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Synthesis, Characterization and Biological Study of Schiff Bases Derived from 4-Amino-3-Hydroxy Benzoic Acid

Atish R. Mehetre[1*], Sandeep R. Deshmukh[2] and Vijay N. Bhosale[3]

[1,3] *Department of Chemistry, Shivaji Arts, Commerce and Science college Kannad, 431103(MS), INDIA*
[2] *Department of Chemistry,R.M.G. Arts, Commerce and Science College Saoli, 441225(MS), INDIA*

[*] *Correspondence: E-mail: atishm912@gmail.com*

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ABSTRACT: Schiff bases were prepared by using 4-amino-3-hydroxy benzoic acid, namely (E)-3-hydroxy-4-((4-nitrobenzylidene)amino)benzoic acid, (E)-3-hydroxy-4-((2-hydroxy-3-methoxybenzylidene)amino)benzoic acid, (E)-4-((5-bromo-2-hydroxybenzylidene)amino)-3-hydroxybenzoic acid and (E)-3-hydroxy-4-((4-hydroxy-3-methoxy benzylidene)amino)benzoic acid. 4-nitrobenzaldehyde, Ortho-vanillin, 5-Bromosalicylaldehyde and vanillin are used for the preparation of Schiff bases. 4-amino-3-hydroxy benzoic acid(0.002m) was dissolved in ethanol and mixed with aldehyde (0.002m) dissolved in ethanol ,mixed solution stirred for 15 minutes at 60o c and gives solid products. Synthesized Schiff bases were structurally characterized based on IR, NMR, HRMS and elemental analysis. The synthesized Schiff bases were screened for their biological activity against bacterial species Gram –ve bacteria (Escherichia coli), Gram +ve bacteria (Staphylococcus aureus) and fungi (aspergillusniger).

Keywords: 4-amino-3-hydroxy benzoic acid; Synthesis; Schiff base and Antimicrobial activity.

INTRODUCTION: Schiff bases have been prepared by using 4-Amino-3-hydroxybenzoic acid which is used in the preparation of various pharmaceutical compounds such as sphingosine kinase inhibitors (1). Schiff bases of aromatic aldehydes having conjugated system which is effective and more stable in the comparison of aliphatic aldehydes are relatively unstable and are readily polymerizable, Schiff bases are the basic units in certain dyes and some of them are used as liquid crystals . In the synthesis for making carbon – nitrogen bonds Schiff bases reactions are useful. It is found that Schiff bases appear to be important intermediates in many enzymatic reactions involving interaction of an enzyme with an amino or carbonyl group of the substrate (5). In the coordination of metal complexes, N and O atoms play a key role at the active sites of numerous metallo-biomolecules(2). Schiff bases of amino acids have shown excellent antiaflatoxigenic activity (3). It has been shown that the tri or more dentate Schiff base ligands with a flexible donor atom in comparison with the bidentate ligands are more suitable ligand systems for stabilizing Schiff base metal complexes because of the coordination of the supporting donor atom and such complexes have good biological activity(4). By keeping this view Schiff bases were prepared by using 4-amino-3-hydroxy benzoic acid with different aldehyde vanillin, 5-Bromosalicylaldehyde, Ortho-vanillin and 4-nitrobenzaldehyde.

MATERIALS AND METHODS: All the chemicals purchased from S. D. Fine chemicals were used. 1H NMR spectra were recorded on a Bruker-AV 400 MHz spectrometer using chloroform-d as solvents and TMS as an internal standard. The infrared spectra (4000–400 cm^{-1}) were recorded by using KBr pellet on Shimadzu IR Affinity-1.Microorganism used in antimicrobial activity, its Strain Name and Strain reference are as [1. Gram positive bacteria Staphylococcus aureus (NCIM 2079)], [2.Gram negative bacteria Escherichia coli (NCIM 2109)] and [3.Fungi (yeast) Aspergillusniger (NCIM 545)].[NCIM: National Collection of Industrial Microorganisms, National Chemical Laboratory (NCL), Pune 411008 (India)]

Synthesis: Schiff base *(E)-3-hydroxy-4-((4-nitro benzylidene)amino)benzoic acid (S1)* was prepared by using 4-amino-3-hydroxy benzoic acid (0.002m) was dissolved in ethanol and mixed with 4-nitrobenzaldehyde (0.002m) dissolved in ethanol, mixed solution stirred for 15 minutes at 60° C. A yellow solid product was obtained, washed with metha-

nol and filtered, dried and melting point was taken (6-9).

Yield-80%, M.P.295-297, H^1NMR: 8.88δ (1H,S,HC=N), 12.87 (1H,S,OH-COOH), 9.70(1H,S,Ar-OH),8.25-8.40 (4H,M,Ar-H), 7.26-7.49 (3H,Ar-H), IR(cm-1): 1626(-HC=N-), 1692 (-C=O,COOH), 3433 (Ar-OH) Elemental Analysis calcd: C, 58.74; H, 3.52; N, 9.79; O, 27.95,Found: C, 58.44; H, 3.82; N, 9.75; O, 27.99,HRMS for $C_{14}H_{10}N_2O_5$ [M+H]calcd:287.0668, found: 287.0664.

E)-3-hydroxy-4-((2-hydroxy-3-methoxybenzylidene) amino)benzoic acid (S2) was prepared by using 4-amino-3-hydroxy benzoic acid(0.002m) was dissolved in ethanol and mixed with Ortho-vanillin(0.002m) dissolved in ethanol ,mixed solution stirred for 15 minutes at 60° C. A red color solid product was obtained, washed with methanol and filtered, dried and melting point was taken (6-9).

Yield-85%, M.P.280-283, H^1NMR: 9.00δ (1H,S,HC=N), 13.73 (1H,S,OH-COOH), 12.85 (1H,S,Ar-OH), 10.15 (1H,S,Ar-OH), 7.41-7.55 (3H,Ar-H), 6.88-7.13 (3H,Ar-H), 3.81(3H,S,OCH_3). IR (cm-1): 1627(-HC=N-), 1697(-C=O, COOH), 2833 and 2953(Ar-OH). Elemental Analysis calcd: C, 62.72; H, 4.56; N, 4.88; O, 27.85, Found: C, 62.81; H, 4.47; N, 4.84; O, 27.88, HRMS for $C_{15}H_{13}NO_5$ [M+H] calcd: 288.0872, Found:288.0865

Schiff base *(E)-4-((5-bromo-2-hydroxybenzylidene) amino)-3-hydroxybenzoic acid (S3)* was prepared by using 4-amino-3-hydroxy benzoic acid (0.002m) was dissolved in ethanol and mixed with 5-Bromosalicylaldehyde (0.002m) dissolved in ethanol ,mixed solution stirred for 15 minutes at 60° c. A orange color solid product was obtained, washed with methanol and filtered, dried and melting point was taken (6-9).

Yield-80%,M.P.270-273,H^1NMR:9.002δ(1H,S,HC=N),13.46(1H,S,OH-COOH),12.90(1H,S,Ar-OH), 10.12(1H,S,Ar-OH),7.49-7.90(3H,Ar-H),6.94-7.48(3H,Ar-H).IR(cm-1):1627(-HC=N-),1674(-C=O,COOH), 2850 and 2960(Ar-OH) Elemental Analysis calcd: C, 50.02; H, 3.00; Br, 23.77; N, 4.17; O, 19.04,Found:C, 50.08; H, 2.24.00; Br, 23.66; N, 4.28; O, 19.04. HRMS for $C_{14}H_{10}BrNO_4$ [M+H] calcd: 335.9871, Found: 335.9868.

Schiff base *(E)-3-hydroxy-4-((4-hydroxy-3-methoxy benzylidene)amino)benzoic acid (S4)* was prepared by using 4-amino-3-hydroxy benzoic acid (0.002m) was dissolved in ethanol and mixed with vanillin (0.002m) dissolved in ethanol ,mixed solution stirred for 15 minutes at 60° c. A yellow color solid product was obtained, washed with methanol and filtered, dried and melting point was taken (6-9).

Yield-80%, M.P. 248-250, H^1NMR: 8.52δ (1H,S,HC=N), 12.66 (1H,S,OH-COOH), 9.80 (1H,S,Ar-OH), 9.29(1H,S,Ar-OH), 7.38-7.68 (3H,Ar-H), 6.89-7.36(3H,Ar-H). IR (cm-1): 1626(-HC=N-), 1673(-C=O, COOH), 2846 and 3417. (Ar-OH) Elemental Analysis Calcd: C, 62.72; H, 4.56; N, 4.88; O, 27.85, Found: C, 62.67; H, 4.61; N, 4.78; O, 27.95. HRMS for $C_{15}H_{13}NO_5$ [M+H] calcd: 288.0872, Found: 288.0857

Antimicrobial Activity: Schiff bases were screened for their biological activity against bacterial species, Gram positive bacteria Staphylococcus aureus (NCIM 2079),Gram negative bacteria Escherichia coli (NCIM 2109),Fungi (yeast) Aspergillusniger (NCIM 545).[NCIM: National Collection of Industrial Microorganisms, National Chemical Laboratory (NCL), Pune 411008 (India)].Agar diffusion assay (Disc diffusion method, Disc size 6 mm).Stock solution [1000 microgram per ml] of each compound was prepared in DMSO. Assay carried out by taking concentration 100 microrgram per disk. Hi-media antibiotics disk: Chloramphenicol (10 microgram/disk, Amphotericin-B (100 units/disk) moistened with water are used as standard. Microbiological media used for bacteria (Staphylococcus aureus, Escherichia coli) is Nutrient agar (Hi-media)Composition (gL^{-1}) Sodium chloride, 5.0; Beef extract 10.0; Peptone 10.0 (pH 7.2).Microbiological media used for fungi (Aspergillusniger) Potato Dextrose agar is used (all ingredients of Hi media).Composition (gL^{-1}): Potatoes infusion, 200; Dextrose, 20; Agar, 15; Final pH (at 25°C) 5.6±0.2. (16-17)

Table 1: Antimicrobial activity of Schiff bases testing (Disc Diffusion Assay).

Sr. No.	Sample code	*Staphylococcus aureus*	*Escherichia coli*	*Aspergillusniger*
1	S1	14.40	14.56	-
2	S2	16.23	15.48	-
3	S3	19.87	20.11	-
4	S4	-	-	-
Control	Chloramphenicol	25.11	25.44	NA
Control	Amphotericin B	NA	NA	12.58

Diameter in mm calculated by Vernier Caliper, '-' means no zone of inhibition, NA- Not applicable

RESULTS AND DISCUSSION:

NMR: Product S1 formation was confirmed by NMR, a singlet pick observed at 8.88 δ assigned to Schiff base proton (14) and aromatic OH proton observed at 9.70 δ. Carboxylic acidic group proton observed at 12.87 δ(15). Four protons of aromatic ring attached to nitro group observed at 8.25-8.40 δ and second aromatic ring protons observed at 7.26-7.49 δ(13).Product S2 formation was confirmed by NMR, a singlet pick observed at9.0 δ assigned to Schiff base proton(14) and aromatic OH protons observed at 10.15 and 12.85 δ. Carboxylic acidic group proton observed at 13.73 δ(15). Three protons of aromatic ring observed at 7.41-7.55 δ and second aromatic ring protons observed at 6.88-7.13 δ(13). A singlet observed at 3.81 assigned to methyl group of OCH_3(15).Product S3 formation was confirmed by NMR, a singlet pick observed at9.0 δ assigned to Schiff base proton (14) and aromatic OH protons observed at 10.12 and 12.90 δ. Carboxylic acidic group proton observed at 13.46 δ(15). Three protons of aromatic ring observed at 7.49-7.90 δ and second aromatic ring protons observed at 6.94-7.48 δ (13).Product S4 formation was confirmed by NMR, a singlet pick observed at 8.52 δ assigned to Schiff base proton(14) and aromatic OH protons observed at 9.80 and 9.29 δ. Carboxylic acidic group proton observed at 12.66 δ(15). Three protons of aromatic ring observed at 7.38-7.68 δ and second aromatic ring protons observed at 6.89-7.36 δ(13). A singlet observed at 3.87 assigned to methyl group of OCH_3(15).

IR: The IR spectrum of the ligand [S1] shows a strong absorption band around 1626(11) cm^{-1}, which may be attributed to the azomethine group[HC=N],carbonyl[C=O] of COOH group observed at 1692(12) and a broad band at 3433cm-1 which is attributed to the stretching frequencies of aromatic hydroxyl substituent hydroxyl substituent(10). The IR spectrum of the ligand [S2] shows a strong absorption band around 1627 (11) cm-1, which may be attributed to the azomethine group[HC=N], carbonyl[C=O] of COOH group observed at 1697 (12)and a broad band at 2833 and 2953 cm-1 which is attributed to the stretching frequencies of aromatic hydroxyl substituent(10).The IR spectrum of the ligand [S3] shows a strong absorption band around 1627 cm^{-1}, which may be attributed to the azomethine group[HC=N](11),carbonyl[C=O] of COOH group observed at 1674(12) and a broad band at 2850 and 2960 cm-1 which is attributed to the stretching frequencies of aromatic hydroxyl substituent(10). The IR spectrum of the ligand [S4] shows a strong absorption band around 1626 cm^{-1}, which may be attributed to the azomethine group[HC=N],carbonyl[C=O] of COOH group observed at 1673 and a broad band at 2846 and 3417 cm-1 which is attributed to the stretching frequencies of aromatic hydroxyl substituent.

Elemental analysis: In the elemental analysis found data are in good agreement with calculated data.

HRMS: In high resolution mass spectroscopy observed values are in good agreement with theoretical values.

Antimicrobial Activity: Schiff bases are active against Gram –ve bacteria (Escherichia coli), Gram +ve bacteria (Staphylococcus aureus) and inactive against fungi(aspergillusniger).In the comparison bacteria are active but have less zone of inhibition as shown by standard. Gram +ve and Gram –ve bacteria shows nearly similar zone of inhibition.

CONCLUSION: Schiff bases were prepared by using 4-amino-3-hydroxy benzoic acid shows formation imine bridge between aldehydes and amine. Synthesized compounds were confirmed by IR, NMR, HRMS and elemental analysis and all the characterization data were in good agreement with illustrated structures. Prepared Schiff bases were evaluated for antibacterial activity and Schiff bases are active against Gram –ve bacteria (Escherichia coli), Gram +ve bacteria (Staphylococcus aureus) and inactive against fungi (aspergillusniger).

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