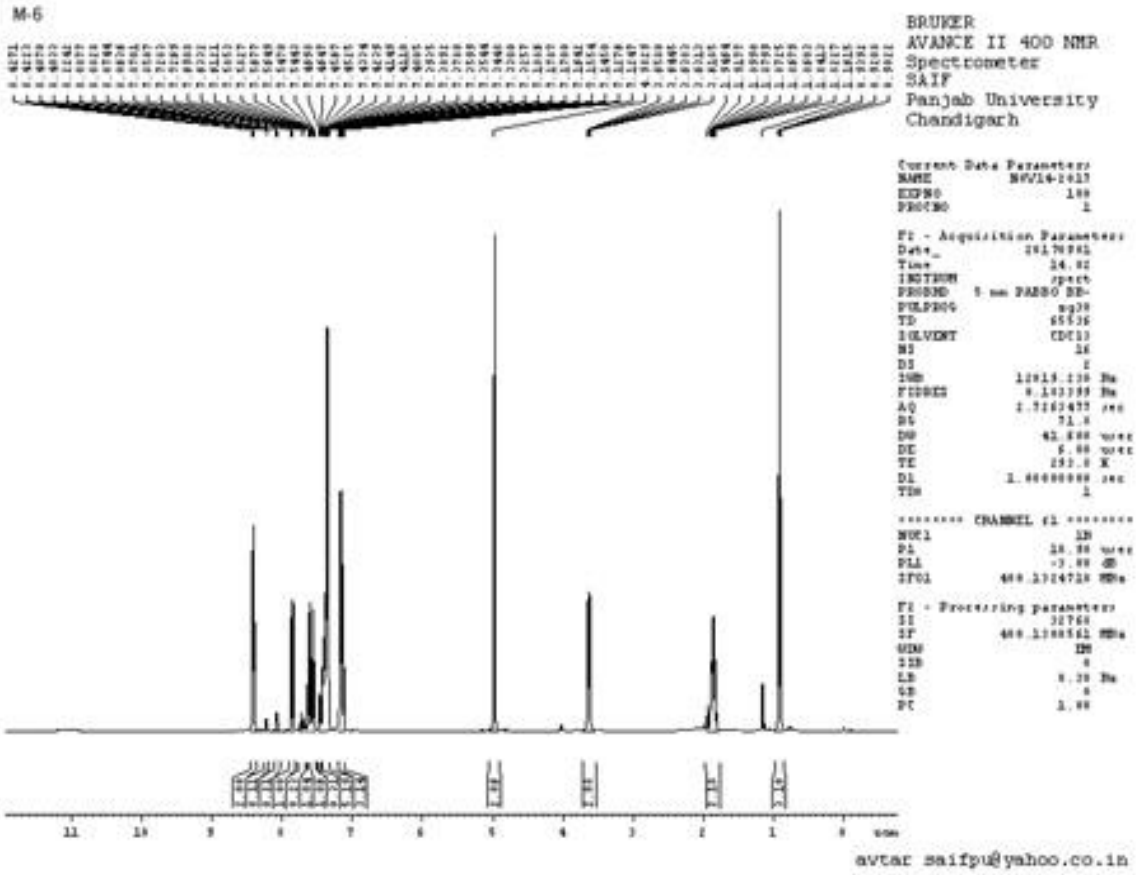
Spectra No.1 - NMR Spectra of compound VI –M_4



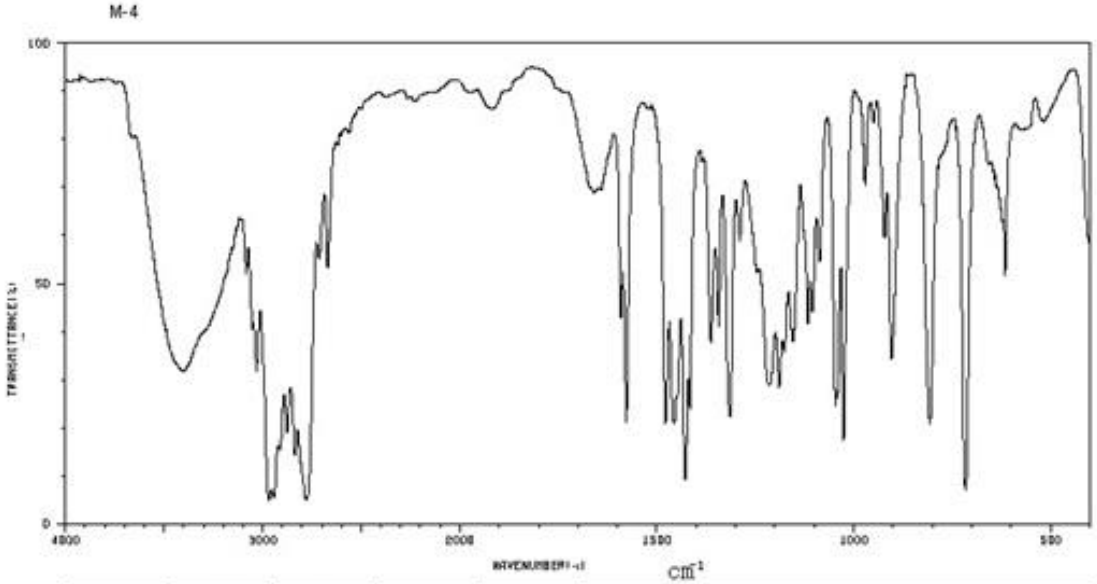
Spectra no 2. NMR spectra of compound VI –M_5



Spectra no 3. NMR spectra of compound VI –M_6

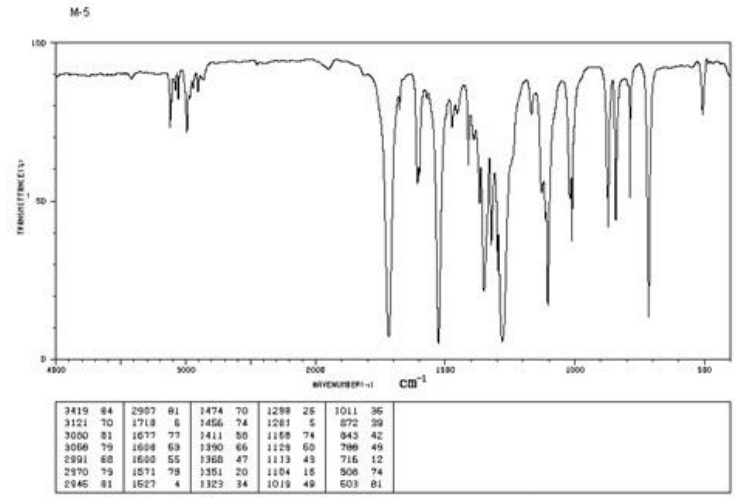


Spectra No 4. IR spectra of Compound VI- M_4



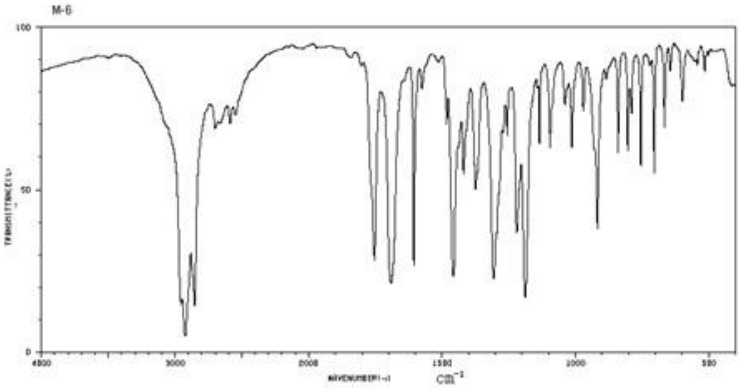
3402	30	2877	18	1470	20	1315	21	1087	62		
3084	50	2834	13	1456	20	1214	27	1045	23		
3051	38	2778	4	1428	6	1190	27	1026	16		
3031	30	2716	69	1417	23	1177	34	904	39		
2969	4	2670	52	1363	36	1155	36	807	20		
2956	6	1591	41	1351	55	1116	59	717	6		
2942	6	1477	20	1344	39	1105	42	616	60		

Spectra No 5. IR spectra of Compound VI- M_5



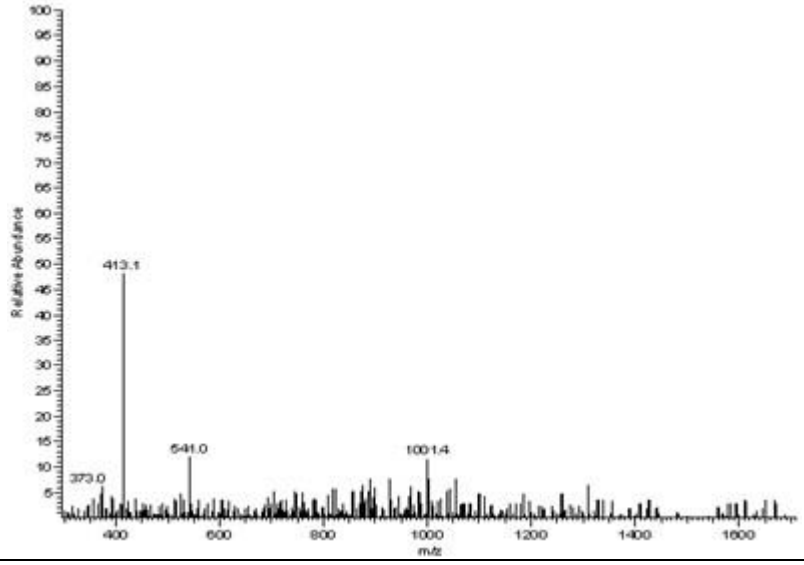
3419	64	2907	81	1474	70	1298	26	1011	36
3121	70	1718	6	1456	74	1261	5	872	39
3050	81	1677	77	1411	58	1168	74	843	42
3006	79	1606	69	1390	66	1129	60	788	49
2991	68	1580	55	1368	47	1113	43	716	12
2970	79	1571	78	1351	20	1104	15	506	74
2946	81	1527	4	1323	34	1019	49	603	81

Spectra No 6. IR spectra of Compound VI- M_6

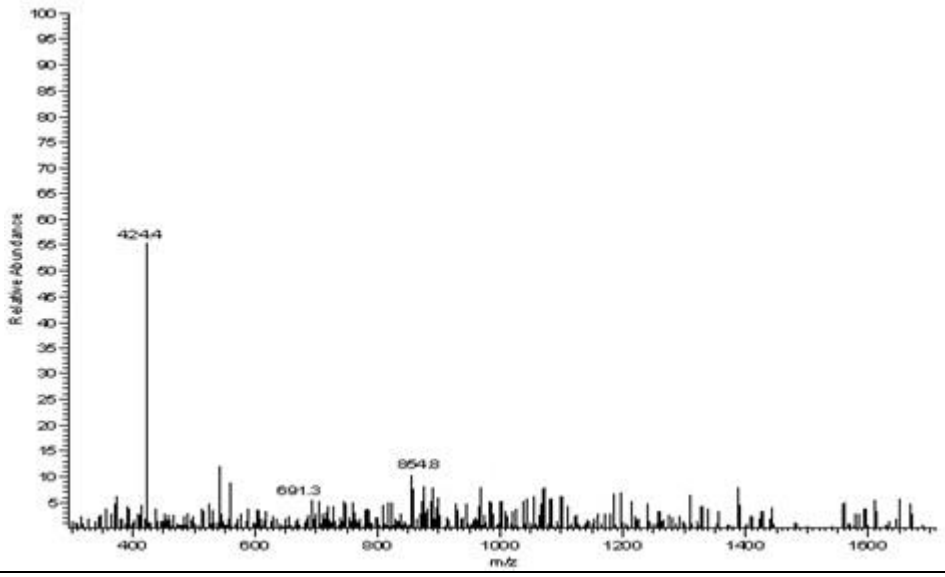


2966	13	2647	70	1420	53	1096	60	841	58
2924	4	1753	26	1375	47	1040	72	805	58
2854	13	1692	20	1307	21	1025	79	790	70
2701	66	1606	26	1267	64	1014	60	766	66
2682	68	1575	79	1221	35	971	79	705	53
2665	69	1483	68	1186	16	918	35	667	86
2686	69	1468	22	1136	62	886	81	600	74

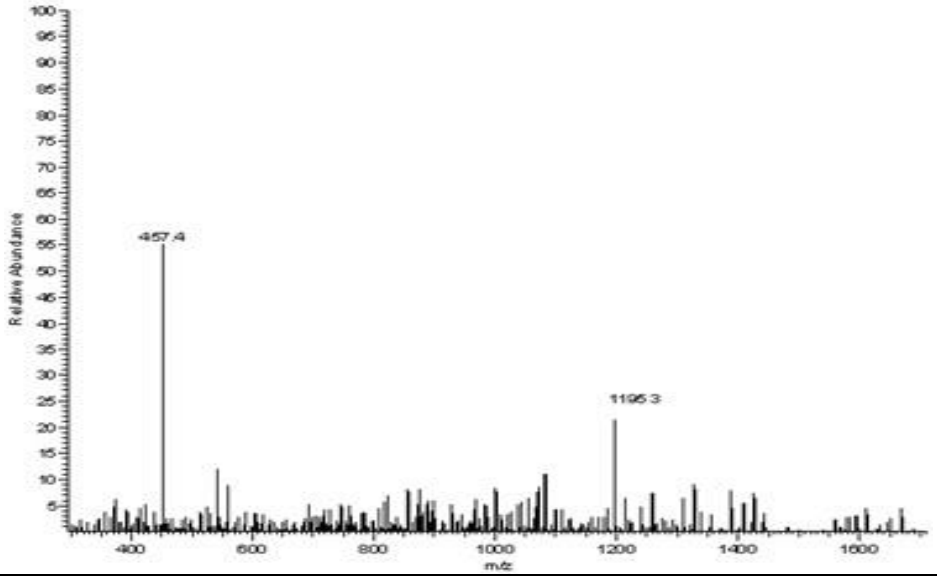
Spectra No 7. GC-MS spectra of Compound VI- M_4



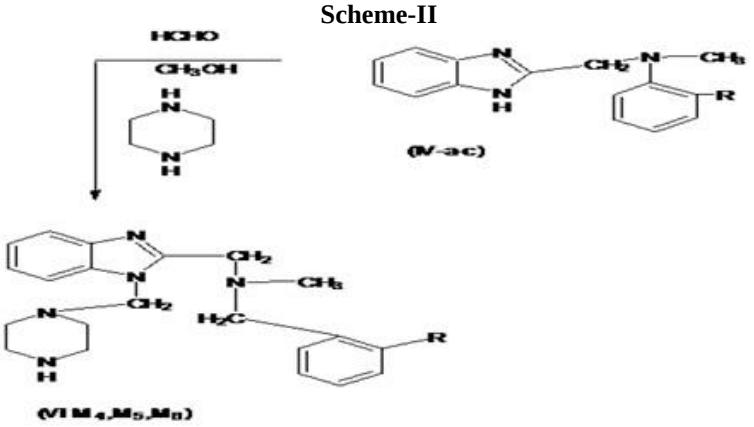
Spectra No 8. GC-MS spectra of Compound VI- M_5



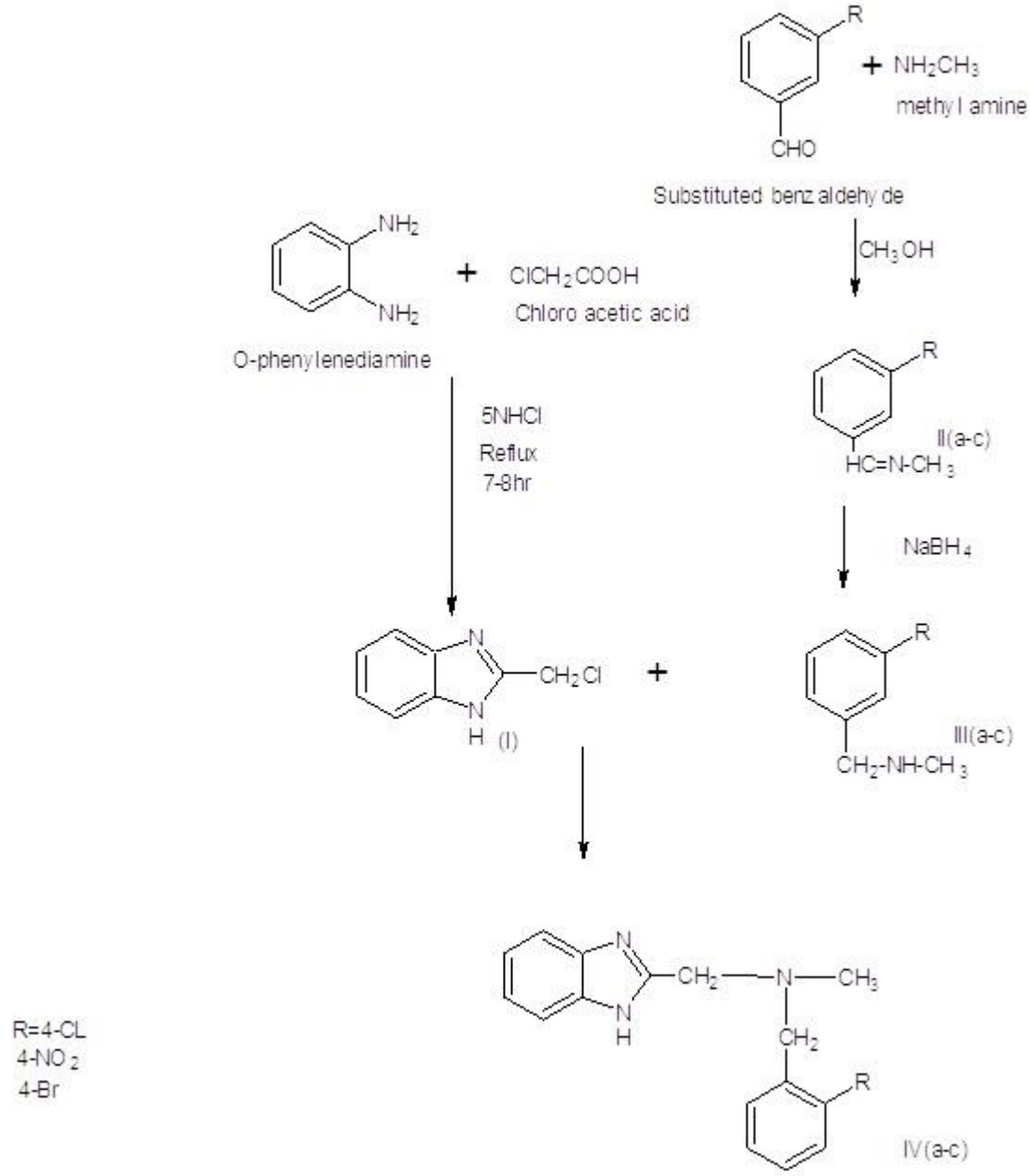
Spectra No 9. GC-MS spectra of Compound VI- M_6



Scheme-II



Scheme-I



DISCUSSION

All the benzimidazole derivatives are Confirmed by spectral data i.e (IR, NMR & GC-MS). The NMR spectra of the synthesized compound VI-M_4 (CH_3) 1.57 indicate the presence of methyl group.the range i.e(R-CH_2-R) 1.62 indicate the presence of alkyl chain .the range that (N-CH_2-R) 4.66 that shows presence of alkyl group.(Ar-H) (6.7-7.16) indicate that the presence of aromatic compound. VI-M5 (CH_3) 0.91 indicate the presence of methyl group.the range i.e(R-CH_2-R) 1.22 indicate the presence of alkyl chain .the range that (N-CH_2-R) 2.89 that shows presence of alkyl group.(Ar-H) 6-7.49 indicate that the presence of aromatic compound

The IR spectra of the compound VI-M_4showed mainly stretching band absorption (1465cm^{-1}) &(742cm-1) assign to (C-Cl) . And the compound VI-M_5 shows stretching band absorption 1421 cm^{-1}(N-O) .andVI-M_6 compound show stretching band absorption 678cm^{-1} (C-Br).

The assessment of Anti-Bacterial action of all the compound(M_4-M_6) was performed by Serial Dilution Method Method by using Standard DrugCiprofloxacin (10μg). The anti-bacterial (E.coli) organisam compound VI-M4 are more significant compound because it show their activity at MIC 1.6mg\ml concentration and other compound VI-M5&VI-M6are sensitive 3.12 mg\ml

concentration. Or these compound show resistance. The anti bacterial (*S.aureus)* organism compound VI-M5 show their activity at MIC 0.8 mg/ml concentration and the compound VI-M4, VI-M6 shows resistance at 12.5mg\ml concentration.

CONCLUSION

The conclusion of research work is that i have been synthesised 3 compounds and that are confirmed by IR, NMR. GC-MS. The synthesised compound shows more, most and significant activity towords the *E.coli* and *S.aureus* microorganisam. Hence the compound are beneficial to treat the bacterial infection.

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CONFLICT OF INTEREST

Nil

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