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Optimization of pyridine based Schiff bases: Design, synthesis and determination of anti-inflammatory, antioxidant and antimicrobial activity

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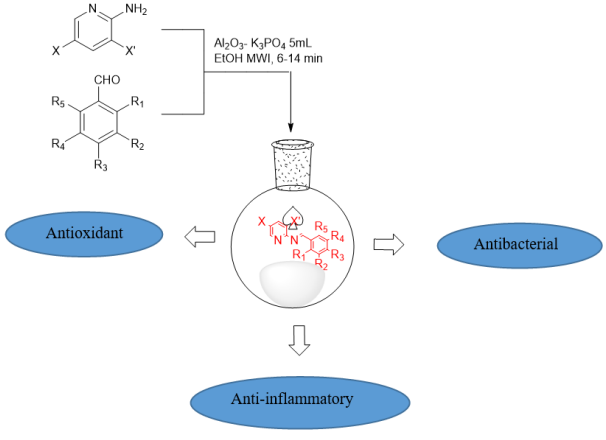
A B S T R A C T

The present paper proposed the tri-potassium phosphate catalyzed synthesis of some novel series of Schiff bases (3a to 3l) derived from 2-aminopyridine using microwave technique. All synthesized Schiff bases were confirmed by different spectroscopic methods. Further all synthesized imines have been evaluated to screen for antimicrobial, antioxidant and anti-inflammatory activity. The compound **3c** displays potent anti-inflammatory activity with 42.307%±1.322 inhibition of inflammation. The antioxidant activity data revealed that all synthesized Schiff bases exhibit potency to scavenge DPPH and nitric oxide radicals. Antimicrobial properties of these compounds showed moderate to excellent growth of inhibitory activity against various tested pathogens. Our observations and examination for pharmacological study towards the presence of active pharmacological functional groups present in the main structural nucleus of synthesized imines. Therefore, present study may lead to the development of a new class of anti-inflammatory and antioxidant drugs with structural modification.



Graphical Abstract

1. Introduction

The organic functionality imines commonly known as Schiff bases. The imines are widely used for the synthesis of five membered heterocycles such as azetidinone and thiazolidinone.[1] These heterocyclic compounds derived from imines have wide range of pharmacological applications, also play important role in quantum mechanical determination to study reactivity and reaction mechanisms for synthesis of biologically active drugs.[2-7] The Schiff base are also known for various

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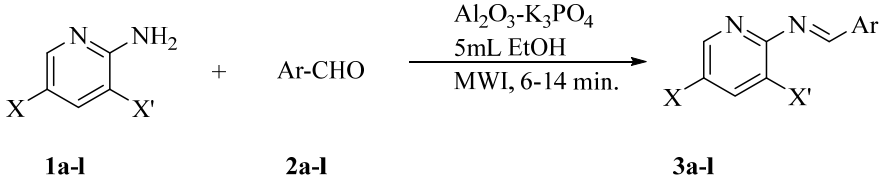
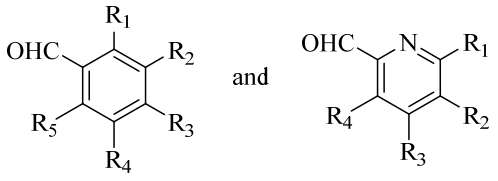
biological properties such as antimicrobial, anti-inflammatory, antioxidant, antitumor, antitubercular and anticonvulsant activity.[8-10] The microwave assisted synthesis has gained popularity in organic synthesis.[11] Using this technique, organic compounds can be prepared very fast with high purity of samples.[12] Therefore, this technique presently known as e-technique as easy, ecofriendly, environmentally being and economic.[13] Following these observations and with the aim of our research has concerned on achieving reasonable yields of the synthesized Schiff bases from 2-aminopyridine. In the present work we plan to synthesize novel series of Schiff bases (3a to 3l) derived from 2-amino pyridine with different substituted benzaldehydes by using alumina-anhydrous tripottasium phosphate under microwave irradiation. These synthesized new series Schiff bases are valuable in development of new, potent, selective and less toxic antimicrobial, anti-inflammatory and antioxidant agents.



1a-l **2a-l** **3a-l**

Where, Ar is



Scheme 1. Alumina-K_3PO_4 Catalyzed synthesis of 2-amino pyridine based Schiff Bases (3a-l).

2. Results and Discussion

2.1. Chemistry and Synthesis

Schiff bases are versatile intermediate for the syntheses of different heterocyclic compounds. These heterocyclic compounds have a wide range of biological applications and medicinal uses. The use of microwave synthesis has advantages to reduce environmental pollution and minimize or eliminate the generation of chemicals that are hazardous to the environment. In view of these observations, we plan to synthesize a new series of Schiff bases derived from 2-amino pyridines and different substituted benzaldehyde using alumina-anhydrous tripottasium phosphate under microwave irradiation.

The various catalyst and reaction conditions such as using water,[14] TiO_2 $(SO)_4$,[15] Au@TiO_2,[16] CaO-MWI,[17] MCM-41-SO_3H,[18] EtOH-Ac_2O,[19] $NaSO_4$[20] are reported for the syntheses of imines. The use of catalyst for the synthesis of imine suffers from several demerits such as harsh reaction conditions, tedious work up in the separation of product and catalyst. Recently the use of K_3PO_4[13] encouraged us to use it for the syntheses of imines in combination with microwave irradiation methods. The tripottasium phosphate faces the reaction between 2-amino pyridine and different substituted benzaldehyde to give Schiff bases **(Scheme 1).**

The experimental reaction procedure involves the equimolar sample of 2-amino pyridine and different substituted benzaldehyde thoroughly mixed in 5mL of ethanol. The resultant solution was absorbed on 5gm of solid alumina- K_3PO_4 catalyst and irradiated under a well-equipped microwave for a given short interval of time **(Table 5).** The completion of reaction was indicated by TLC using the mobile phase of hexane and ethyl acetate as a mobile phase ratio (8:2). The obtained product was collected by workup procedure and unreacted inorganic solid removed using 50mL ethanol. Finally, the pure solid product obtained by evaporation of filtrate and recrystallize from ethanol **(Scheme 1).**

The reported method for syntheses of imines is efficient in terms of simple reaction procedure, environment eco-friendly, and short period (6-14 min.) of time and high yield of product (70-86 %) **(Table 5).** The inorganic tripottasium phosphate is a readily available and inexpensive catalyst compared to other reported expensive catalysts. Therefore, the present method is useful for the synthesis of imine in comparison to other reported methods.

Structures of all newly synthesized Schiff bases were confirmed by spectral data such as IR, ^{1}H NMR, ^{13}C NMR, Mass and Elemental analysis. IR analysis showed the characteristic bands at 3379-3436 cm^{-1}, 1607-1683 cm^{-1} and 1446-1580 cm^{-1} for the corresponding –OH, C=N and C=C bond stretch respectively. The ^{1}H NMR spectrum of synthesized imines showed the characteristic singlet at 7.92-9.42 ppm suggested the attribution of proton of CH=N group, the singlet signal at 13.44-13.37 revealed the presence of OH proton *ortho* to imine and 5.38 ppm suggested that OH group present at *para* position to imine. The multiplets of aromatic protons observed in the range 6.46-9.38 ppm.

2.2 Antimicrobial Activity

The synthesized Schiff bases were screened for their antibacterial activity. The test was conducted on two usual microorganisms such as *Escherichia coli and Staphylococcus aureus* which are the characteristic types of grams -ve and gram +ve bacteria respectively. The antibacterial activity of the Schiff bases was performed by using the disc diffusion method, Ciprofloxacin was used to determine the zone of inhibition. The 50 µg/ml concentration of the compounds were prepared using Methanol as solvent, the *zone of inhibition* given in **(Table 1).**

The tested imines showed potent antibacterial assay against gram +ve as well as gram –ve pathogen. The compounds **3b**, **3e**, **3j** and **3l** showed a good *zone of inhibition* towards all the tested imines. Most of the screened azomethines exhibited a potent zone of inhibition against gram –ve pathogenic than gram +ve pathogen **(Table 1)**. The compounds **3b**, **3e**, **3j** and **3l** exhibited zones of inhibition due to presence of pharmacological active substituents such as **-OCH_3, -Cl, -Br** and **–CH_3** present in the main structural nucleus. Among these compounds **3j** and **3l** have exhibited good antibacterial activity due to bearing chlorine and methoxy groups.

Table 1. Antimicrobial activity (inhibition zone in mm) of synthesized compounds (3a-l).

Compounds	Antibacterial activity	
	Gram positive bacteria	Gram negative bacteria
	S. aureus	***E. coli***
3a	-	06
3b	05	06
3c	-	05
3d	-	03
3e	08	06
3f	-	-
3g	-	-
3h	-	-
3i	-	-
3j	10	10
3k	-	04
3l	08	09
Ciprofloxain (14)		

* (-ve) indicate no zone of inhibition

2.3 In Vitro Anti-Inflammatory Assays

The procedure outlined by Padmanabhan and Jangle,[21] Elias and Rao,[22] was followed, with a few minor adjustments, to assess the synthetic compounds' anti-inflammatory activities. Bovine Serum Albumin (BSA, 5%) was homogenized with a volume of 1 ml of drugs (aqueous and ethanolic) or diclofenac (positive control) at concentrations of 1 mg/ml before incubating at 27 °C for 15 minutes. The control tube was created by using distilled water and BSA. By putting the mixture in a water bath at 70 °C for 10 minutes, the proteins were denatured. Each mixture's activity was monitored at 660 nm as it cooled inside an environment of room temperature. Three times were given to each test and the calculations were made using the formula below.

$$(\%)\text{inhibition} = \frac{[\text{Absorbance of control (Ac)} - \text{Absorbance of sample(As)}]}{\text{Absorbance of control (Ac)}} \times 100$$

Table 2. Anti-inflammatory activity of some synthesized compounds (3a-d and 3j).

Sr. No.	Compounds (Concentration 1mg/mL)	Inflammatory Potential (In %) (Mean±SD)
1	3a	-
2	3b	-
3	3c	**42.307±1.322**
4	3d	-
5	3j	
	+Ve Control (diclofenac)	**46.153±2.705**

*All the data statistically analyzed with mean±SD (n=3)

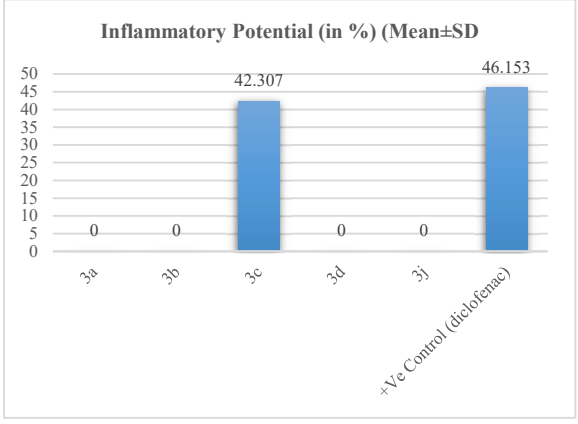


Fig. 1. Graphical representation of anti-inflammatory activity of Compounds **(3a-d and 3j).**\

The diclofenac is used as positive control. From the above tested compounds only compound **3c** has been exhibited better percentage of inhibition **(42.307±1.322)** as compared to standard. The compound **3c** exhibited potency in inflammatory activity is attributed due to presence of pharmacophore two pyridine rings with substituent present at 3-methyl and 5-bromo positions in main structural nucleus of imines. The remaining compounds didn't display the expected anti-inflammatory properties compare to standard drug. Therefore, present study may lead to find out the more potent anti-inflammatory agents by structural modification of imines.

2.4. Antioxidant Assay

2.4.1 DPPH Scavenging activity

The 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) test was used to calculate the free-radical scavenging activity. The reaction mixture included 2.9 ml of a methanolic solution of 0.1 mM DPPH radical and 100 µl of test extracts with various concentrations. The mixture was then vigorously shaken and incubated for 30 minutes at 37 °C. At 517 nm, the absorbance was measured using ascorbic acid acting as the positive control. The reaction mixture's lower absorbance indicated a higher level of free radical scavenging activity, which was determined using the following equation.

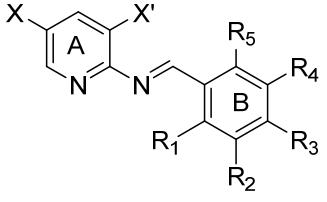
$$(\%)\ \text{DPPH scavenging effect} = \frac{[\text{Absorbance of control} - \text{Absorbance of sample}]}{\text{Absorbance of control}} \times 100$$

2.4.2 Nitric oxide scavenging assay

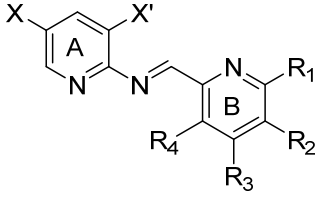
The Griess Illosvory reaction was used to measure nitric oxide radical inhibition.[23,24] In this study, 1-napthylamine (5%), instead of napthyl ethylene diamine dihydrochloride (0.1% w/v) was used to generally change the Griess Illosvory reagent. The reaction mixture (3 ml) was incubated at 25°C for 150 minutes. It contained 2 ml of 10 mM sodium nitroprusside, 0.5 ml of saline phosphate buffer, and 0.5 ml of standard solution or aqueous and ethanolic extracts (500–1000 g/ml). After the reaction mixture is incubated for 0.5 ml of it was combined with 1 ml of the sulfanilic acid reagent (0.33% in 20% glacial acetic acid), and the combination was let to stand for 5 minutes to allow the diazotization reaction to finish. The napthyl ethylene diamine dihydrochloride was then added in a further 1 ml, stirred and allowed to stand for 30 minutes at 25°C. Nitrite concentration was measured at 546 nm and estimated using the standard nitrite solution's control absorbance (without extracts or standards, but the same procedure should be performed). Ascorbic acid and quercetin were utilized in this instance as the standard solution while buffer was used as the blank solution. The following formula was used to get the % inhibition.

$$\%\ \text{Scavenging activity} = \frac{\text{Acontrol} - \text{Atest or Astd}}{\text{AControl}} \times 100$$

For the anti-oxidant studies, the absorbance of DPPH radical was decreased and is monitored for imines at 517 nm. It reveals that, **-OH** and **CH_3** substituted imines exhibited potent scavenging activity. The compounds **3a, 3d, 3f, 3h** and **3i** display more potent DPPH anti-oxidant activity compared to other remaining compounds. The compound **3e**, **3f**, **3h**, **3i**, **3j**, **3l** didn't display the NO scavenging properties respectively. Therefore, the imines moiety carrying a **–OH** substituent at 2nd, 3rd and 4th position of aromatic ring B show better antioxidant activity. Exceptionally the imine derivative **3k** don't display anti-oxidant properties carrying **–OH** substituents at 3rd and 4th position of ring B. This decrease in scavenging activity might be due to the presence of electron withdrawing –Cl substituent in ring A.



3a,3b,3d-f,3h-l



3c and 3g

3a: X', CH_3; X, R_4, Br; R_1, OH; R_2, R_3, R_5, H

3b: X', CH_3; X, Br; R_1, R_5 H; R_2, R_3, R_4, OCH_3

3c: X', CH_3; X, Br; R_1, R_2, R_3, R_4, H

3d: X', CH_3; X, Br; R_3, OH; R_1, R_2, R_4, R_5, H

3e: X', CH_3; X, Br; R_3, OCH_3; R_1, R_2, R_4, R_5, H

3f: X', CH_3; X, Br; R_2, R_3, OH; R_1, R_4, R_5, H

3g: X', X, Cl; R_1, R_2, R_3, R_4, H

3h: X', X, Cl; R_1, OH; R_4, Br; R_2, R_3, R_5, H

3i: X', X, Cl; R_1, OH; R_2, R_3, R_4, R_5, H

3j: X', X, Cl; R_1, R_5 H; R_2, R_3, R_4, OCH_3

3k: X', X, Cl; R_2, R_3 OH; R_1, R_4, R_5, H

3l: X', X, Cl; R_3, OCH_3; R_1, R_2, R_4, R_5, H

Table 3. DPPH and NO Scavenging activity based antioxidant activity of synthesized compounds 3a-l.

Compound (Conc. 1 mg)	DPPH Test	NO Test
3a	32.56±2.13	22.56±2.52
3b	59.23±1.25	39.23±1.25
3c	55.93±1.02	35.93±1.02
3d	30.66±2.23	19.66±2.23
3e	18.47±1.85	-
3f	19.65±1.55	-
3g	44.25±2.30	24.55±1.35
3h	16.98±1.20	-
3i	20.13±2.08	-
3j	25.33±1.45	-
3k	60.25±2.68	40.25±2.68
3l	26.63±1.58	-
Ascorbic Acid	86.56±2.39	66.50±1.55

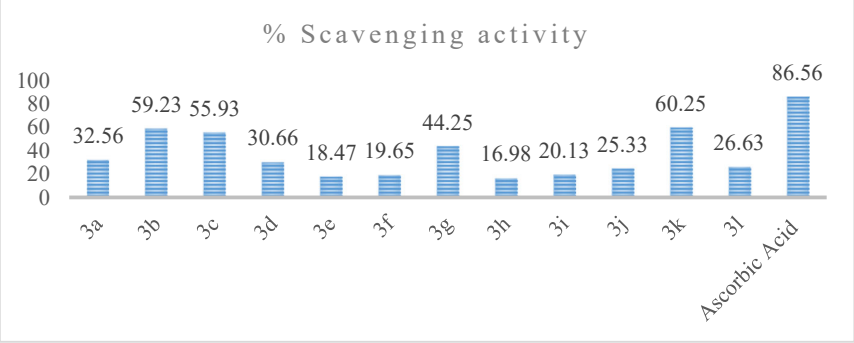


Fig. 2. Graphical representation of scavenging activity

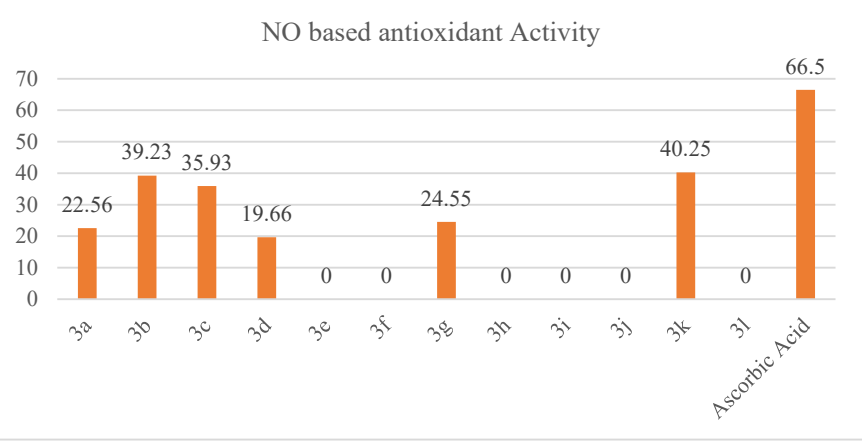


Fig. 3. Graphical representation of NO based scavenging activity

Table 4. DPPH % Scavenging activity of standard drug Ascorbic acid.

Conc. of Ascorbic Acid (in mg)	DPPH % Scavenging Activity
0.2	56.24±2.15
0.4	68.36±3.55
0.6	75.65±1.58
0.8	78.88±1.47
1.0	86.58±2.52

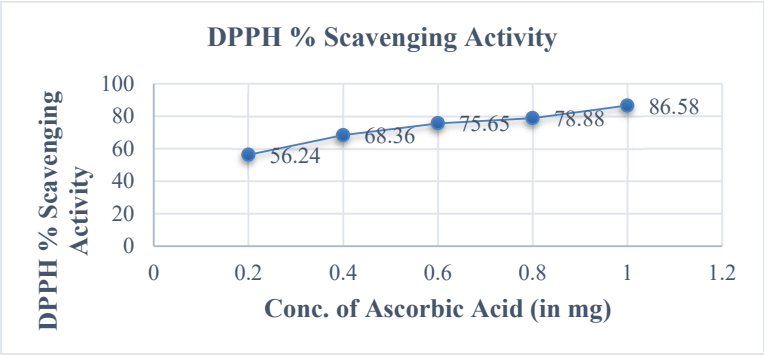


Fig. 4. Graphical representation of DPPH % scavenging activity of Ascorbic acid

3. Conclusions

In conclusion, we have synthesized and evaluated the antibacterial, anti-inflammatory and antioxidant activity of a novel series of imines. All the synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR, IR and Mass spectral data. The compound **3c** was found to have promising anti-inflammatory activity 42.307%±1.322 inhibition of inflammation due to presence of two pyridine rings as a biologically active nucleus. All imines have been screened for antibacterial activity, among these **3b**, **3e**, **3j** and **3l** showed good antibacterial activity. The presence of **-OCH$_3$, -Cl, -Br** and **–CH$_3$** as pharmacophore substituents on in the main structural nucleus of imines is found to be an effective antibacterial agent. The compounds **3b**, **3e**, **3j** and **3l** exhibit the common **-OCH$_3$** substituent functionality on benzaldehyde. Also, all the novel Schiff bases were evaluated for their antioxidant activity. Out of all imines **3a**, **3d**, **3f**, **3h** and **3i** display more potent DPPH anti-oxidant activity compared to other remaining imines. These imines **(3a**, **3d** and **3f)** exhibit antioxidant activity due to presence of electron donating functional groups (-OH and -CH$_3$) and compound **3h and 3i** exhibited DPPH anti-oxidant activity due to **–OH** functional group except compound **3k** due to presence of electron withdrawing –Cl substituent on pyridine ring, similarly compound **3e**, **3f**, **3h**, **3i**, **3j**, **3l** didn't display the NO scavenging properties respectively.

Supporting information

Supplementary data to this article can be found at the journal website.

Acknowledgements

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4. Experimental

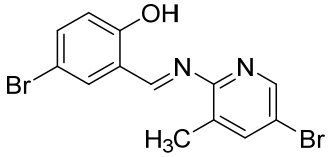
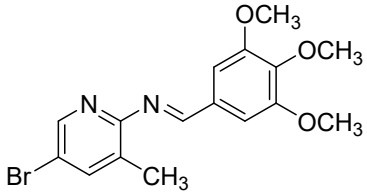
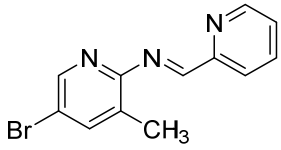
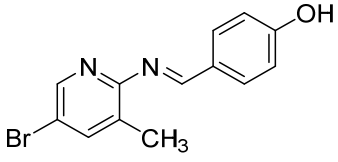
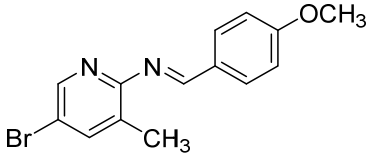
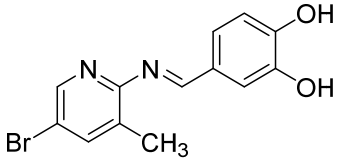
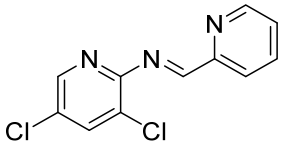
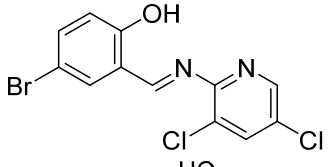
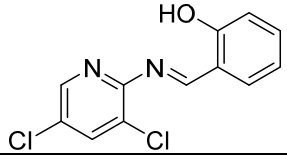
4.1. Materials and Methods

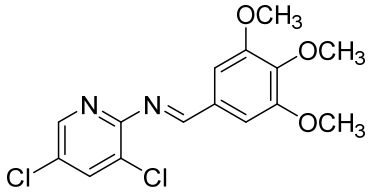
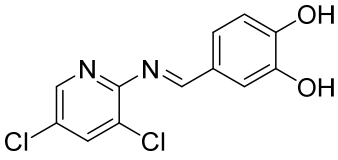
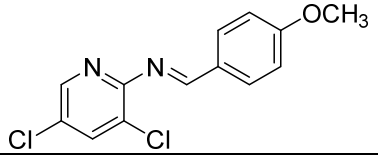
Starting Material Commercially available aldehydes, amines, solvents, and reagents were used without purification. TLC provided information about the purity of each processed sample. The synthesis was carried out in a laboratory Oven (Ragatech, Scientific microwave system, 700w). Open glass capillaries were used to determine melting points using the uncorrected digital Veego, VMP-D, melting point method. On Bruker and PerkinElmer infrared spectrometers, FT-IR spectra are recorded as KBr pellets. ^{1}H-NMR and ^{13}C NMR spectra were recorded in DMSO and $CDCl_3$ on Bruker Avance NEO500 Spectrometer at 400, 500 MHz with internal standard TMS and LC-MAS spectrometer records mass spectra.

4.2. General Procedure for Synthesis of Schiff Bases (3a-l)

A mixture of 3, 5 di-substituted 2-amino pyridine (0.01 moles) and Substituted Benzaldehyde (0.01 moles) were dissolved in 5mL ethanolic solution. The solution was absorbed on the equivalent mixture (5 gm each) of basic alumina and anhydrous tripottasium phosphate and air-dried. The reaction mixture was exposed to microwave irradiation at 500 watts with 30 intervals of seconds for overall irradiation at 6-14 minutes. The progress of the reaction was monitored by TLC using mobile phase Hexane: ethyl acetate (8:2). After completion of the reaction, mixture was cooled and poured in cold water, obtained solid was filtered and washed with sufficient amount of water (25mL). Product was separated by dissolving in 50 mL of ethanol to remove the insoluble inorganic material and filtered off. Solid product was obtained by the evaporation of filtrate. Desired Schiff Base compounds are obtained up to 86% yield. After that, the structure of the synthesized Schiff Base was confirmed by spectral analysis.

Table 5. Reaction condition and Physical Data of Synthesized Schiff Bases 3a-l by Microwave irradiation method.

Compound	Structure of Synthesized Schiff base	Reaction condition used for Microwave Irradiation		Yield (%)
		Reaction Temperature (^{0}C)	Reaction Time (min.)	
3a		80	8	70
3b		80	9	75
3c		80	7	86
3d		80	6	71
3e		80	6	73
3f		80	9	74
3g		80	11	72
3h		80	10	82
3i		80	8	80

3j		80	10	76
3k		80	9	73
3l		80	14	72

4.3. Spectral Analytical Data of the Synthesized Compounds

4.3.1(E)-4-bromo-2-(5-bromo-3-methylpyridin-2-yl)imino)methyl) phenol(3a)

Orange colour Solid; Recrystallized from ethanol; m.p 178 ºC; ^{1}H NMR (400 MHz, DMSO) δ 13.44 s (1H, OH), 9.38 – 6.91 m (5H, Ar-H), 8.36 s (1H, CH=N), 2.45 s (3H, CH_3); ^{13}C NMR (DMSO, δ ppm) 193.18(C=N Ar), 160.89 (C=N), 160.29-110.59 (Aromatic Carbon), 17.38(CH_3); IR Spectrum, *v* cm^{-1}: 3429.18 (OH), 1605.85 (C=N), 1546.79 (ring C=C), 822.54 (C-Br); EIMS m/z = 368.7 [M^{+}]; Anal.Calc for $C_{13}H_{10}Br_2N_2O$, %: C, 42.20; H, 2.72; N, 7.57. Found: C, 42.01; H, 2.65; N, 7.71.

4.3.2 (E)-5-bromo-3-Methyl-N-(3,4,5-trimethoxybenzylidene) pyridin-2-amine(3b)

Brown colour Solid; Recrystallized from ethanol; m.p 103 ºC; ^{1}H NMR (400 MHz, DMSO) δ 8.95 s (1H, CH=N), 8.30-7.17 m (4H, Ar-H), 3.88 s (9H, OCH_3), 2.45 s (3H, CH_3); ^{13}C NMR (DMSO, δ ppm) δ191.19 (C=N Ar), 161.80 (C=N), 156.94-106.78(Aromatic carbon), 60.71, 56.27(OCH_3), 17.28(CH_3); IR Spectrum, *v* cm^{-1}: 2942.21-2839.56 (OCH_3)\, 1683.57 (C=N), 1329.30 (aromatic C-N), 845.61 (C-Br); EIMS m/z = 364.8 [M^{+}].

4.3.3 (E)-5-bromo-3-Methyl-N-(pyridin-2-ylmethylene) pyridin-2-amine (3c)

White colour Solid; Recrystallized from ethanol; m.p 160 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.81 s (1H, CH=N)), 8.80 - 7.31 m (6H, Ar-H), 2.45 s (3H, CH_3); ^{13}C NMR ($CDCl_3$, δ ppm) 193.47(C=N Ar), 162.94(C=N), 155.80-108.39 (Aromatic carbon), 17.00 (CH_3); IR Spectrum, *v* cm^{-1}: 2922.97 (=CH), 1647.03 (C=N), 1465.62, 1391.53 (CH_3), 752.78, 827.62 (C-Br); EIMS m/z = 275.70; Anal. Calc for $C_{12}H_{10}BrN_3$, %: C, 52.20; H, 3.65; N, 15.22. Found: C, 52.14; H, 3.80; N, 15.25.

4.3.4 (E)-4-(((5-bromo-3-methylpyridin-2-yl) imino) methyl) phenol(3d)

Yellow colour Solid; Recrystallized from ethanol; m.p 140 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 s (1H, CH=N), 8.37-6.95 m (6H, Ar-H), 5.38 s (1H, OH), 2.45 s (3H, CH_3); ^{13}C NMR (DMSO, δ ppm) 174.74 (C=N Ar), 166.37 (C-OH), 159.38 (C=N), 152.24-121.87 (Aromatic Carbon), 22.20(CH_3); IR Spectrum, *v* cm^{-1}: 3436.26 (OH), 2924.34 (=CH), 1607.62 (C=N), 831.14, 752.64 (C-Br); EIMS m/z = 290.70 [M^{+}].

4.3.5 (E)-5-bromo-N-(4-methoxybenzylidene)-3-methyl pyridin-2-amine (3e)

Brown colour Solid; Recrystallized from ethanol; m.p 135 ºC; ^{1}H NMR (400 MHz, DMSO) δ 8.96 s (1H, CH=N), 8.31-7.16 m (6H, Ar-H), 3.89 s (3H, OCH_3), 2.58 s (3H, CH_3); ^{13}C NMR (DMSO, δ ppm) δ191.19 (C=N Ar), 161.80 (C=N), 156.94-116.83(Aromatic carbon), 56.27(OCH_3), 17.19(CH_3); IR Spectrum, *v* cm^{-1}: 2942.21-2839.56 (O-CH_3), 1683.57 (C=N), 1329.30 (aromatic C-N), 845.61 (C-Br); EIMS m/z = 305 [M^{+}].

4.3.6 (E)-4-(((5-bromo-3-methylpyridin-2-yl) imino) methyl) benzene-1,2-diol(3f)

Yellow colour Solid; Recrystallized from ethanol; m.p 109 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 s (1H, CH=N), 8.37-6.95 m (6H, Ar-H), 5.38 s (1H, OH), 2.45 s (3H, CH_3); ^{13}C NMR (DMSO, δ ppm) 174.74 (C=N Ar), 166.37 (C-OH), 159.38 (C=N), 152.24-121.87 (Aromatic Carbon), 22.20(CH_3); IR Spectrum, *v* cm^{-1}: 3436.26 (OH), 2924.34 (=CH), 1607.62 (C=N), 831.14, 752.64 (C-Br); EIMS m/z = 307 [M^{+}].

4.3.7 (E)-3, 5-dichoro-N-(pyridin-2-ylmethylene) pyridin-2-amine (3g)

White colour Solid; Recrystallized from ethanol; m.p 112 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.60 s (1H, CH=N), 7.99-7.14 m (6H, Ar-H); ^{13}C NMR ($CDCl_3$, δ ppm) 165.85 (C=N Ar), 162.44 (C=N), 145.63-119.66 (Aromatic Carbon), 118.04 (C-Cl); IR Spectrum, *v* cm^{-1}: 3288.41, 3049.01 (=CH), 1587.85 (C=N), 881.17, 776.40 (C-Cl); EIMS m/z = 251.5 [M$^+$]; Anal. Calc for $C_{11}H_7Cl_2N_3$, %: C, 52.41; H, 2.80; N, 16.67. Found: C, 52.18; H, 2.39; N, 16.92.

4.3.8 (E)-4-bromo-2-(((3,5-dichoropyridin-2-yl) imino) methyl) phenol (3h)

Orange colour Solid; Recrystallized from ethanol; m.p 160 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 13.37 s (1H, OH), 9.36 s (1H, CH=N), 8.38-6.98 (5H, Ar-H); ^{13}C NMR ($CDCl_3$, δ ppm) 164.24 (C=N Ar), 161.28 (C=N), 151.85 (C-OH), 145.76-110.83 (Aromatic Carbon); IR Spectrum, *v* cm^{-1}:3379.50 (OH), 3034.08, 2943.73 (=CH), 1640.39 (C=N), 826.44, 761.57 (C-Br, C-Cl); EIMS m/z = 343.91 [M$^+$]; Anal. Calc for $C_{12}H_7BrCl_2N_2O$, %: C, 41.65; H, 2.04; N, 8.10. Found: C, 41.61; H, 2.08; N, 8.16.

4.3.9 (E)-2-(((3,5-dichoropyridin-2-yl) imino) methyl) phenol (3i)

Orange colour Solid; Recrystallized from ethanol; m.p 165 ºC; ^{1}H NMR (500 MHz, DMSO) δ 13.37 s (1H, OH), 9.42 s (1H, CH=N), 8.34-6.95 (6H, Ar-H); ^{13}C NMR ($CDCl_3$, δ ppm) 157.88 (C=N Ar), 151.58 (C=N), 149.34 (C-OH), 144.86-115.96 (Aromatic Carbon); IR Spectrum, *v* cm^{-1}:3381.25(OH), 3036.87, 2937.92 (=CH), 1635.81 (C=N) 757.81 (C-Cl); EIMS m/z = 266 [M$^+$].

4.3.10 (E)-3, 5-dichoro-N-(3,4,5-trimethoxybenzylidene) pyridine-2-amine (3j)

White colour Solid; Recrystallized from ethanol; m.p 117 ºC;^{1}H NMR (500 MHz, DMSO) δ 9.88 (1H, Ar N=CH), 7.92 s (1H, CH=N), 7.92-7.24(3H, Ar-H), 3.86 (s 9H); ^{13}C NMR (DMSO, δ ppm) 191.75 (C=N Ar), 161.80 (C=N), 157.63-106.79 (Aromatic Carbon), 60.73, 56.28 (OCH_3); IR Spectrum, *v* cm^{-1}: 2895.53, 2830.58 (OCH_3), 1676.46 (C=N) 1580.8, 1499.58 (ring C=C), 717 (C-Cl); EIMS m/z = 340 [M$^+$]; Anal. Calc for $C_{15}H_{14}Cl_2N_2O_3$, %: C, 52.80; H, 4.14; N, 8.21. Found: C, 52.74; H, 4.29; N, 8.41.

4.3.11 (E)-4-(((3, 5-dichoropyridin-2-yl) imino) methyl) benzene-1, 2-diol (3k)

Black colour Solid; Recrystallized from ethanol; m.p 102 ºC; ^{1}H NMR (500 MHz, DMSO) δ 8.7621 s (1H, Ar N=CH), 8.6303 s (1H, CH=N), 7.9374-6.5035 (4H, Ar-H),5.3303 s (2H, OH); ^{13}C NMR ($CDCl_3$, δ ppm) 165.27(C=N Ar), 162.82(C=N), 155.52, 148.24 (C-OH), 142.76-118.37 (Aromatic Carbon); IR Spectrum, *v* cm^{-1}: 3383.75 (OH), 3044.22, 2939.85(=CH), 1642.58(C=N); EIMS m/z = 282 [M$^+$].

4.3.12 (E)-3, 5-dichoro-N-(4-methoxybenzylidene) pyridine-2-amine (3l)

White colour Solid; Recrystallized from ethanol; m.p 145 ºC; ^{1}H NMR (500 MHz, DMSO) δ 9.70 s (1H, Ar N=CH), 8.020 s (1H, CH=N), 8.0157-6.4644 (5H, Ar-H), 3.3392 s (3H, OCH_3); ^{13}C NMR ($CDCl_3$, δ ppm) 172.33 (C=N Ar), 162.17(C-O-C), 157.20 (C=N), 146.04-106.77 (Aromatic Carbon), 55.85 (OCH_3); IR Spectrum, *v* cm^{-1}: 3072.63(=CH), 2979.06 (OCH_3), 1622.04 (C=N), 1566.29, 1446.58 (ring C=C), 728.63 (C-Cl); EIMS m/z = 283 [M$^+$].

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