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MICROWAVE ASSISTED RAPID SYNTHESIS OF SOME SCHIFF BASES AND THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT

A microwave promoted simple, clean and efficient synthesis of Schiff bases of 2-hydroxybenzaldehyde and substituted 2–amino pyridine as precursors was done and its derivatives were prepared. Microwave reactions under solvent-free conditions or minimal solvent usage are attractive in offering reduced pollution, low cost and offer high yields together with simplicity in processing and handling. The isolated compounds obtained, were purified and characterized by IR and NMR spectral data and were subsequently subjected to the in-vitro antimicrobial activity against few pathogenic strains of microbes.

***Keywords*:** Schiff base, 2-hydroxybenzaldehyde, substituted 2–amino pyridine, microwave irradiation, antimicrobial.

1. Introduction

Two third part of the entire organic compounds deals with heterochemistry. They have high synthesis flexibility, varied coordinating ability and medicinal utility. Condensation of a carbonyl compound with varied primary amine or amino compound with elimination of water molecule forms a weak basic compound known as imine or an anil or azomethine, which is commonly known as Schiff base[1]. These compounds have their own identity and importance in pharmaceutical, co-ordination and drug chemistry along with medicinal, biotechnological sciences[2-3] and hence it was thought interesting to investigate the reactions. Decent number of Schiff bases has been synthesized and reported for their bactericidal[4-6], fungicidal[7-8], antipyretic[9-10], antitubercular[11], antitumor[12] and anticancer activity[13-14].

Synthesis of Schiff base is often carried out by acid-catalysis or generally by refluxing the mixture of aldehyde (or ketone) and amine in organic medium[15-17]. However, with the assistance of microwave irradiation, it was found that the condensation reaction could proceed fast and efficiently without solvent. The products could be purified simply by recrystallization in an appropriate solvent or a mixture of solvents with high yield of products. Thus microwave assisted reactions offers environmentally benign synthetic methods in terms of pollution, cost, yield and handling[18-23].

Synthetic route of Schiff base using 2-hydroxy benzaldehye and 2-amino pyridine by refluxing it conventionally in an acid catalysed organic medium has been reported by Asiri et-al[15]. However the present paper reports the synthesis of Schiff bases by the reaction of 2-hydroxybenzaldehyde and substituted 2–amino pyridine as precursors under microwave irradiations and its derivatives were then further studied for their antimicrobial potential. Structure of the synthesized compounds was confirmed by IR and ^{1}H-NMR spectroscopy. An enormous amount of work had been carried out and reported on Schiff bases showing Schiff base as promising microbial active agent[24-28].

2. Materials and Methods

2.1 Materials

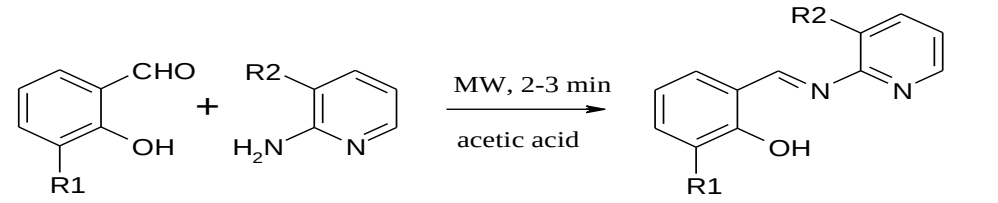
The chemicals used for synthesis were of L.R. grade and purity of synthesized compounds was checked by TLC. All the working solutions were freshly prepared from the deionized water. To avoid any ionic contamination, deionized water was used for all the purpose in this study. The melting points of all synthesized compounds were recorded in precision digital melting point apparatus Model MP-D and are uncorrected. All experiments under microwave irradiation were carried out in unmodified domestic microwave oven model MC-7148MS manufactured by LG Electronics India Pvt. Ltd, Noida, India having maximum power output of 800W and 2450 MHz frequency. All the weighing was made on Citizen CY 104 one pan digital balance, (± 0.0001gm). The IR spectrum of the synthesized compounds was recorded on Bruker Avance II 400 NMR spectrometer at Punjab University, Chandigarh.^{1}H-NMR spectra were recorded on a Bruker Avance II 400 NMR spectrometer at Punjab University, Chandigarh in DMSO and $CDCl_3$ as a solvent and TMS as internal standard. Peak values are shown in δ ppm.

The various microbial strains used in the study were *E. coli, S. typhi, S. paratyphi* and *S. aureus*. They were procured from M/s. Hi-Media Pvt. Ltd., Mumbai, India.

2.2 Synthesis of Schiff bases under microwave irradiations

Equimolecular mixture of 2- hydroxybenzaldehyde and 2- aminopyridine in presence of catalytic amount of glacial acetic acid was taken in borosilicate glass beaker. The reaction mixture was then irradiated inside a microwave oven for 2 min at a power of P60. The reaction mixture was cooled and poured into ice cold water. Product formed, was separated by filtration and crystallized with alcohol to afford the title compound. The formation of ligand was confirmed by M.P and spectral studies.

Following is the scheme of the synthesis-



2 - hydroxy benzaldehyde | 2 - amino pyridine | N - (2 - hydroxybenzylidene) pyridine - 2 - amine

Where,

R_1 = -H, $-NO_2$

R_2 = -H, -OH, $-NO_2$, $-CH_3$

Thus with the substitution of R_1 and R_2, following 8 Schiff bases were synthesised and screened for their antimicrobial activity against the above mentioned microorganisms.

1. N-(2'-hydroxybenzylidene) pyridine-2-amine (A_1)
2. N-(2'-hydroxy-3'-nitrobenzylidene) pyridine-2-amine (B_1)
3. N-(2'-hydroxybenzylidene)-3-hydroxy pyridine-2-amine (A_2)
4. N-(2'-hydroxy-3'-nitrobenzylidene)-3-hydroxy pyridine-2-amine (B_2)
5. N-(2'-hydroxybenzylidene)-3-nitropyridine-2-amine (A_3)
6. N-(2'-hydroxy-3'-nitrobenzylidene)-3-nitropyridine-2-amine (B_3)
7. N-(2'-hydroxybenzylidene)-3-methylpyridine-2-amine (A_4)
8. N-(2'-hydroxy-3'-nitrobenzylidene)-3-methylpyridine-2-amine (B_4)

3. Result and Discussion

3.1 Synthesized Schiff bases

The details of the substituted ligands synthesized under microwave irradiations are summarized in the following table.

Ligand	R_1	R_2	Molecular formula	Molecular weight	Colour	Melting point (^{0}C)	Yield (%)	R_f Value
A_1	-H	-H	$C_{12}H_{10}ON_2$	198	Yellow	63	83	0.68
A_2	-H	-OH	$C_{12}H_{10}O_2N_2$	214	Crimson Brown	272	98	0.7
A_3	-H	$-NO_2$	$C_{12}H_9O_3N_3$	243	Pale Yellow	168	66	0.82
A_4	-H	$-CH_3$	$C_{13}H_{12}ON_2$	212	Yellow	78	80	0.72
B_1	$-NO_2$	-H	$C_{12}H_9O_3N_3$	243	Amber	52	88	0.85
B_2	$-NO_2$	-OH	$C_{12}H_9O_4N_3$	259	Crimson Brown	258	92	0.88
B_3	$-NO_2$	$-NO_2$	$C_{12}H_8O_5N_4$	288	Chrome Yellow	166	55	0.84
B_4	$-NO_2$	$-CH_3$	$C_{13}H_{11}O_3N_3$	257	Yellow Ochre	66	61	0.86

3.2 Spectral Data Analysis

1) N-(2'-hydroxybenzylidene) pyridine-2-amine (A_1)

IR in cm^{-1} (KBr pellets): 3001.43 (Ar-H str), 2976.44 (Ar-CH str), 3051.42 (Ar-OH str), 1453.17 (-C=N str (Schiff base)).

^{1}H-NMR (δ ppm): 13.45 (s, 1H, -OH str), 9.39 (s, 1H, -CH str), 6.89 to 8.47 (m, 8H, Ar-H str).

2) N-(2'-hydroxybenzylidene)-3-hydroxy pyridine-2-amine (A_2)

IR in cm^{-1} (KBr pellets): 3165.10 (Ar-H str), 2972.34 (Ar-CH str), 3334.9 (Ar-OH str), 1455.9 (-C=N str (Schiff base)).

^{1}H-NMR (δ ppm): 13.102 (s, 1H, -OH str), 11.438 (s, 1H, -OH str), 8.70 (s, 1H, -CH str), 6.29 to 7.44 (m, 8H, Ar-H str).

3) N-(2'-hydroxybenzylidene)-3-nitropyridine-2-amine (A_3)

IR in cm^{-1} (KBr pellets): 3091.99 (Ar-H str), 2919.05 (Ar-CH str), 3462.90 (Ar-OH str), 1431.88 (-C=N str (Schiff base)), 1331.22 (-C-NO_2 str).

^{1}H-NMR (δ ppm): 10.17 (s, 1H, -OH str), 8.37 (s, 1H, -CH str), 6.71 to 7.85 (m, 7H, Ar-H str).

4) N-(2'-hydroxybenzylidene)-3-methylpyridine-2-amine (A_4)

IR in cm^{-1} (KBr pellets): 3046.39 (Ar-H str), 2973.36 (Ar-CH str), 3432.47 (Ar-OH str), 1452.9 (-C=N str (Schiff base)).

^{1}H-NMR (δ ppm): 13.75 (s, 1H, -OH str), 9.41 (s, 1H, -CH str), 6.91 to 8.32 (m, 7H, Ar-H str), 2.4337 (s, 3H, -CH_3 str).

5) N-(2'-hydroxy-3'-nitrobenzylidene) pyridine-2-amine (B_1)

IR in cm^{-1} (KBr pellets): 3002.27 (Ar-H str), 2860.27 (Ar-CH str), 3052.26 (Ar-OH str), 1454.13 (-C=N str (Schiff base)), 1349.2 (-C-NO_2 str).

^{1}H-NMR (δ ppm): 13.45 (s, 1H, -OH str), 9.45 (s, 1H, -CH str), 6.9 to 8.48 (m, 7H, Ar-H str).

6) N-(2'-hydroxy-3'-nitrobenzylidene)-3-hydroxy pyridine-2-amine (B_2)

IR in cm^{-1} (KBr pellets): 3072.28 (Ar-H str), 2855.43 (Ar-CH str), 3328.27 (Ar-OH str), 1454.29 (-C=N str (Schiff base)), 1338.34 (-C-NO_2 str).

^{1}H-NMR (δ ppm): 13.405 (s, 1H, -OH str), 11.207 (s, 1H, -OH str), 8.164 (s, 1H, -CH str), 6.81 to 7.49 (m, 6H, Ar-H str).

7) N-(2'-hydroxy-3'-nitrobenzylidene)-3-nitropyridine-2-amine (B_3)

IR in cm^{-1} (KBr pellets): 3094.10 (Ar-H str), 2627.26 (Ar-CH str), 3277.19 (Ar-OH str), 1458.21 (-C=N str (Schiff base)), 1334.3 (-C-NO_2 str).

^{1}H-NMR (δ ppm): 10.45 (s, 1H, -OH str), 8.37 (s, 1H, -CH str), 6.72 to 8.23 (m, 6H, Ar-H str).

8) N-(2'-hydroxy-3'-nitrobenzylidene)-3-methylpyridine-2-amine (B_4)

IR in cm^{-1} (KBr pellets): 3046.47 (Ar-H str), 2855.47 (Ar-CH str), 3338.51 (Ar-OH str), 1452.22 (-C=N str (Schiff base)), 1350.38 (-C-NO_2 str).

^{1}H-NMR (δ ppm): 13.74 (s, 1H, -OH str), 9.38 (s, 1H, -CH str), 6.9 to 8.31 (m, 6H, Ar-H str), 2.41 (s, 3H, -CH_3 str).

3.3 Antimicrobial activity

Schiff base compounds possess a wide range of biological activities which are associated with different substituents and the unsaturation of -C=N moiety in between the aryl rings. Hence, it is intended to examine their antimicrobial activities against their respective microbes-bacterial strains. For testing the antimicrobial activity, the compounds were assayed against *E. coli, S. typhi, S. paratyphi and S. aureus*. All these compounds were dissolved in DMSO and the ligand solutions were prepared with 20 mg/ml concentration. Gentamycin drug solution was used as the standard. The zone of inhibition was determined by disk diffusion method or zone inhibition method (ZIM) and minimum inhibitory concentration values (MIC values) were determined by serial dilution method.

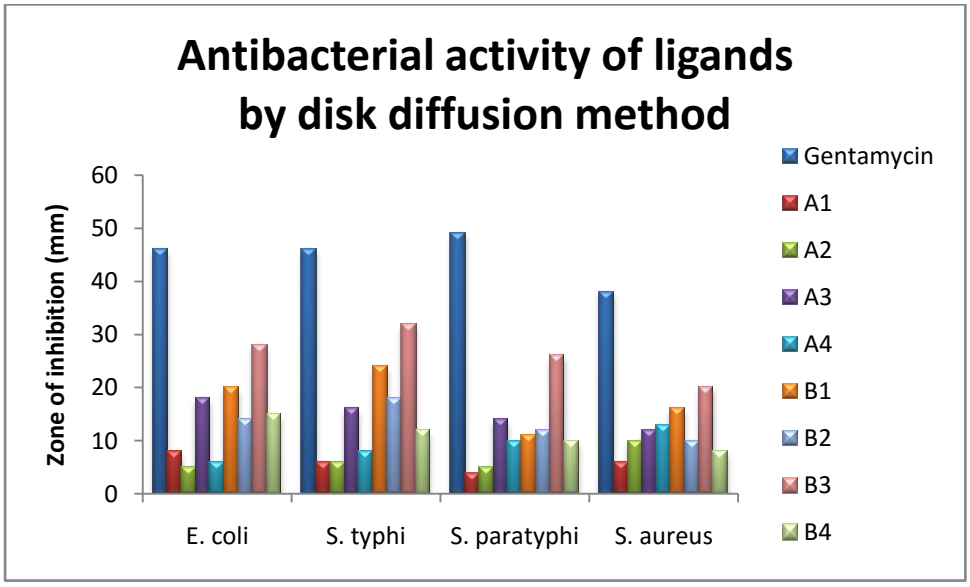
3.3.1 Disk diffusion method

A 24 h fresh diluted culture of organisms under study was spread evenly over the entire surface of each petriplate containing solidified nutrient agar medium, using sterile cotton swab. Then the discs with 6mm diameter made up of Whatmann filter paper No.42, impregnated with the solution of the compounds (20mg/ml) was placed on the surface of inoculated plate. They were allowed to diffuse in the media and then the plates were incubated at 37°C for 24 h. The diameter of inhibition zone was observed and measured with the help of ruler[29-30].

Table 1

Compounds	Zone of inhibition (mm)			
	E. *coli*	**S. *typhi***	**S. *paratyphi***	**S. *aureus***
Gentamycin	46	46	49	38
A_1	8	6	4	6
A_2	5	6	5	10
A_3	18	16	14	12
A_4	6	8	10	13
B_1	20	24	11	16
B_2	14	18	12	10
B_3	28	32	26	20
B_4	15	12	10	8

Fig. 1



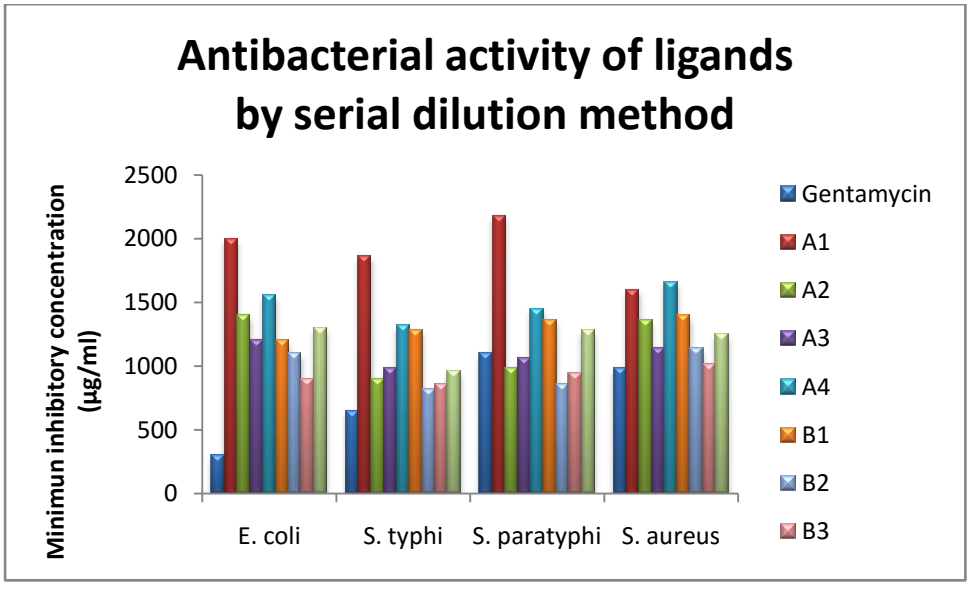
3.3.2 Serial dilution method

The 5 ml of the nutrient broth medium was distributed in each sterile test tube. The 0.01 M stock solution of the test compounds, prepared in DMSO solvent was aseptically added to the various nutrient broth test tubes. Fresh culture of the test bacterium were inoculated in each test tube (0.2 ml culture) and were incubated at 37°C for 24 h and then were observed for minimum inhibitory concentration (MIC) against test bacterium[31].

Table 2

Compounds	Minimum inhibitory concentration (µg/ml)			
	E. *coli*	**S. *typhi***	**S. *paratyphi***	**S. *aureus***
Gentamycin	300	650	1100	980
A1	2000	1860	2180	1600
A2	1400	900	980	1360
A3	1200	980	1060	1140
A4	1550	1320	1450	1660
B1	1200	1280	1360	1400
B2	1100	820	860	1140
B3	900	860	940	1020
B4	1300	960	1280	1250

Fig. 2



From the table 1 and 2 above, it is evident that all the synthesized Schiff base compounds are biologically active against *E. coli, S. typhi, S. paratyphi and S. aureus*. Especially ligand B_3 had shown good activity against all the four pathogens.

Different substituents have different effects on the range of biological activities. $–NO_2$ group being electron withdrawing is shown to enhance antimicrobial activity. Ligand B_3

containing two -NO_2 groups shows highest value among all. Thus B_3, B_1, A_3 and B_2 have significant to moderate activity than A_4, A_2 and A_1 due to presence of electron withdrawing nitro group. However, A_4 and B_4 has lower values of zone inhibition due to the presence of electron donating –CH_3 group. Lower values for A_2 can be justified due to the presence of electron donating –OH group (Fig. 1 and 2).

3.4 Conclusion

The present report gives the successful synthesis of the eight Schiff base ligands assisted by microwave irradiation which offers short reaction time, simplicity in handling, efficiency over conventional process and is a clean method. The synthesized compounds were purified and characterized by IR and NMR spectral data.

The antibacterial activity of all the synthesized ligands was studied and the data shows that both the methods – disk diffusion and serial dilution method gives comparable results of the ligands against pathogens under study.

Thus, the present study helps us to accomplish that ligand B_3 and to some extent ligand B_1, B_2 and B_4 show good activity against *E. coli, S. typhi, S. paratyphi and S. aureus* as compared to other synthesized ligands.

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REFERENCES

1. Schiff H., (1864), *Ann. Chem.*, 131, 118.
2. Shirodkar S.G., Mane P.S. and Chondhekar T.K., (2001), *Ind. J. Chem*., 40, 1114 - 17.
3. Raghuwanshi P.B., (2012), *Asian J. Chem. Env. Res*., 5(3-4), 11.
4. Kareem A., Laxmi, Arshad M., Nami S.A., Nishat N., (2016), *J. Photochem. Photobiol. B.*, 160, 163-71.
5. Al-Shemary R.K., Ali M.A. Al-khazraji, Ali N.N., (2016), *The Pharma. Inno. J.*, 5(1), 81-86.

6. Rakesh K.P., Shiva Prasad K., Shridhara Prasad K., (2012), *Int. J. Res. Chem. Environ*. 2, 221.
7. Singh V.P., Sharma J.R., Manrao M.R., (2008), *J. Ind. Council Chem.*, 25(1), 7-9.
8. Sridhar S.K., Saravan M., Ramesh A., (2001), *Eur. J. Med. Chem*., 36, 615.
9. Panditrao P.R., Samani S.D., Deodhar L.D., (1981), *Ind. J. Chem*., 20(B), 929.
10. Merchand J.R., Chotia D.S., (1970), *J. Med. Chem*., 40, 194.
11. Tajudeen S.S., Kannappan G., (2016), *Ind. J. of Advances in Chem. Sci*., 4(1), 40-48.
12. Walsh O.M, Meegan M.J, Prendergast R.M, Nakib T.A, (1996), *Eur. J. Med. Chem*., 31, 989.
13. Gwaram N.S., Hassandarvish P., (2014), *J. of App Pharm Sci*., 4 (10), 75-80.
14. Mahmoud W.H., Deghadi R.G., Mohamed G.G., (2016)., *App. Organometallic Chem*., 30 (4), 221–230.
15. Asiri A.M., Badahdah K.O., (2007), *Molecules*, 12, 1796-1804.
16. Rajendra G., Sreeletha G.S., (2001), *Asian J. Chem.*, 13 (3), 1142.
17. Kumar S., Niranjan M.S., Chaluvaraju K.C., Jamakhandi C.M., Kadadevar D., (2010), *J. Pharma. Res.*, 01, 39-42.
18. Savalia R.V., Patel A.P., Trivedi P.T., Gohel H.R., Khetani D.B, (2013), *Res. J. Chem. Sci.*, 3 (10), 97-99.
19. Zhaoqi Yang, Pinhua Sun, (2006), *Molecular Diversity Preservation Int.*, M514.
20. Mhaske G., Nilkanth P., Auti A., Davange S., Shelke S., (2014), *Int. J. of Inno. Res. in Sci, Eng and Tech*, 1(1).
21. Naglah A.M., Awad H.M., Bhat M.A., Al-Omar M.A., El-Galil Amr A.E., (2015), *J. of Chem.*, 2015, Article ID 364841.
22. Naser Al-Shamkhani Z.A., Al-Hazam H.A., Al-Mosawi S.K., (2015), *Chem. and Mat. Res.*, 7 (6), 50-56.
23. Miglani S., Mishra M., Chawla P., (2012), *Sch. Res. Lib. Der Pharma Chemica*, 4 (6), 2265-2269.
24. Gupta A.S., Barhate V.D., (2012), *Res. J. Pharm., Bio. and Chem. Sci*., 3 (3), 1013.
25. Waghamare B.Y., Kumbhar D.D., Pathade A.G., Pardeshi S.K., (2016), *Int. J. of Inno. Res. in Sci., Eng. and Tech*., 5 (1), 194-202.
26. Udayagiri M.D., Yernale N.G., Mruthyunjayaswam B.H.M., (2016), *Int. J. of Pharm. and Pharmaceu. Sci*., 8 (3).

27. Da Silva C.M., Da Silva D.L., Modolo L.V., Alves R.B., De Resende M.A., Martins C.B., Fatima A.D., (2011), *J. Adv. Res.*, 2 (1), 1-8.
28. Al-Shemary R.K., Zaidan B.A., (2016), *Sch. Acad. J. Biosci*., 4 (1), 18-26.
29. Ananthanarayan R. and Panikar C.K.J., (2003), "Textbook of Microbiology", 6th Ed., Orient Longmann, Hyderabad.
30. Cappuceino J.C., Sherman N., (1983), "Microbiology: A Laboratory Manual", Weslex Publishing Co.
31. Aneja K.R., (2005), "Experiments in Microbiology, Plant Pathology and Biotechnology", New Age Publishers, 69.