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Synthesis of Benzimidazole Derivatives Containing Schiff Base Exhibiting Antimicrobial Activities

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Abstract: *A series of 2-substituted Benzimidazole having imine linkage were synthesized by two step reactions. In the first step, o-phenylenediamine was condensed with p-amino benzoic acid in xylene and poly phosphoric acid to give 2-amino Benzimidazole. In the second step, 2-amino Benzimidazole is treated with different substituted aldehydes and ketones to form substituted Benzimidazole having imine linkage. The synthesized compounds were evaluated for anti-bacterial activity against Staphylococcus aureus and Escherichia coli by tube dilution method. The compounds SAM-2 and SAM-10 were found to be more potent than standard drug vancomycin against Gram (+) ve and Gram (–) ve bacteria. The compounds SAM-4 and SAM-9 had anti-microbial activity comparable to standard drugs against both the microorganisms. The aims of this study to synthesize novel benzimidazole derivatives containing Schiff's base and evaluate the antimicrobial activity of the synthesized derivatives against S. aureus and E. coli.*

Keywords: *Benzimidazole, Schiff base, Antimicrobial activity, Staphylococcus aureus, Synthesized*

1. INTRODUCTION

Benzimidazole is a heterocyclic aromatic organic compound. It is an important pharmacophore and a fortunate structure in medicinal chemistry. This bicyclic compound consists of the fusion of benzene and imidazole ring [1, 20]. The general synthesis of benzimidazole involves condensation of phenylenediamine with formic acid. By using different derivative of carboxylic acid, this method is generally able to afford 2-substituted benzimidazoles [5].

The different benzimidazole derivatives are associated with a wide range of biological activities such as anti-cancer [13], anti-viral [21], anti-bacterial [7, 9, 22], anti-fungal [18], anti-helmintic [23], anti-inflammatory [10], anti-histaminic [15], proton pump inhibitor [8], anti-oxidant [2], anti-hypertensive [14] and anti-coagulant [17] properties. Literature review shows that along with the benzimidazole derivatives, 2-substituted ones are found to be pharmacologically more important and hence syntheses of 2-substituted benzimidazoles are the reasonable area of research [4, 19]. Prevalent biochemical and pharmacological studies have confirmed that its derivatives are capable against a variety of strains of microorganisms [6, 11, and 12].

Imines are formed by the reaction of a primary amine with aldehydes or ketones with the simultaneous removal of water e.g. by azeotropic distillation, by addition of anhydrous sodium sulfate, by addition of molecular sieves or by use of titanium chloride. When one, or both, of reactant is aliphatic, the imine is quite stable and usually known as a Schiff base [5]. In case of completely aliphatic reactants, the imines tend to decompose or polymerize; in these cases their further reaction is carried out without delay [5].

The formation of a Schiff base from aldehydes of ketones is a reversible reaction and generally takes place under acid or base catalysis. The formation is generally driven to the completion by separation

of the product or removal of water or both. Many Schiff bases can be hydrolyzed back to their aldehydes or ketones and amines by aqueous acid or base. The mechanism of formation of Schiff base is another variation on the theme of nucleophilic addition to the carbonyl group. In this case, the nucleophile is the amine. In the first part of the mechanism, the amine reacts with the aldehyde or ketone to give an unstable carbinolamine compound [16].Schiff bases have also exhibited a broad range of biological activities including anti-fungal, anti-bacterial, anti-malarial, anti-proliferative, anti-inflammatory, anti-viral, and anti-pyretic properties [3].

2. MATERIALS AND METHODS

2.1. General Methodology

The experiment was conducted in the Department of Pharmaceutical Chemistry, Lovely Professional University, Punjab, India. The melting points of compounds were determined by capillary method. The slurry for the preparation of TLC plates was prepared by mixing the adsorbent (Silica gel G) in water. The TLC plates were prepared by pouring method. IR spectra were recorded on FTIR spectrophotometer Shimadzu, Singapore as KBr disks. H-NMR spectra were acquired using a Bruker Avance-2 NMR spectrometer (400 MHz) using TMS as internal standard. The following abbreviations were used to indicate the peak multiplicity: s = singlet, d = doublet, t = triplet, m = multiplet. ^{1}H-NMR spectra were assigned relative to the TMS peak at 0.0 ppm.

The percentage yield of the reaction was calculated by the following formula:

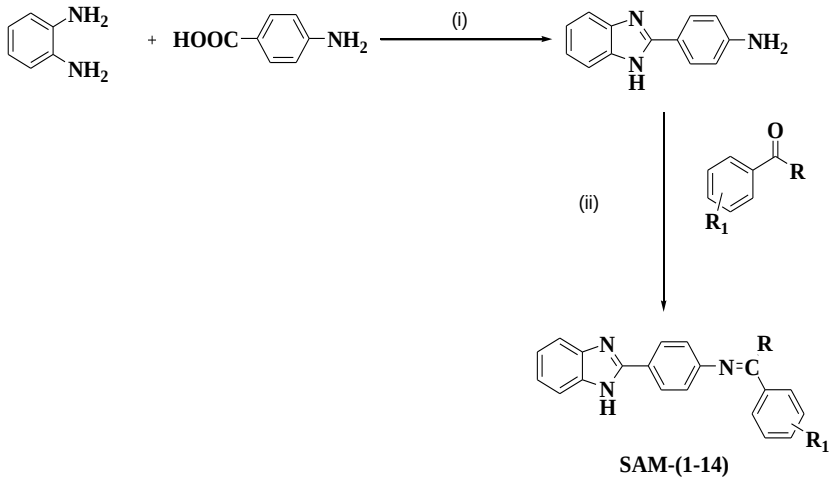
$$\text{Percentage Yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

2.2. Synthesis of 2-(4-Aminophenyl)-1H-Benzimidazole

A suspension of polyphosphoric acid (0.08mol) in xylene (0.2mol) at 60°C, *o*-phenylenediamine (0.03mol) and 4-aminobenzoic acid (0.03mol) were added. The temperature was raised to 130°C for 1 hr before it was raised to 150°C and stirred for 6 h. The reaction mixture was cooled and diluted with hot water with stirring. The hot reaction mixture was filtered through a Buchner funnel and solid was isolated. Mother liquor also afforded additional solid. Both solids were taken in water (500 ml) and neutralized with solid $NaHCO_3$. The yellowish green solid was filtered. These observed characteristics were- Melting point : 297-299^{o}C, Yield: 51% , Solubility: Soluble in DMSO, Mobile phase - Ethyl acetate: hexane (4:6), R_f value for *O*-Phenylendiamine– 0.33, R_f value for 2- (4 aminophenyl)-1*H*-benzimidazole– 0.12, R_f value for 4-amino benzoic acid– 0.15.

2.3. Synthesis of Imine Derivatives of Benzimidazole

2-(4-aminophenyl)-1*H*-benzimidazole (0.002mol) was mixed with different substituted aldehydes / ketones (0.002mol) in 15 ml absolute ethanol. The reaction mixture was refluxed till the completion of reaction (as indicated by TLC using silica as stationary phase and (Toluene: Ethyl acetate: Formic acid: 5:4:1 as mobile phase). Then the reaction mixture was concentrated and kept overnight for crystallization. The solid separated was filtered and recrystallized from 95% ethanol.



SAM-(1-14)

Scheme: Reagents: *(i) PPA/Xylene, 150°C, 6hr Reflux (ii) Ethanol*

Table1. *List of synthesized benzimidazole derivatives containing Schiff base*

Serial No.	Compound codes	R	R_1
1	SAM – 1	-H	2-Cl
2	SAM – 2	-H	-H
3	SAM – 3	-H	4-F
4	SAM – 4	-H	4-NO_2
5	SAM – 5	-H	2-NO_2
6	SAM – 6	-CH_3	4-NO_2
7	SAM – 7	-CH_3	-H
8	SAM – 8	-H	4-OH
9	SAM – 9	-CH_3	4-OH
10	SAM – 10	-H	2-OH
11	SAM – 11	-H	4-OCH_3
12	SAM – 12	-H	4-Cl
13	SAM – 13	-H	3-OCH_3-4-OH
14	SAM – 14	-CH_3	2,4-OH

*N-(2-chlorobenzylidene)-4-(1H-benzo[d]imidazol-2-yl)benzenamine (**SAM-1**):*Melting point : 326-330 ºC, Yield : 50% **,** Solubility : Soluble in DMSO, R_f value for 2-(4-aminophenyl)-1*H*-benzimidazole– 0.46**,** R_f value for Compound (SAM-1)– 0.26,R_f value for 2- Chlorobenzaldehyde– 0.82**.** IR (KBr) cm-1; 3344 (N-H), 3219 (aromatic C-H), 3059(C-H imine linkage), 1627 (C=N), 1600 and 1462 (aromatic C=C); 1H- NMR

*4-(1H-benzo[d]imidazol-2-yl)-N-benzyl idene benzenamine (**SAM-2**):* Melting point: 328-330 ºC, Yield: 48%, Solubility: Soluble in DMSO, R_f value for 2-(4-aminophenyl)-1*H*-benzimidazole– 0.47, R_f value for Compound (SAM-2)– 0.58, R_f value for Benzaldehyde– 0.78.

*N-(4-fluorobenzylidene)-4-(1H-benzo[d]imidazol-2-yl)benzenamine (**SAM-3**):*Melting point : 287-291 ºC, Yield : 62%, Soluble in DMSO, R_f value for 2-(4-aminophenyl)-1*H*-benzimidazole– 0.47, R_f value for Compound (SAM-3)– 0.80, R_f value for 4-Flurobenzaldehyde– 0.89.

*N-(4-nitrobenzylidene)-4-(1H-benzo[d]imidazol-2-yl)benzenamine (**SAM-4**):*Melting point : 270-280 ºC, Yield : 65%**,** Soluble in DMSO, R_f value for 2-(4-aminophenyl)-1*H*-benzimidazole– 0.46**,** R_f value for Compound (SAM-4)– 0.64**,** R_f value for 4-Nitrobenzaldehyde – 0.76.

*N-(2-nitrobenzylidene)-4-(1H-benzo[d]imidazol-2-yl) benzenamine (**SAM-5**):*Melting point : 284-289 ºC, Yield : 61%, Soluble in DMSO**,** R_f value for 2-(4-aminophenyl)-1*H*-benzimidazole– 0.46, R_f value for Compound **(**SAM-5**)**– 0.48,R_f value for 2-Nitrobenzaldehyde – 0.77.

*4-(1H-benzo[d]imidazol-2-yl)-N-(1-(4-nitrophenyl)ethylidene) benzenamine (**SAM-6**):*Melting point : 286-291, Yield : 50%**,** Soluble in DMSO, R_f value for 2-(4-aminophenyl)-1*H*-benzimidazole– 0.48,R_f value for Compound (SAM-6)– 0.37**,** R_f value for 4-Nitroacetophenone – 0.81.

*4-(1H-benzo[d]imidazol-2-yl)-N-(1-phenylethylidene) benzenamine (**SAM-7**):* Melting point: 276-281 ºC, Yield: 45%, Soluble in DMSO, R_f value for 2-(4-aminophenyl)-1*H*-benzimidazole– 0.46, R_f value for Compound (SAM-7)– 0.26, R_f value for Acetophenone – 0.84.

*4-((4-(1H-benzo[d]imidazol-2-yl) phenylimino) methyl) phenol (**SAM-8**):*Melting point : 284-289 ºC, Yield : 50%, Soluble in DMSO**,** R_f value for 2-(4-aminophenyl)-1*H*-benzimidazole – 0.45, R_f value for Compound (SAM-8)– 0.26,R_f value for 4-Hydroxybenzaldehyde – 0.70.

*4-(1-(4-(1H-benzo[d]imidazol-2-yl)phenylimino)ethyl) phenol (**SAM-9**):*Melting point : 284-289 ºC, Yield : 55%**,** Soluble in DMSO, R_f value for 2-(4-aminophenyl)-1*H*-benzimidazole– 0.46,R_f value for Compound (SAM-9)– 0.71, R_f value for 4-Hydroxyacetophenone– 0.80.

*2-((4-(1H-benzo[d]imidazol-2-yl) phenylimino) methyl) phenol (**SAM-10**):*Melting point : 284-288 ºC, Yield : 62%**,** Soluble in DMSO, R_f value for 2-(4-aminophenyl)-1*H*-benzimidazole– 0.44,R_f value for Compound (SAM-10)– 0.65, R_f value for 2-Hydroxybenzaldehyde– 0.78.

*N-(4-methoxybenzylidene)-4-(1H-benzo[d]imidazol-2-yl)benzenamine (**SAM-11**):*Melting point : 274-278 ºC, Yield : 54%**,** Soluble in DMSO, R_f value for 2-(4-aminophenyl)-1*H*-benzimidazole– 0.47**,** R_f value for Compound (SAM-11)– 0.64,R_f value for 4-Methoxybenzaldehyde– 0.72

*N-(4-chlorobenzylidene)-4-(1H-benzo[d]imidazol-2-yl)benzenamine (**SAM-12**):*Melting point : 274-278 ºC, Yield : 51%**,** Soluble in DMSO, R_f value for 2-(4-aminophenyl)-1*H*-benzimidazole– 0.46**,** R_f value for Compound (SAM-12)– 0.58,R_f value for 4-Chlorobenzaldehyde – 0.88.

*4-((4-(1H-benzo[d]imidazol-2-yl) phenylimino) methyl) -2 methoxy phenol. (**SAM-13**):*Melting point : 274-278 ºC, Yield : 64%**,** Soluble in DMSO, R_f value for 2-(4-aminophenyl)-1*H*-benzimidazole– 0.46**,** R_f value for Compound (SAM-13)– 0.23,R_f value for Vanillin– 0.82.

*4-(1-(4-(1H-benzo[d]imidazol-2-y) phenylimino)ethyl)benzene-1,3-diol (**SAM-14**):*Melting point : 274-278 ºC, Yield : 64%**,** Soluble in DMSO, R_f value for 2-(4-aminophenyl)-1*H*-benzimidazole– 0.45,R_f value for Compound (SAM-14)– 0.64,R_f value for 2,4-Dihydroxyacetophenone – 0.82.

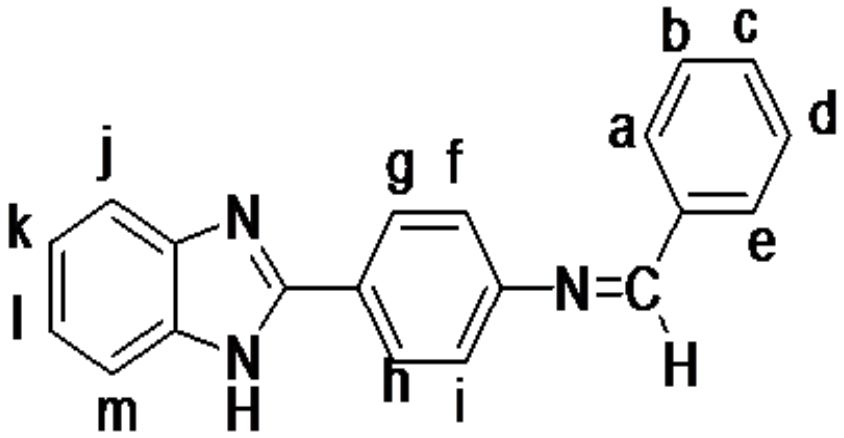
2.4. Estimation of Anti-Bacterial Activity

The anti-bacterial activity of compounds was studied by employing a tube-dilution method, using two different culture media: Mueller–Hinton broth and Luria Bertania (LB). Compounds were dissolved in DMSO (10% of the final volume) and diluted with culture broth to a concentration of 2 mg/mL. Further 1:2 serial dilutions were performed by addition of culture broth to reach concentrations ranging from 2 to 0.0156 mg/mL,100 µL of each dilution were distributed in 96 well plates/tubes, as well as a sterility control and a growth control (containing culture broth plus DMSO, without antimicrobial substance).

All experiments were performed in triplicate and the micro dilution trays were incubated at 36ºC for 18 h. Bacterial growth was detected former by optical density and after by addition of 20 µL of an INT alcoholic solution (0.5 mg/mL). The trays were again incubated at 36ºC for 30 min, and in those wells, where bacterial growth occurred. MIC values were defined as the lowest concentration of each compound, which completely inhibited microbial growth [24].

3. RESULTS AND DISCUSSION

3.1. 4-(1*H*-Benzo[d]Imidazol-2-yl)-*N*-Benzylidenebenzenamine (SAM-2)



(SAM-2)

3.2. IR Spectra

IR spectra of the compound showed one medium intensity band of secondary N-H stretching vibrations at 3473 cm^{-1}. A medium band at 3385 cm^{-1} indicated aromatic C-H stretching vibrations. A weak band observed at 1641 cm^{-1} due to the presence of C=N. Two bands at 1610 cm^{-1} & 1464 cm^{-1} indicated the presence of C=C ring structure. A medium band observed at 1200 cm^{-1} due to the presence of C-N. The N-H bending vibrations were observed at 1512 cm^{-1}. The para disubstituted ring was confirmed by a band at 940 cm^{-1} (fig 1).

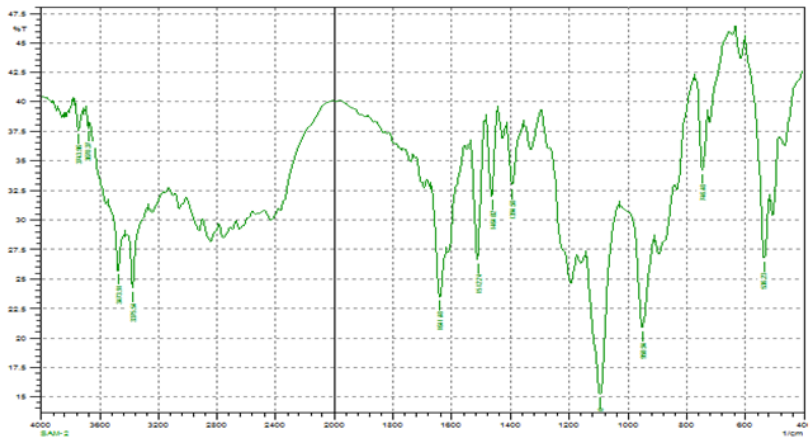


Fig1. *IR Spectra of 4-(1H-benzo[d]imidazol-2-yl)-N-benzylidenebenzenamine*

3.3. NMR Spectra

NMR spectra of the compound showed a triplet at 7.29 indicate three aromatic methine protons designated as b, c & d. One triplet were observed at 7.62 due to the presence of two aromatic methine protons designated as a & e. A doublet at 6.7 indicates two aromatic methine protons designated as f & i. One triplet was observed at 7.2 due to the presence of two aromatic methine protons designated as g & h. Similarly another doublet was observed at 8.27 indicate two aromatic methine protons designated as j & m. One triplet was observed at 7.96 due to the presence of two aromatic methine protons designated as k & l. A singlet was observed at 8.39 due to the aliphatic methine proton designated as N=CH. A singlet was observed at 9.69 due to the N-H proton of the benzimidazole ring (fig 2).

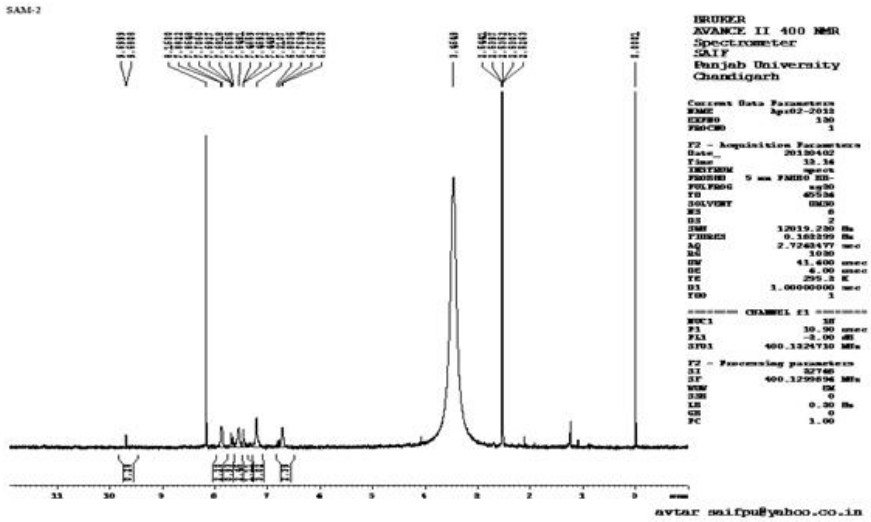


Fig2. *NMR Spectra of 4-(1H-benzo[d]imidazol-2-yl)-N-benzylidenebenzenamine*

3.4. Anti-Microbial Screening of the Synthesized Compounds

The synthesized compounds were screened for their anti-bacterial activity using micro dilution method against *S. aureus* & *E. coli*. The reference standard and control used vancomycin and DMSO respectively. The compounds SAM-2, SAM-4, SAM-9 & SAM-10 were found to be most promising antimicrobial agents among the synthesized compound (table 2).

Table2. *MIC values (µg/ml) of benzimidazole derivatives against gram positive & gram negative bacteria*

Compound	*Staphylococcus aureus* Gram (+ve)	*Escherichia coli* Gram (-ve)
SAM- 1	29	25
SAM- 2	10	13
SAM- 3	52	46
SAM- 4	21	17
SAM- 5	31	29
SAM- 6	44	55

SAM- 7	58	38
SAM- 8	51	29
SAM- 9	21	18
SAM- 10	10	11
SAM- 11	38	42
SAM- 12	32	30
SAM- 13	60	45
SAM- 14	50	39
Vancomycin	17	16
DMSO	00	00

3.5. Structure Activity Relationship Studies

Structure activity relationship (SAR) studies revealed that different substitutions on the benzimidazole schiff bases and its azetidinone and thiazolidinone derivatives exerted varied biological activities. The electronic nature of the substituent groups at 4' positions in the benzimidazole nucleus, 7" azetidinone and 2"' thiazolidinone led to significant variation in antimicrobial activity. Among the series of compounds substituted by electron-withdrawing (-NO2 and -Cl) and electron-donating (-OCH3, -OH and -CH3) groups are enhanced biological activity.

4. CONCLUSION

A series of benzimidazole derivatives having imine linkage were synthesized by using o-phenylenediamine as starting material. In first step, o-phenylenediamine is treated with 4-amino benzoic acid to give 2-aminobenzimidazole which was then treated with different substituted aldehydes or ketones to give benzimidazole containing Schiff base. The synthesized compounds were screened for anti-microbial activity against Gram (+) ve (*Staphylococcus aureus*) and Gram (–) ve (*Escherichia coli*) bacteria. The compound SAM-2, SAM-4, SAM-9 & SAM-10 had MIC values in the range of 10-21 µg/ml while the standard drug vancoycin had MIC value of 17 µg/ml.

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