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Synthesis and *in vitro* biological activity of 6-chloro-pyridin-2-yl-amine derivatives

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ABSTRACT

*6-Chloro-pyridin-2-yl-amine derivatives **3(a-h)** were synthesized by using 2-amino-6-chloropyridine (**1**) as starting material. The newly synthesized compounds were characterized by elemental analyses, ^{1}H NMR and mass spectral studies. The synthesized compounds were screened for antibacterial and antifungal activities. All the synthesized compounds showed the antibacterial activity against four pathogenic strains of Bacillus subtilis (MTCC 121), Staphylococcus aureus (MTCC 7443), Xanthomonas campestris (MTCC 7908), Escherichia coli (MTCC 7410) and antifungal activity against pathogenic strains of Fusarium oxysporum (MTCC 2480). All compounds showed good to moderate antimicrobial activity. However, compounds **3a**, **3f** and **3h** showed potent antibacterial activity against pathogenic strains used in the study.*

Keywords: 2-Amino-6-chloropyridine, Aldehyde, Antibacterial activity, Antifungal activity

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INTRODUCTION

Throughout history, there has been a continual battle between humans and the multitude of microorganisms that cause infection and disease. Diseases caused by microbial infection are a serious menace to the health of human beings, and often have connection to some other diseases whenever the body system gets debilitated. During the 20th century, vaccines for bacterial toxins and many other common acute viral infections were developed and made widely available. In recent years, considerable interest has been devoted to find a new methodology for the synthesis of pyridine building blocks. Number of different classes of antibacterial [1, 2] and antifungal agents [3] have been discovered. The incidence of fungal infections has increased significantly in the past two decades [4]. Some antifungal drugs could also help to kill fungi which infect the human body. Many of the drugs currently available have undesirable side effects and might be toxic.

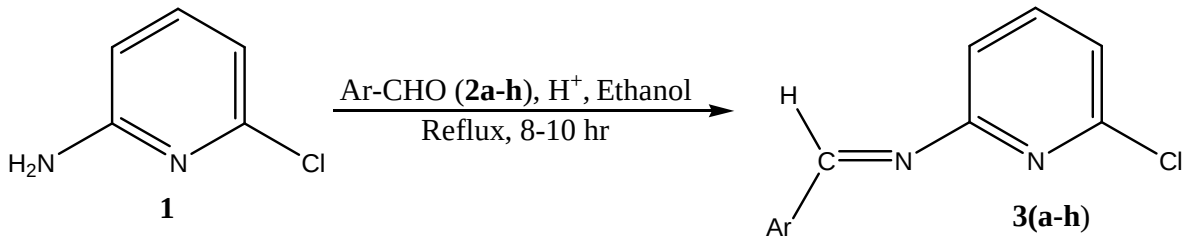
Chemical modifications of existing antibacterial agents in order to generate novel molecules with better therapeutic properties are necessary because of the emergence of multidrug resistant bacteria [5]. Pyridine, a heterocyclic nucleus, played a pivotal role in the development of different medicinal agents and in the field of agrochemicals. Pyridine derivatives have shown potent pharmacological properties like antimicrobial [6-8], insecticidal [9] etc. In connection with such studies, the present paper reporting for the first time on the synthesis of 6-chloro-pyridin-2-yl-amine derivatives **3(a-h)** which are formed during the reaction of 2-amino-6-chloropyridine **(1)** with different aldehydes, **2(a-h)**. These synthesized compounds were characterized by elemental analyses, FT-IR, ^{1}H NMR and mass studies. Antibacterial and antifungal activities were reported and structural activity relationship was also discussed in this paper.

MATERIALS AND METHODS

Melting range was determined by Veego Melting Point VMP III apparatus. Elemental analyses were recorded on VarioMICRO superuser V1.3.2 Elementar. ^{1}H NMR spectra were recorded on Bruker DRX -500 spectrometer at 400 MHz using DMSO-d_6 as solvent and TMS as an internal standard. Mass spectral data were obtained by LC/MSD Trap XCT. Silica gel column chromatography was performed using Merck 7734 silica gel (60–120 mesh) and Merck-made TLC plates. All solvents and reagents were purchased from Sigma Aldrich Chemicals Pvt Ltd.

General procedure for the synthesis of 6-chloro-pyridin-2-yl-amine derivatives 3(a-h)

Equimolar concentrations of aryl aldehydes (**2a**) and 2-amino-6-chloropyridine (**1**) and 2-3 drops of concentrated sulfuric acid was added. The reaction mixture was refluxed for 8-10 hr at room temperature in absolute ethanol (25 ml). The progress of the reaction was monitored by TLC until the reaction was complete. It was cooled to 0 °C, and the precipitate was filtered, washed with diethyl ether and the residue was recrystallized from ethanol. 2-Amino-6-chloropyridine derivatives **3(a-h)** was synthesized by the method summarized in Scheme 1.



Scheme 1

Synthesis of 4-[(6-chloro-pyridin-2-ylimino)-methyl]-2-methoxy-phenol (3a)

The general experimental procedure described above afforded **3a**, and the product obtained from 2-amino-6-chloropyridine (**1**) (0.1062 g) and 4-hydroxy-3-methoxy-benzaldehyde (**2a**) (0.1205 g). Yield: 78 %. ^{1}H-NMR (400 MHz, DMSO-d_6): 11.3 (s, br, 1H, OH), 8.13 (s, 1H, CH), 7.81 (t, 1H, Py-H), 7.60-7.53 (d, 2H, Py-H), 7.45 (s, 1H, Ar-H), 7.21 (d, 1H, Ar-H), 7.18 (d, 1H, Ar-H), 3.75 (s, 3H, OCH_3). MS (ESI) *m/z*: 263.7. Anal. Calcd. for $C_{13}H_{11}ClN_2O_2$ (in %): C, 59.44; H, 4.22; N, 10.66. Found: C, 59.21; H, 4.32; N, 10.75.

Synthesis of (6-chloro-pyridin-2-yl)-(1H-indol-3-ylmethylene)-amine (3b)

The general experimental procedure described above afforded **3b**, and the product obtained from 2-amino-6-chloropyridine (**1**) (0.2014 g) and indole-3-carboxaldehyde (**2b**) (0.2210 g). Yield: 65 %. ^{1}H-NMR (400 MHz, DMSO-d_6): 10.0 (s, 1H, NH), 8.10 (s, 1H, CH), 8.01 (t, 1H, Py-H), 7.65-7.58 (d, 2H, Py-H), 7.40 (s, 1H), 7.35-7.26 (d, 2H, Ar-H), 7.18-7.05 (m, 2H, Ar-H). MS (ESI) *m/z*: 256.7. Anal. Calcd. for $C_{14}H_{10}ClN_3$ (in %): C, 65.76; H, 3.94; N, 16.43. Found: C, 65.94; H, 4.02; N, 16.69.

Synthesis of (6-chloro-pyridin-2-yl)-(4-dimethylamino-benzylidene)-amine (3c)

The general experimental procedure described above afforded **3c**, and the product obtained from 2-amino-6-chloropyridine (**1**) (0.2040 g) and 4-dimethylamino-benzaldehyde (**2c**) (0.2328 g). Yield: 75 %. ^{1}H-NMR (400 MHz, DMSO-d_6): 8.15 (s, 1H, CH), 7.83 (t, 1H, Py-H), 7.62-7.54 (d, 2H, Py-H), 7.40 (d, 2H, Ar-H), 6.74 (d, 2H, Ar-H), 2.90 (s, 6H, CH_3). MS (ESI) *m/z*: 260.1. Anal. Calcd. for $C_{14}H_{14}ClN_3$ (in %): C, 64.74; H, 5.43; N, 16.18. Found: C, 64.53; H, 5.62; N, 16.37.

Synthesis of (4-chloro-benzylidene)-(6-chloro-pyridin-2-yl)-amine (3d)

The general experimental procedure described above afforded **3d**, and the product obtained from 2-amino-6-chloropyridine (**1**) (0.2021 g) and 4-chloro-benzaldehyde (**2d**) (0.2189 g). Yield: 72 %. ^{1}H-NMR (400 MHz, DMSO-d_6): 8.20 (s, 1H, CH), 8.04 (t, 1H, Py-H), 7.70-7.62 (d, 2H, Py-H), 7.52 (d, 2H, Ar-H), 7.45 (d, 2H, Ar-H). MS (ESI) *m/z*: 252.1. Anal. Calcd. for $C_{12}H_8Cl_2N_2$ (in %): C, 57.40; H, 3.21; N, 11.16. Found: C, 57.32; H, 3.10; N, 11.27.

Synthesis of (6-chloro-pyridin-2-yl)-(4-nitro-benzylidene)-amine (3e)

The general experimental procedure described above afforded **3e**, and the product obtained from 2-amino-6-chloropyridine (**1**) (0.2012 g) and 4-nitro-benzaldehyde (**2e**) (0.2362 g). Yield: 76 %. ^{1}H-NMR (400 MHz, DMSO-

d_6): 8.16 (s, 1H, CH), 8.01 (t, 1H, Py-H), 7.95 (d, 2H, Ar-H), 7.83-7.72 (d, 2H, Ar-H), 7.60 (d, 2H, Py-H). MS (ESI) *m/z*: 262.5. Anal. Calcd. for $C_{12}H_8ClN_3O_2$ (in %): C, 55.08; H, 3.08; N, 16.06. Found: C, 55.13; H, 2.97; N, 15.98.

Synthesis of (6-chloro-pyridin-2-yl)-(3,5-dinitro-benzylidene)-amine (3f)

The general experimental procedure described above afforded **3f**, and the product obtained from 2-amino-6-chloropyridine (**1**) (0.2012 g) and 3,5-dinitro-benzaldehyde (**2f**) (0.3591 g). Yield: 78 %. ^{1}H-NMR (400 MHz, DMSO-d_6): 9.10 (s, 1H, Ar-H), 8.94 (s, 1H, Ar-H), 8.90 (s, 1H, Ar-H), 8.10 (s, 1H, CH), 8.00 (t, 1H, Py-H), 7.95 (d, 2H, Ar-H). MS (ESI) *m/z*: 307.6. Anal. Calcd. for $C_{12}H_7ClN_4O_4$ (in %): C, 47.00; H, 2.30; N, 18.27. Found: C, 46.96; H, 2.37; N, 18.19.

Synthesis of benzylidene-(6-chloro-pyridin-2-yl)-amine (3g)

The general experimental procedure described above afforded **3g**, and the product obtained from 2-amino-6-chloropyridine (**1**) (0.2080 g) and benzaldehyde (**2g**) (0.1700 g). Yield: 68 %. ^{1}H-NMR (400 MHz, DMSO-d_6): 8.14 (s, 1H, CH), 8.05 (t, 1H, Py-H), 7.90 (d, 2H, Ar-H), 7.65 (d, 2H, Ar-H), 7.40 (m, 3H, Ar-H). MS (ESI) *m/z*: 217.7. Anal. Calcd. for $C_{12}H_9ClN_2$ (in %): C, 66.52; H, 4.19; N, 12.93. Found: C, 66.35; H, 4.21; N, 12.78.

Synthesis of (6-chloro-pyridin-2-yl)-(4-methylsulfanyl-benzylidene)-amine (3h)

The general experimental procedure described above afforded **3h**, and the product obtained from 2-amino-6-chloropyridine (**1**) (0.2020 g) and 4-methylsulfanyl-benzaldehyde (**2h**) (0.2200 g). Yield: 75 %. ^{1}H-NMR (400 MHz, DMSO-d_6): 8.12 (s, 1H, CH), 8.00 (t, 1H, Py-H), 7.84 (d, 2H, Py-H), 7.65 (d, 2H, Ar-H), 7.25 (d, 2H, Ar-H), 2.50 (s, 3H, CH_3). MS (ESI) *m/z*: 263.1. Anal. Calcd. for $C_{13}H_{11}ClN_2S$ (in %): C, 59.42; H, 4.22; N, 10.66. Found: C, 59.58; H, 4.40; N, 10.73.

Antibacterial activity

Antibacterial activity of the synthesized compounds was determined against Gram-positive bacteria (*Bacillus subtilis* MTCC 121, *Staphylococcus aureus* MTCC 7443) and Gram-negative bacteria (*Xanthomonas campestris* MTCC 7908 and *Escherichia coli* MTCC 7410) in DMF by disc diffusion method on nutrient agar medium [10]. The sterile medium (Nutrient Agar Medium, 15 ml) in each petri-plates was uniformly smeared with cultures of Gram positive and Gram negative bacteria. Sterile discs of 10 mm diameter (Hi-Media) was placed in the petriplates, to which 50 µl (1 mg/ml i.e., 50 µg/disc) of the different synthesized compounds were added. The treatments also included 50 µl of DMF as negative, bacteriomycin and gentamycin as positive control for comparison. For each treatment, three replicates were maintained. The plates were incubated at 37 ± 2 ºC for 24 h and the zone of inhibition was determined.

Antifungal activity

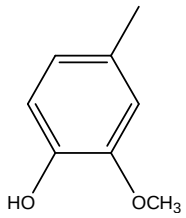
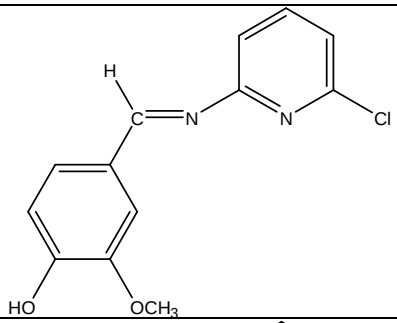
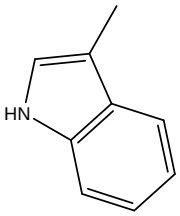
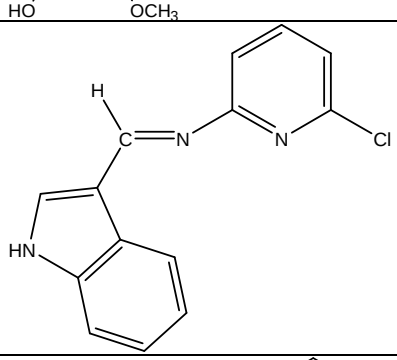
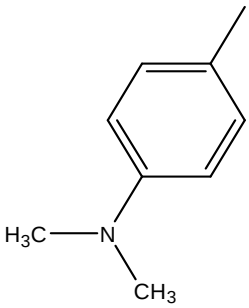
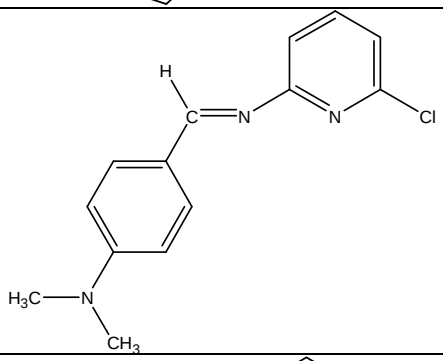
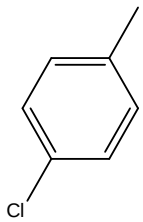
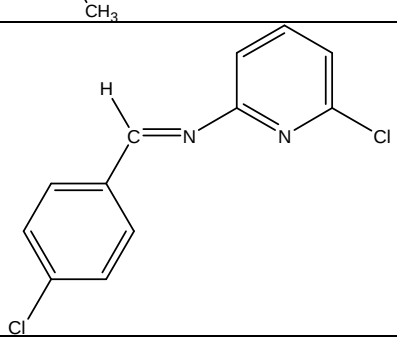
The synthesized compounds were screened for their antifungal activity against *Fusarium oxysporum* MTCC 2480 in DMF by poisoned food technique [11]. Potato Dextrose Agar (PDA) media was prepared and about 15 ml of PDA was poured into each petriplate and allowed to solidify. 5 mm disc of seven days old culture of the test fungi was placed at the center of the petriplates and incubated at 26 ± 2 °C for 7 days. After incubation the percentage inhibition was measured and three replicates were maintained for each treatment. Nystatin was used as standard. All the synthesized compounds were tested (at the dosage of 500 µl of the novel compounds/petriplate, where concentration was 0.1 mg/ml) by poisoned food technique.

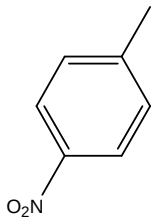
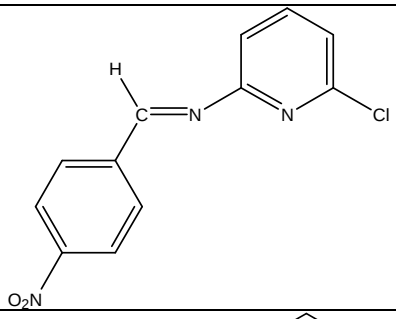
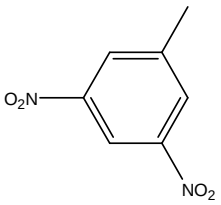
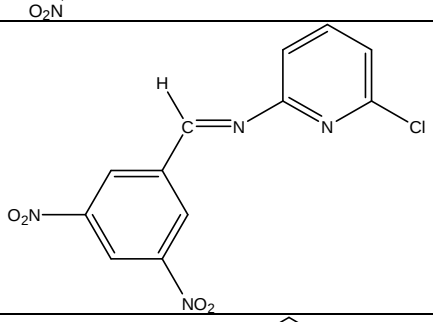
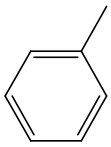
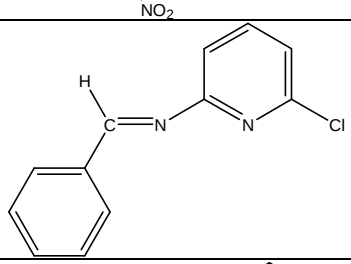
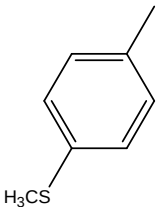
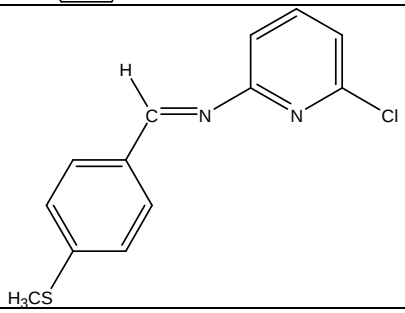
RESULTS AND DISCUSSION

Chemistry

Formation of 6-chloro-pyridin-2-yl-amine derivatives **3(a-h)** was confirmed by recording their elemental analyses, ^{1}H NMR and mass spectra. The ^{1}H NMR spectrum of **3a** and **3f** showed singlet in the region of δ, 8.13 and 8.10, respectively. The mass spectra of **3a** showed molecular ion peak at m/z 263.7, which is in agreement with the molecular formula $C_{13}H_{11}ClN_2O_2$. The elemental analyses data showed good agreement between the experimentally determined values and the theoretically calculated values within ± 0.4 %. The chemical structures and melting point of all the synthesized compounds are tabulated in Table 1.

Table 1: Chemical structure and melting range of compounds 3(a-h)

Compound	R	Structure	mp (°C)
3a			140-144
3b			102-104
3c			-
3d			-

3e			-
3f			-
3g			88-91
3h			116-119

In vitro antimicrobial activity

The investigation of antibacterial screening data revealed that all tested compounds showed antibacterial activity against four pathogenic bacterial strains. Among the series **3(a-h)**, compound **3a**, **3f** and **3h** exhibited a good antibacterial activity against Gram positive and Gram negative bacteria. Compounds **3b**, **3c**, **3d**, **3e** and **3g** showed moderate zone of inhibition against tested bacterial strains in comparison to standard drugs. The results were compared with standard drugs bacteriomycin and gentamycin as depicted in Table 2.

The *in vitro* antifungal activity of the synthesized compounds **3(a-h)** was studied against *Fusarium oxysporum*. The results were compared with the standard drug nystatin as in Table 2. Compounds **3a**, **3f** and **3h** showed good antifungal activity when compared with other compounds in the series against *F. oxysporum*. Other compounds in the series were found to be moderately active against tested fungal strain.

Table 2: *In vitro* antimicrobial activity of compounds 3(a-h)

Compound	Zone of inhibition in diameter (mm)				% Inhibition
	B. subtilis	*S. aureus*	*X. campestris*	*E. coli*	*F. oxysporum*
3a	27	26	28	27	87.3
3b	18	17	17	16	63.0
3c	16	15	16	15	62.2
3d	20	18	19	18	64.0
3e	23	20	21	19	65.5
3f	26	25	26	24	84.6
3g	14	15	16	15	60.0
3h	26	24	25	23	82.9
Bacteriomycin	-	-	34	-	-
Gentamycin	32	30	-	33	-
Nystatin	-	-	-	-	100

The close survey of antimicrobial efficacy indicated that the inhibition values of all the compounds exhibited a varied range (14 - 28 mm and 60.0 % - 87.3 %) of antibacterial and antifungal activities against all the tested microbial strains. The methoxy and phenol groups in **3a** produce good activity probably when compared to other compounds in the series. The electron withdrawing nitro group in **3f** produces good antimicrobial activity. The above studies reveal that, the nature of the linkage (substituent on aromatic ring) influences the antimicrobial activity. It is important to note from the biological data that compound **3h** having thio group showed good antimicrobial activity, whereas different groups in compounds **3b**, **3c**, **3d**, **3e** and **3g** exhibited moderate activity against all the tested microbial strains. The compounds **3a-h** showed antimicrobial activity in the order: **3a** > **3f** > **3h** > **3e** > **3d > 3b > 3c** > **3g** against tested microbial strains. However, the activities of the tested compounds are much less than those of standard antibacterial agents used.

CONCLUSION

In conclusion, series of novel 6-chloro-pyridin-2-yl-amine derivatives **3(a-h)** were synthesized in good yield, characterized by different spectral studies and their antimicrobial activity have been evaluated. Overall observation from the results of antimicrobial activity reveals that most of the synthesized compounds are found to have moderate activity against corresponding species. Compounds **3a**, **3f** and **3h** produced significant changes in activity against tested microbial strain. Therefore, this work presents a novel class of potent, wide-spectrum antimicrobial activity of the compounds.

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REFERENCES

[1] Appelbaum, P. C.; Hunter, P. A. *Int. J. Antimicrob. Agents* **2000**, 16, 5-15.
[2] Ball, P. *J. Antimicrob. Chemother.* **2000**, 46, 17-24.
[3] Andriole, V. T. *J. Antimicrob. Chemother.* **1999**, 44, 151-162.
[4] Enoch, D. A.; Ludlam, H. A.; Brown, N. M. *J. Med. Microbiol.* **2006**, 55, 809-818.
[5] Chu, D. T. W.; Plattner, J. J.; Katz, L. *J. Med. Chem.* **1996**, 39, 3853-3874.
[6] Patrick, G. L.; Kinsman, O. S.; *Eur. J. Med. Chem.* **1996**, 31, 615-624.
[7] Hishmat, O. H.; Abdel, Galil, F. M.; Farrag, D. S. *Pharmazie* **1990**, 45, 793-795.
[8] Doshi, R.; Kagthara, P.; Parekh, H. *Indian J. Chem.* **1999**, 38, 348-352.
[9] Holla, B. S.; Prasanna, C. S.; Poojary, B.; Rao, K. S.; Shridhara, K.; Bhatt, U. G. *Indian J. Chem.* **2004**, 43B, 864-869.
[10] Bauer, A. W.; Kirby, W. M.; Sherris, J. C.; Turck, M. *Am. J. Clin. Pathol.* **1966**, 45, 493-496.
[11] Satish, S.; Mohana, D. C.; Raghavendra, M. P.; Raveesha, K. A. *J. Agri. Techn.* **2007**, 3, 109-119.