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In vitro antimicrobial activity of new 2-amino-4-chloropyridine derivatives: A structure-activity relationship study

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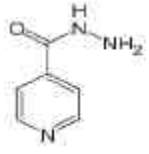
ABSTRACT

In the present study, a series of new Schiff bases of 2-amino-4-chloropyridine derivatives **3(a-p)** were synthesized from the building blocks of 2-amino-4-chloropyridine **(1)** and different aldehydes **(2a-p)**. Newly synthesized compounds were characterized by different spectral studies. These new compounds were evaluated for their *in-vitro* antimicrobial effect using the agar-well diffusion method against three Gram positive bacteria (*Staphylococcus aureus, Bacillus cereus* and *Bacillus licheniformis*), three Gram negative bacteria (*Escherichia coli, Acetobacter sp.* and *Pseudomonas aeruginosa*) and three fungi (*Penicillium expansum, Asperigillus flavus and Pichia anomola*). The structure-activity relationships of the synthesized compounds were also discussed. Variable and modest activity was observed against the investigated strains of bacteria and fungi. However, the results revealed that, compounds **3b, 3c, 3d, 3f** and **3g** have exhibited significant biological activity against the tested microorganisms.

KEYWORDS: 2-Amino-4-chloropyridine; Aldehydes; Antimicrobial activity; SAR Studies.

1. INTRODUCTION

A pyridine moiety plays a vital role in the development of drug discoveries. Many biologically significant compounds used in pharma industries contain pyridine nucleus such as isoniazide (**1**), clopidogrel (**2**), Prasugrel (**3**), Esomeprazole (**4**). Pyridine and its derivatives exhibit broad spectrum of biological activities such as antimicrobial[1], anti-inflammatory[2], analgesic[3], anticonvulsant[4] and antimalarial[5]. Other applications of the pyridine are used in agrochemicals[6, 7,] corrosion inhibitors[8] and dyes[9].



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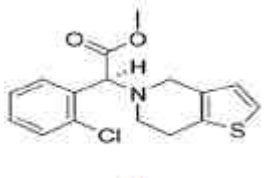

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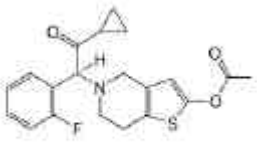

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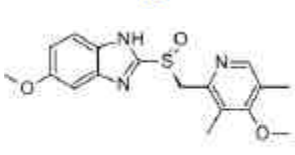

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Over the past few decades, life threatening infections caused by the multidrug resistant microbes creates a serious challenge to the scientist. Commonly used antibiotics have become less effective against these microbes. Compound containing azomethine functional groups which are known as Schiff base. These Schiff bases shown to be promising leads for the design of efficient antimicrobial agents as a result of the broad range of biological activities such as antimicrobial[10], antioxidant[11], antitumor[12], anti-inflammatory[13], analgesic and anti-tubercular[14]. In view of these, we have attempted to incorporate schiff base with pyridine nucleus by reacting 2-amino-4-chloropyridine **(1)** and different aldehydes **(2a-p).** These synthesized compounds were characterized by elemental analyses, UV-visible, FT-IR, Mass and ^{1}H NMR studies. Antibacterial and antifungal activities were reported and structural activity relationship was also discussed in this paper.

2. MATERIALS AND REAGENTS

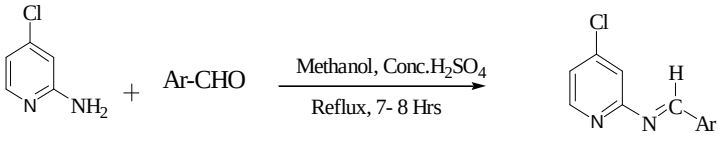
2.1. Chemistry

All solvents and reagents were purchased from Merck and Sigma Aldrich Chemicals. The elemental analyses of the compounds were performed on a Perkin Elmer 2400 elemental analyser. The UV-Visible spectrum was recorded on UV-1800 SHIMADZU UV spectrometer

with quartz cell of 1.0 cm path length. The FT-IR spectrum was recorded using KBr discs on Jasco FT-IR 4100 infrared spectrophotometer. The ^{1}H NMR spectra was recorded using Bruker DRX 400 spectrometer at 300 MHz with tetramethylsilane as the internal standard. Mass spectral data was obtained by LC/MSD Trap XCT.

2.2. General procedure for the synthesis of 3-fluoro-5-(trifluoromethyl) benzylamine derivatives 3(a-p)

Equimolar concentration of 2-amino-4-chloropyridine (**1**) with different aryl aldehydes (**2a-p**) in methanol (25 ml) and then catalytic amount of conc. sulfuric acid was added to the mixture and stirred for 7-8 hr at room temperature. The reaction completion was confirmed by TLC. The solvent was concentrated and the solid was dried and recrystallized from methanol. 2-amino-4-chloropyridine derivatives **3(a-p)** were synthesized by the method summarized in Scheme 1.



1 **2(a-p)** **3(a-p)**

Scheme 1

2.2.1. Synthesis of N-benzylidene-4-chloropyridin-2-amine (3a)

FT-IR (KBr, cm^{-1}): 3051 (Ar-H), 1641 (HC=N), 1463 (C=C), 788 (C-Cl). ^{1}H NMR (DMSO-d$_6$) δ ppm: 8.60 (d, 1H, Py-H), 8.10 (s, 1H, CH=N), 7.60 (d, 2H, Ar-H), 7.42 (s, 1H, Py-H), 7.40 (d, 1H, Py-H), 7.30 (t, 3H, Ar-H). MS (ESI) *m/z*: 216.67. Anal. Calcd. for $C_{12}H_9ClN_2$ (in %) C, 66.52; H, 4.19; N, 12.93. Found: C, 66.55; H, 4.15; N, 12.99.

2.2.2. Synthesis of N-(4-chlorobenzylidene)-4-chloropyridin-2-amine (3b)

FT-IR (KBr, cm^{-1}): 3078 (Ar-H), 1670 (HC=N), 1463 (C=C), 741 (C-Cl). ^{1}H NMR (DMSO-d$_6$) δ ppm: 8.60 (s, 1H, Py-H), 8.10 (s, 1H, CH=N), 7.60 (d, 2H, Ar-H), 7.42 (s, 1H, Py-H), 7.40 (d, 1H, Py-H), 7.29 (d, 2H, Ar-H). MS (ESI) *m/z*: 251.11. Anal. Calcd. for $C_{12}H_8Cl_2N_2$ (in %): C, 57.40; H, 3.21; N, 11.16. Found: C, 57.34; H, 3.19; N, 11.12.

2.2.3. Synthesis of N-(4-bromobenzylidene)-4-chloropyridin-2-amine (3c)

FT-IR (KBr, cm^{-1}): 3048 (Ar-H), 1672 (HC=N), 1465 (C=C), 715 (C-Cl), 597 (C-Br). ^{1}H NMR (DMSO-d6) δ ppm: 8.60 (d, 1H, Py-H), 8.10 (s, 1H, CH=N), 7.50 (d, 4H, Ar-H), 7.42 (s, 1H, Py-H), 7.40 (d, 1H, Py-H). MS (ESI) m/z: 295.56. Anal. Calcd. for $C_{12}H_8BrClN_2$ (in %): C, 48.76; H, 2.73; N, 9.48. Found: C, 48.79; H, 2.71; N, 9.51.

2.2.4. Synthesis of N-(4-fluorobenzylidene)-4-chloropyridin-2-amine (3d)

FT-IR (KBr, cm^{-1}): 3019 (Ar-H), 1661 (HC=N), 1471 (C=C), 1178 (C-F), 736 (C-Cl). ^{1}H NMR (DMSO-d6) δ ppm: 8.60 (d, 1H, Py-H), 8.10 (s, 1H, CH=N), 7.60 (d, 2H, Ar-H), 7.42 (s, 1H, Py-H), 7.40 (d, 1H, Py-H), 7.00(d, 2H, Ar-H). MS (ESI) m/z: 234.66. Anal. Calcd. for $C_{12}H_8ClFN_2$ (in %): C, 61.42; H, 3.44; N, 11.94. Found: C, 61.44; H, 3.41; N, 11.99.

2.2.5. Synthesis of N-(4-nitrobenzylidene)-4-chloropyridin-2-amine (3e)

FT-IR (KBr, cm^{-1}): 3060 (Ar -H), 1640 (HC=N), 1530 (N-O), 1490 (C=C), 720 (C-Cl). ^{1}H NMR (DMSO-d$_6$) δ ppm: 8.60 (d, 1H, Py-H), 8.20 (d, 2H, Ar-H), 8.10 (s, 1H, CH=N), 7.90 (d, 2H, Ar-H), 7.42 (s, 1H, Py-H), 7.40 (d, 1H, Py-H). MS (ESI) *m/z*: 261.66. Anal. Calcd. For $C_{12}H_8ClN_3O_2$ (in %): C, 55.08; H, 3.08; N, 16.06. Found: C, 55.01; H, 3.11; N, 16.12.

2.2.6. Synthesis of N-(4-(trifluoromethyl)benzylidene)-4-chloropyridin-2-amine (3f)

FT-IR (KBr, cm^{-1}): 3071 (Ar-H), 1656 (HC=N), 1458 (C=C), 1124 (C-F), 771 (C-Cl). ^{1}H NMR (DMSO-d6) δ ppm: 8.60 (d, 1H, Py-H), 8.10 (s, 1H, CH=N), 7.60 (d, 2H, Ar-H), 7.50(d, 2H, Ar-H), 7.42 (s, 1H, Py-H), 7.40 (d, 1H, Py-H). MS (ESI) m/z: 284.66. Anal. Calcd. for $C_{13}H_8ClF_3N_2$ (in %): C, 54.85; H, 2.83; N, 9.84. Found: C, 54.81; H, 2.79; N, 9.89.

2.2.7. Synthesis of N-(3,5-dinitrobenzylidene)-4-chloropyridin-2-amine (3g)

FT-IR (KBr, cm^{-1}): 3081 (Ar-H), 1678 (HC=N), 1469 (C=C), 1159 (C-N), 724 (C-Cl). ^{1}H NMR (DMSO-d$_6$) δ ppm: 9.10 (s, 1H, Ar-H), 8.90 (s, 2H, Ar-H), 8.60 (d, 1H, Py-H), 8.10 (s, 1H, CH=N), 7.42 (s, 1H, Py-H), 7.40 (d, 1H, Py-H). MS (ESI) *m/z*: 306.66. Anal. Calcd. for $C_{12}H_7ClN_4O_4$ (in %): C, 47.00; H, 2.30; N, 18.27. Found: C, 47.05; H, 2.33; N, 18.21.

2.2.8. Synthesis of N-(4-methoxybenzylidene)-4-chloropyridin-2-amine (3h)

FT-IR (KBr, cm^{-1}): 3059 (Ar-H), 1658 (HC=N), 1454 (C=C), 725 (C-Cl). ^{1}H NMR (DMSO-d$_6$) δ ppm: 8.60 (d, 1H, Py-H), 8.10 (s, 1H, CH=N), 7.50 (d, 2H, Ar-H), 7.42 (s, 1H, Py-H), 7.40 (d, 1H, Py-H), 6.80 (d, 2H, Ar-H), 3.73 (s, 3H, OCH_3). MS (ESI) *m/z*: 246.69. Anal. Calcd. for $C_{13}H_{11}ClN_2O$ (in %): C, 63.29; H, 4.49; N, 11.36. Found: C, 63.31; H, 4.45; N, 11.31.

2.2.9. Synthesis of 2-((4-chloropyridin-2-ylimino)methyl)phenol (3i)

FT-IR (KBr, cm^{-1}): 3534 (O-H), 3074 (Ar-H), 1679 (HC=N), 1468 (C=C), 703 (C-Cl). ^{1}H NMR (DMSO-d$_6$) δ ppm: 10.53 (s, 1H, Ar-OH), 8.60 (d, 1H, Py-H), 8.10 (s, 1H, CH=N), 7.42 (s, 1H, Py-H), 7.40 (d, 1H, Py-H), 7.40 (d, 1H, Ar-H), 7.10 (t, 1H, Ar-H), 6.80 (m, 2H, Ar-H). MS (ESI) *m/z*: 232.67. Anal. Calcd. for $C_{12}H_9ClN_2O$ (in %): C, 61.95; H, 3.90; N, 12.04. Found: C, 61.91; H, 3.95; N, 12.09.

2.2.10. Synthesis of 4-((4-chloropyridin-2-ylimino)methyl) phenol (3j)

FT-IR (KBr, cm^{-1}): 3584 (O-H), 3065 (Ar-H), 1671 (HC=N), 1458 (C=C), 764 (C-Cl). ^{1}H NMR (DMSO-d_6) δ ppm: 10.08 (s, 1H, Ar-OH). 8.60 (d, 1H, Py-H), 8.10 (s, 1H, CH=N), 7.42 (s, 1H, Py-H), 7.40 (d, 1H, Py-H), 7.40 (d, 2H, Ar-H), 6.80 (d, 2H, Ar-H). MS (ESI) m/z: 232.67. Anal. Calcd. for $C_{12}H_9ClN_2O$ (in %): C, 61.95; H, 3.90; N, 12.04. Found: C, 61.91; H, 3.99; N, 12.09.

2.2.11. Synthesis of 4-((4-chloropyridin-2-ylimino)methyl)-2-methoxyphenol (3k)

FT-IR (KBr, cm^{-1}): 3512 (O-H), 3076 (Ar-H), 1680 (HC=N), 1468 (C=C), 714 (C-Cl). ^{1}H NMR (DMSO-d_6) δ ppm: 10.42(s, 1H, Ar-OH), 8.60 (d, 1H, Py-H), 8.10 (s, 1H, CH=N), 7.42 (s, 1H, Py-H), 7.40 (d, 1H, Py-H), 7.00 (d, 1H, Ar-H), 7.00 (d, 1H, Ar-H), 6.70 (d, 1H, Ar-H), 3.81 (s, 3H, OCH_3). MS (ESI) *m/z*: 262.69. Anal. Calcd. for $C_{13}H_{11}ClN_2O_2$ (in %): C, 59.44; H, 4.22; N, 10.66. Found: C, 59.41; H, 4.28; N, 10.61.

2.2.12. Synthesis of 4-((4-chloropyridin-2-ylimino)methyl)benzoic acid (3l)

FT-IR (KBr, cm^{-1}): 3184 (O-H), 3075 (Ar-H), 1719 (C=O), 1652 (HC=N), 1458 (C=C), 742 (C-Cl). ^{1}H NMR (DMSO-d_6) δ ppm: 11.76 (s, 1H, COOH), 8.60 (d, 1H, Py-H), 8.20 (d, 2H, Ar-H) , 8.10 (s, 1H, CH=N), 7.80 (d, 2H, Ar-H) , 7.42 (s, 1H, Py-H), 7.40 (d, 1H, Py-H). MS (ESI) m/z: 260.68. Anal. Calcd. for $C_{13}H_9ClN_2O_2$ (in %): C, 59.90; H, 3.48; N, 10.75. Found: C, 59.95; H, 3.42; N, 10.71.

2.2.13. Synthesis of N-(4-(dimethylamino)benzylidene)-4-chloropyridin-2-amine (3m)

FT-IR (KBr, cm^{-1}): 3068 (Ar-H), 1675 (HC=N), 1467 (C=C), 1157 (C-N), 727 (C-Cl). ^{1}H NMR (DMSO-d_6)δ ppm: 8.60 (d, 1H, Py-H), 8.10 (s, 1H, CH=N), 7.42 (s, 1H, Py-H), 7.40 (d, 1H, Py-H), 7.40 (d, 2H, Ar-H), 6.60 (d, 2H, Ar-H), 2.85 (s, 6H, CH_3). MS (ESI) *m/z*: 259.73. Anal. Calcd. for $C_{14}H_{14}ClN_3$ (in %): C, 64.74; H, 5.43; N, 16.18. Found: C, 64.79; H, 5.48; N, 16.12.

2.2.14. Synthesis of 4-chloro-N-(-3-phenylallylidene)pyridin-2-amine (3n)

FT-IR (KBr, cm^{-1}): 3025 (Ar-H), 1680 (HC=N), 1459 (C=C), 734 (C-Cl). ^{1}H NMR (DMSO-d6) δ ppm: 8.60 (d, 1H, Py-H), 7.50 (s, 1H, CH=N), 7.42 (s, 1H, Py-H), 7.40 (d, 1H, Py-H), 7.30 (d, 2H, Ar-H), 7.21-7.14 (m, 3H, Ar-H), 6.60 (d, 1H, CH=CH), 5.60 (d, 1H, CH=CH). MS (ESI) m/z: 242.70. Anal. Calcd. for $C_{14}H_{11}ClN_2$(in %): C, 69.28; H, 4.57; N, 11.54. Found: C, 69.31; H, 4.62; N, 11.62.

2.2.15. Synthesis of N-((1H-indol-2-yl)methylene)-4-chloropyridin-2-amine (3o)

FT-IR (KBr, cm^{-1}): 3073 (Ar-H), 1671 (HC=N), 1468 (C=C), 1264 (C-N), 748 (C-Cl). ^{1}H NMR (DMSO-d_6) δ ppm: 10.10 (s, 1H, Ind N-H), 8.60 (d, 1H, Py-H), 7.60 (d, 1H, Ind-H), 7.50 (s, 1H, CH=N), 7.42 (s,1H,Py-H), 7.40 (d, 1H, Py-H), 7.30 (d,1H, Ind-H), 7.10 (t,1H, Ind-H), 7.00 (t, 1H, Ind-H), 6.40 (s,1H, Ind-H). MS (ESI) *m/z*: 255.7. Anal. Calcd. for $C_{14}H_{10}ClN_3$ (in %): C, 65.76; H, 3.94; N, 16.43. Found: C, 65.71; H, 3.91; N, 16.48.

2.2.16. Synthesis of 4-chloro-N-((furan-2-yl)methylene)pyridin-2-amine (3p)

FT-IR (KBr, cm^{-1}): 3071 (Ar-H), 1674 (HC=N), 1466 (C=C), 729 (C-Cl). ^{1}H NMR (DMSO-d_6) δ ppm: 8.60 (d, 1H, Py-H), 7.50 (s, 1H, CH=N), 7.42 (s, 1H, Py-H), 7.40(d, 1H, Py-H), 7.40 (d, 1H, Furan-H), 6.30 (t, 1H, Furan-H), 6.30 (d, 1H, Furan-H). MS (ESI) m/z: 206.63. Anal. Calcd. for $C_{10}H_7ClN_2O$ (in %): C, 58.13; H, 3.41; N, 13.56. Found: C, 58.17; H, 3.48; N, 13.51.

2.3. Biology

2.3.1. In vitro evaluation of antimicrobial assay

2.3.1.1. Determination of Relative Percentage Inhibition:

Synthesized Compounds were evaluated for antibacterial activity by agar diffusion method, followed by methods[16,17] with slight modifications. For preliminary check and to select the efficient compound against three strains of gram positive bacteria (*Staphylococcus aureus* MTCC-7443, *Bacillus cereus* MTCC-430 and *Bacillus licheniformis* MTCC-2465), Three strains of gram negative bacteria (*Escherichia coli* MTCC-7410, *Acetobacter sp.* MTCC-3245 and *Pseudomonas aeruginosa*) and three fungal species (*Penicillium expansum* MTTC-8241, *Aspergillus flavus* MTCC-9606 and *Pichianomala* MTCC-237). The inoculum was adjusted to approximately 5 x 10^5 CFU/ml with sterile saline solution. All synthesized compounds were dissolved 10 mg/mL in Dimethyl Sulfoxide (DMSO) as a stock solution and loaded different concentration ranges 100 µg, 200 µg, 300 µg 400 µg and 500 µg for different wells. The medium used were nutrient agar for Bacteria and Czapek's-Doxagar media for fungal species. DMSO as negative control showed no inhibition zones and as positive control standard antibiotics Rifampicin & Bacitracin were used for bacteria and Fluconazole for fungus. After 24 h of incubation at 37 °C for bacteria and 72 h of incubation at 28 °C for fungi, the diameter of inhibition zone (mm) was measured and relative percent inhibition was calculated.

2.3.1.2. Determination of Minimum Inhibitory Concentration (MIC):

Based on the previous agar diffusion method, synthesized compounds were identified to have potent antimicrobial activity and hence for

these compounds Minimum Inhibitory Concentrations (MIC) were determined by broth dilution method[18] modified with the addition of 0.15% agar as suggested by Mann *et al.*[19]. From the Stock solutions the compounds were serially diluted ranging 0, to 50 µg per ml (w/v) were prepared in DMSO. One hundred and twenty microliters of each dilution was dispensed into rows of wells in microtitre plates (96×320 µl wells). An equal volume of inoculum adjusted to 5 x 10^5 CFU/ml was added into the appropriate wells and was mixed with the growth medium using the micro-pipette. Bacterial plates were incubated at 37 °C for 48 h and fungal plates were incubated at 28 °C for 72h with the lids on. The growth and MICs values were determined as the lowest concentration that inhibits the growth of tested microorganisms. The experiments were repeated three times with the same parameters to calculate standard deviation.

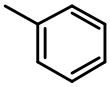
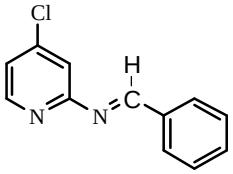
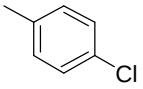
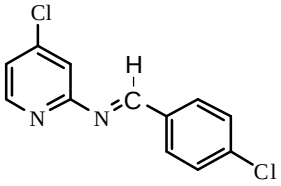
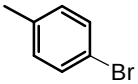
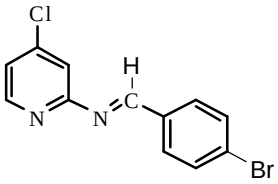
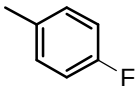
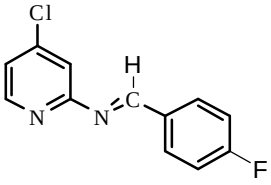
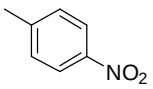
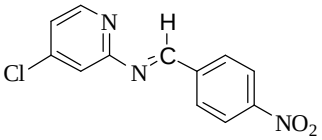
Data of all experiments were performed in triplicates, statistically analyzed and expressed as the mean ± standard deviation of three replicate experiments. The relative percentage inhibition with respect to positive and negative control was calculated by the familiar formula as fallows. Relative percentage inhibition of the compound = [{100 x (a - b)}/(c - b)], Where, 'a'is total inhibition area of the synthesized compounds, 'b' is total inhibition area of the solvent, 'c' is total inhibition area of the standard drug.

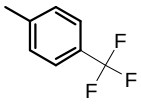
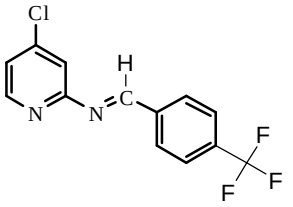
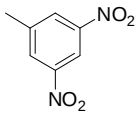
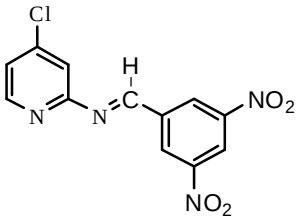
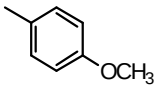
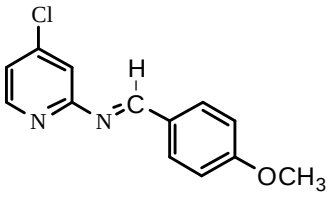
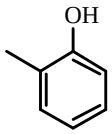
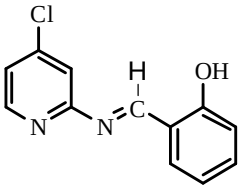
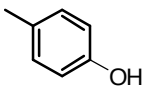
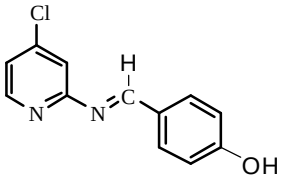
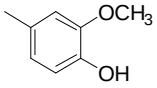
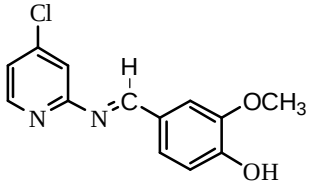
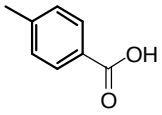
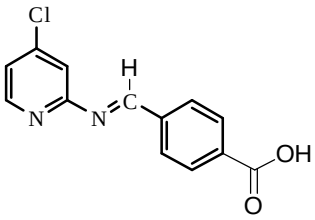
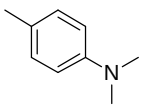
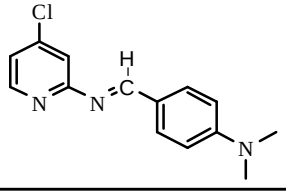
3. RESULTS AND DISCUSSION

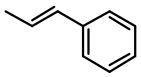
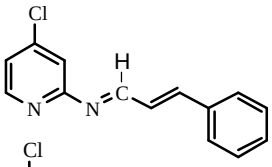
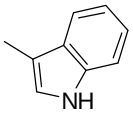
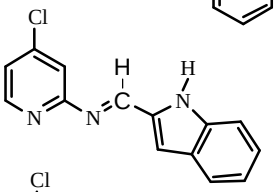
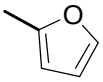
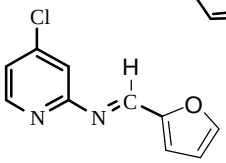
3.1. Chemistry

The chemical structures and physical data of all the synthesized compounds are tabulated in Table 1. Schiff bases of 2-amino-4-chloropyridine derivatives, **3(a-p)** were synthesized from condensation reaction of 2-amino-4-chloropyridine with different aryl aldehydes (Scheme 1). Newly synthesized compounds were confirmed by different spectral studies. The elemental analyses data showed good agreement between the experimentally determined values and the theoretically calculated values within the limits of permissible error. The FT-IR spectra of **3(a-p)** were recorded using KBr pellets in the range of

Table 1. Chemical structure and Physical data of the synthesized compounds 3(a-p)

Compound	R	Structure	UV-visible (nm)	Yield (%)	Solubility
3a			235	85.70	Methanol
3b			340	71.83	Methanol
3c			345	79.59	Methanol
3d			365	76.29	Methanol
3e			360	74.15	Methanol

Compound	R	Structure	UV-visible (nm)	Yield (%)	Solubility
3f			350	81.94	Methanol
3g			360	67.21	Methanol
3h			370	68.78	Methanol
3i			340	68.81	Methanol
3j			365	66.29	Methanol
3k			360	64.98	Methanol
3l			350	80.99	Methanol
3m			360	77.21	Methanol

Compound	R	Structure	UV-visible (nm)	Yield (%)	Solubility
3n			365	76.29	Methanol
3o			360	74.98	Methanol
3p			350	80.99	Methanol

4000 - 400 cm^{-1}. The absence of NH_2 and C=O absorption bands in the IR spectra confirmed that the synthesized compounds. The absorptions around 3019-3081 cm^{-1} in synthesized compounds confirm the aromatic C-H stretching vibrations, and the appearance of a medium to strong absorption bands above 1640-1680 cm^{-1} due to a stretching vibration of the azomethine (C=N) bond formation in synthesized compounds via condensation. The absorption band at 1728 cm^{-1} is due to the C=O stretch in **3l**. New bands appeared at 1124 cm^{-1} and 1178 (**3d & 3f**) corresponding to C-F stretching frequency respectively. The strong bands at 703-771 cm^{-1} are assigned to the C-Cl stretching frequency. The strong bands at 597 cm^{-1} assigned to the C-Br stretch in **3c**. The proton spectral data agree with respect to the number of protons and their chemical shifts with the proposed structures. The proton spectral data of the key intermediate, 2-amino-4-chloropyridine (**1**) shows absorption at d 5.58 ppm (s, 2H, -NH_2). In all the synthesized compounds, **3**(**a-p**) the above absorption disappeared and additional resonances assigned to the –CH=N (d 8.10 – 7.50 ppm) were observed, which confirmed the condensation between the amino group and carbonyl group.

3.2. Biology

The antibacterial activity of compounds, **3**(**a-p**) were evaluated and compared with Rifampicin and Bacitracin as standard drug. Compounds **3b**, **3c**, **3d**, **3f** and **3g** showed good antibacterial properties against four pathogenic bacterial strains compared with other compounds. Compound, **3f** exhibit inhibition in the range of MIC 2-7 µg/ml, other compounds showed moderate antibacterial activity against pathogenic bacterial strains.

Table 2. MIC value of the synthesized compounds 3(a-p)

Compound	MIC								
	Gram +ve Bacteria			Gram –ve Bacteria			Fungus		
	SA	BC	BL	*E Coli*	AS	PA	AF	PE	PA
3a	16±1.0	17±0.8	17±0.9	15±0.7	18±0.8	16±0.6	14±0.5	16±0.6	15±0.5
3b	7±0.4	6±0.3	7±0.8	7±0.7	6±0.7	8±0.8	9±0.9	8±1.0	7±0.8
3c	8±0.9	7±0.9	7±0.8	6±0.5	7±0.6	9±0.6	18±1.0	8±0.1	11±1.0
3d	6±1.5	5±1.8	5±1.9	5±1.6	5±1.5	11±0.9	5±2.0	6±2.3	4±2.6
3e	15±0.9	13±0.8	14±0.7	16±0.8	14±0.9	13±0.8	12±0.9	14±0.7	13±0.7
3f	4±0.9	6±0.8	4±0.9	6±0.7	4±0.6	7±0.4	2±1.0	4±1.0	5±1.0
3g	10±0.7	25±0.9	15±0.8	6±0.4	6±0.6	20±0.8	6±0.4	6±0.5	6±0.5
3h	28±1.1	29±1.2	25±1.2	29±0.9	30±1.0	36±1.0	31±1.8	30±1.7	28±1.7
3i	27±0.8	30±0.9	23±0.7	28±0.6	29±0.7	40±0.5	28±0.7	26±0.7	27±0.8
3j	27±1.1	21±1.1	21±1.0	16±1.0	15±1.2	15±1..2	35±0.9	15±1.2	15±1.2
3k	32±1.9	34±1.9	26±1.6	30±1.7	31±1.7	39±1.7	29±1.5	34±1.4	30±1.5
3l	16±0.9	14±1.0	13±1.1	16±1.1	13±1.0	12±1.0	11±0.8	14±1.1	13±1.1
3m	30±1.0	28±1.0	21±1.0	38±1.1	30±1.1	49±0.6	25±1.0	25±1.0	25±1.0
3n	17±1.0	18±1.1	18±1.1	17±1.2	19±1.2	18±1.0	15±0.4	18±0.8	16±0.9
3o	17±1.2	18±1.3	19±1.1	20±1.2	21±1.2	22±1.2	24±1.5	23±1.4	21±1.3
3p	25±1.0	23±1.0	20±1.0	26±1.0	24±0.8	31±0.7	23±1.1	24±1.1	27±1.1

Table 3. Relative percentage inhibition of the synthesized compounds 3(a-p)

Compound	Relative percentage inhibiton (at 500 µg)								
	Gram +ve Bacteria			Gram –ve Bacteria			Fungus		
	SA	BC	BL	*E Coli*	AS	PA	AF	PE	PA
3a	80±1.1	79±0.7	75±0.9	77±0.7	81±1.2	71±0.4	76±0.5	71±0.9	81±0.5
3b	230±0.8	225±1.1	247±0.5	261±0.7	244±1.0	200±00	258±0.4	272±0.5	268±0.2
3c	189±0.2	189±0.7	214±0.7	200±0.8	289±0.11	130±0.6	165±0.5	246±0.9	225±0.11
3d	79±0.3	324±1.0	204±0.8	130±0.6	361±0.9	165±0.3	204±0.8	269±0.8	246±0.9
3e	142±0.8	134±1.9	141±0.6	112±0.5	115±0.8	101±0.5	120±0.8	175±0.7	182±0.9
3f	192±0.9	400±1.0	246±0.9	225±1.0	576±1.2	61±0.7	555±1.4	344±0.9	400±1.0
3g	169±0.4	156±1.0	165±0.7	147±0.8	200±1.1	100±1.2	130±0.6	184±0.8	205±1.1
3h	37±1.3	36±1.1	37±0.7	39±0.9	36±0.8	36±0.6	38±0.7	39±0.8	41±0.5
3i	49±0.7	48±0.6	46±0.8	48±1.1	47±1.3	46±1.4	59±1.5	59±0.6	49±0.9
3j	45±0.9	41±0.5	43±1.2	44±0.5	42±0.6	41±0.5	43±0.7	46±0.9	41±0.7
3k	34±0.7	34±0.8	32±0.9	33±1.1	32±1.2	31±0.7	34±0.7	31±0.5	39±0.6
3l	108±0.6	102±0.8	118±0.5	98±1.1	101±1.0	95±0.7	103±0.6	119±0.8	123±0.6
3m	22±0.8	24±0.9	28±0.5	27±0.9	26±0.9	29±1.1	26±0.5	25±0.7	29±1.2
3n	71±0.5	65±0.8	64±0.9	68±0.6	69±0.7	64±0.8	72±0.5	70±0.9	69±0.6
3o	59±0.8	51±0.9	56±0.7	51±0.8	54±0.8	59±0.7	61±0.8	65±0.7	61±0.8
3p	55±1.1	50±0.6	51±0.5	69±0.7	51±0.5	56±0.6	59±1.2	60±1.0	59±0.9

All the synthesized compounds **3(a-p)** were also tested against fungus and found that compounds showed varying degree of percentage inhibition. The compounds **3b**, **3c**, **3d**, **3f** and **3g** showed good antifungal activity than other compounds in the series against tested strain. From the results, it is evident that most of the compounds are moderately active. The antimicrobial activity results of synthesized compounds were compared with standard drugs as depicted in Table 2. Relative percentage inhibition of the compounds were depicted in Table 3.

3.3. SAR Studies

In the present study, different electron withdrawing and electron donating groups attached to phenyl ring as substituent were linkage to azomethine group. The close survey of antimicrobial efficacy indicated that the inhibition values of all the compounds exhibited a varied rang of antibacterial and antifungal activities against all the tested microbial strains. The electron withdrawing group of phenyl ring in **3f** (MIC 2-7 µg/ml) shows more potency against microbes. This indicates the positional requirement of fluoro group of phenyl ring for enhanced activity. The compound **3d** (MIC 5-11 µg/ml), **3b** (MIC 6-11 µg/ml), **3c** (MIC 6-18 µg/ml) amd **3g** (MIC 6-25 µg/ml) containing fluoro, chloro, bromo and dinitro groups showed stronger antibacterial activity against all tested pathogens. Compound **3a** (MIC 14-18 µg/ml) and **3n** (MIC 16-18 µg/ml) revealed relatively moderate antimicrobial activity due to the unsubstituted phenyl ring. Compound **3l** and **3e** containing carboxylic and nitro group shows moderate activity may be the electron withdrawing nature. However **3j** (MIC 15-35 µg/ml) showed better activity compared to **3i** (MIC 23-40 µg/ml) probably due to the presence in para position of hydroxy group in **3j**. The elctron donating methoxy group in **3h** (MIC 25-36 µg/ml) and **3k** (MIC 26-39 µg/ml) showed lower antimicrobial activity. The indole group in **3o** (MIC 17-24 µg/ml) and furan group in **3p** (MIC 20-31 µg/ml) produces mild activity.

4. CONCLUSION

In conclusion, a series of new 2-amino-4-chloropyridine derivatives **3(a-p)** were synthesized in good yield, characterized by different spectral studies and their antimicrobial activities were determined against clinically important pathogens. Compounds **3b**, **3c**, **3d**, **3f** and **3g** demonstrated good inhibition against bacterial and fungal strains tested. Therefore, we are found some important details about antimicrobial property of pyridine compounds, these compounds could be beneficial for antimicrobial drug synthesis.

5. ACKNOWLEDGEMENTS

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