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A Facile, Catalyst-free Green Synthesis for Schiff's Bases in Aqueous Medium under Ultrasonic Irradiation Conditions and Their Antimicrobial Activity

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ABSTRACT

A libraries of Schiff's base derivatives (12 compounds) such as N'-(substituted- benzylidene)nicotinohydrazides ***3(a-l)*** *were synthesized by reacting nicotinohydrazide* ***(1)*** *with various aryl/heterocyclic aldehydes containing pharmacological active functional groups* ***2(a-l)*** *under conventional conditions in ethanol as well as ultrasonic conditions in aqueous medium without using any catalyst. It was observed that the reaction in ultrasonic irradiation afforded excellent yields in a simple work-up procedure with in short reaction time and the reaction is an environmentally benign process. The compounds were screened for their antibacterial and antifungal activities at two different concentrations 100, 250 µg/disc as well as minimum inhibitory concentrations against the growth of microbial stains. Most of the synthesized compounds exhibited potential activity against tested microorganisms and low MIC values.*

Key words: Nicotinohydrazide, Aqueous medium, Ultrasonic irradiation, Antibacterial activity, Antifungal activity, MIC values.

INTRODUCTION

The chemistry of heterocyclic compounds has been an interesting field of study for a long time, which is due to these molecules are commonly used scaffolds in various fields such as medicinal, agricultural, pharmaceutical and provide potent and selective drugs. Nitrogen heterocyclic compounds mostly pyridine derivatives are prevalent in numerous natural products, functional materials [1], medicinal chemistry and they have been intensively used as scaffolds for drug development and chemistry of biological systems [2]. Pyridine derivatives containing multi-functional groups such as streptonigrin, lavendamycin are reported as anticancer drugs and cerivastatin is reported as HMG-CoA enzyme inhibitor [2], as well as the derivatives of pyridine exhibiting wide range of biologically active agents such as anti-inflommatory [3, 4], antimicrobial [5] and antituberculosis [6].

The reaction of primary amines with carbonyl compounds to give imines-also called Schiff's bases or azomethines is a reaction which is well known [7]. In nature it serves to interconvert amino acids and α-ketoacids into one another with the help of vitamin B_6 (pyridoxamine, pyridoxal as coenzyme in transaminase [8] and also which accumulate great importance as intermediate for designing various biologically active or intermediate heterocyclic compounds in organic and medicinal chemistry for numerous purposes since 1800s. The Schiff's base derivatives have been extensively investigated for more than a century and employed in different aspects including biological

studies, magneto chemistry [9], non-linear optics [10], photo physical studies [11], catalysis [12], materials chemistry [13], chemical analysis [14], absorption and transport of oxygen [15]. Schiff's bases have a wide variety of biological properties including their antibacterial [16-22], antifungal [17-22], antitumour [23-25], antioxidant [26,27], anti-inflammatory [28], antihypertensive [29,30], anti HIV [31], antiproliferative [32], anticonvulsant [33], herbicidal, insecticidal and anthelmintic [34], anticancer activities and they are used as pigments and dyes, catalysts, intermediates in organic synthesis, and as polymer stabilizers [35]. Recently, the researchers have been focused to design Schiff's base metalized complexes as better anticancer drugs and chemotherapeutic agents, due to the Schiff's base metal complexes have potent binding nature with DNA [36]. QSAR study was proved the efficient biological activity [37].

Schiff's base derivatives in biological and chemical processes, prompted the researchers towards the design of novel aryl/heterocyclic Schiff's base derivatives and the development of the new technologies for an environmentally benign processes (green chemistry) [38], which are both economically and technologically feasible [39,40]. An important area of green chemistry deals with aqueous medium based reactions, solvent-free, solid state reaction may be carried out by using the reactants alone and using of an environmentally benign solvents. As part of our research programme, we herein report the synthesis of N'-(substituted- benzylidene)nicotinohydrazide Schiff's base derivatives **3(a-l)** from nicotinohydrazide **(1)** and substituted aldehydes **2(a-l)** using a facile, green protocol in aqueous medium without using any catalyst under ultrasonic irradiation. The title compounds were screened for their antimicrobial activity. All the compounds showed moderate to potent activity against tested microorganisms.

MATERIALS AND METHODS

Melting points were determined using Guna melting point apparatus in open capillaries and are uncorrected. IR spectra (in KBr pellets) were recorded on JASCO-5300, FT-IR spectrometer. ^{1}H and ^{13}CNMR spectra were recorded on a BRUKER 400 MHz Spectrometer using 5mm tubes at 400 MHz and 100.6 MHz respectively. Chemical shift values are given in δ scale. Mass spectra were recorded on Mass instrument using (M^{+}+H) mode and Perkin-Elmer 240C (Flash EA-1112 series) was used for C, H, N elemental analysis. The progress of the reactions was checked by thin layer chromatography (TLC) on silica gel coated aluminum sheets. The chemicals, reagents and solvents were procured from Aldrich, USA and from SD fine, Bombay were used without further purification.

General procedure for synthesis of Schiff's base derivatives of nicotinohydrazide 3(a-l) under conventional conditions:

Nicotinohydrazide (**1**) (274 mg, 2 mmol) and m-nitro benzaldehyde (**2g**) (304 mg, 2 mmol) were taken in round bottom flask containing 10 mL of dry ethanol. The reaction mixture was stirred at 60 ^{o}C for 3 h. The progress of the reaction was monitored by TLC at regular intervals. After the completion of the reaction, the crude mixture was cooled and poured in ice cold water, resulted solid was collected by filtration. The obtained product was recrystallized from ethanol to obtain 84% yield of N'-(3-nitrobenzylidene)nicotinohydrazide (**3g**). All the title compounds were synthesized by using same procedure as shown in **Table 1**. The Schiff's base derivatives **3(a-l)** were confirmed by spectroscopic data and physical methods and some of the products were consistent with previously reported data [44].

General procedure for the synthesis of Schiff's bases of nicotinohydrazide 3(a-p) under catalyst-free ultrasonic irradiation conditions:

In this typical synthetic protocol, nicotinohydrazide (**1**) (274 mg, 2 mmol), m-nitro benzaldehyde (**2g**) (304 mg, 2 mmol) and 15 mL of water were taken in 50 mL round bottom flask and subjected to ultrasonic irradiation at 60 ^{o}C for 14 min. The progress of the reaction was monitored by TLC for each 5 min. After completion of the reaction, the solid product was filtered off and purified by recrystallization from ethanol to afford 92% yield of N'-(3-nitrobenzylidene)nicotinohydrazide (**3g**). All the remaining title compounds were synthesized by adopting same procedure as shown in **Table 1**. The Schiff's base derivatives **3(a-l)** were confirmed by spectroscopic data and physical methods and some of the products were consistent with previously reported data [44].

N'-(3-Hydroxybenzylidene)nicotinohydrazide (3a). White solid, Yield 90%, Mp 202-204 ^{o}C; IR (ν_{max}, cm^{-1}): 3427 (-OH, str), 3351 (-NH, str), 3058 (=C-H, str), 1661 (-C=O, str), 1592 (-C=N, str), 1438 (-C-N, str). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 6.84 (dd, J = 6 Hz, 2 Hz, 1H, Ar-H), 7.11-7.29 (m, 3H, Ar-H), 7.57 (q, J= 5.6, 1H, Ar-H), 8.24 (d, J = 7.6 Hz, 1H, Ar-H), 8.35 (s, 1H, -CH=N), 8.76 (d, J = 8.8 Hz, 1H, Ar-H), 9.07 (s, 1H, pyridine-N=CH), 9.68 (s, 1H, Ar-OH), 11.98 (s, 1H, -CO-NH). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ_C 112.6 (C_{16}), 117.6 (C_{14}), 118.9 (C_{12}),

123.6 (C_1), 129.2 (C_{13}), 129.9 (C_5), 135.3 (C_{11}), 135.4 (C_6), 148.4 (C_{10}), 148.5 (C_2), 152.3 (C_4), 157.7 (C_{15}), 161.6 (C_7). MS, *m/z*: 242.13 (M+H); Anal. Calcd. for $C_{13}H_{11}N_3O_2$: C, 64.72; H, 4.60; N, 17.42; O, 13.26; found: C, 64.60; H, 4.53; N, 17.38.

N'-(4-Hydroxybenzylidene)nicotinohydrazide (3b). White solid, Yield 85%, Mp 231-232 ^oC. IR (ν_{max}, cm^{-1}): 3433 (-OH, str), 3354 (-NH, str), 3059 (=C-H, str), 1661 (-C=O, str), 1590 (-C=N, str), 1441 (-C-N, str). ^1H NMR (400 MHz, DMSO-d_6): δ_H 6.86 (d, J = 8 Hz, 2 Hz, 2H, Ar-H), 7.35-7.38 (m, 1H, Ar-H), 7.59 (d, J= 7.6 Hz, 2H, Ar-H), 8.15 (m, 2H, Ar-H), 8.38 (s, 1H, -CH=N), 9.1 (s, 1H, pyridine-N=CH), 9.65 (s, 1H, Ar-OH), 12.05 (s, 1H, -CO-NH). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ_C 114.6 ($C_{13,}C_{15}$), 123.8 (C_1), 125.4 (C_{11}), 130.1 (C_{12} C_{16}), 130.9 (C_5), 134.7 (C_6), 148.1 (C_2), 149.5 (C_4), 152.1 (C_{10}), 159.2 (C_{14}), 162.5 (C_7). MS *m/z*: 242.03 (M+H); Anal. Calcd. for $C_{13}H_{11}N_3O_2$: C, 64.72; H, 4.60; N, 17.42; O, 13.26; found: C, 64.61; H, 4.50; N, 17.33.

N'-(2-Hydroxybenzylidene)nicotinohydrazide (3c). White solid, Yield 87%, Mp 172-174 ^oC. IR (ν_{max}, cm^{-1}): 3431 (-OH, str), 3352 (-NH, str), 3059 (=C-H, str), 1660 (-C=O, str), 1585 (-C=N, str), 1439 (-C-N, str). ^1H NMR (400 MHz, DMSO-d_6): δ_H 7.41-7.44 (m, 5H, Ar-H), 8.42 (s, 1H, -CH=N), 8.51-8.53 (m, 2H, Ar-H), 9.05 (s, 1H, pyridine-N=CH), 10.6 (s, 1H, Ar-OH), 12.15 (s, 1H, -CO-NH). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ_C 115.3 (C_{15}), 118.9 (C_{11}), 120.6 (C_{13}), 123.9 (C_1), 126.1 (C_{12}), 131.0 (C_5), 136.2 (C_{14}), 137.4 (C_6), 146.8 (C_2), 149.1 (C_4), 149.6 (C_{10}), 154.5 (C_{16}), 161.4 (C_7). MS *m/z*: 242.27 (M+H); Anal. Calcd. for $C_{13}H_{11}N_3O_2$: C: 64.72, H, 4.60; N, 17.42; O, 13.26; found: C, 64.63; H, 4.51; N, 17.36.

N'-(4-Fluorobenzylidene)nicotinohydrazide (3d). Light yellow solid, Yield 92%, Mp 182-183 ^oC. IR (ν_{max}, cm^{-1}): 3348 (-NH, str), 3062 (=C-H, str), 1651 (-C=O, str), 1589 (-C=N, str), 1440 (-C-N, str), 1120 (Ar-F, str). ^1H NMR (400 MHz, DMSO-d_6): δ_H 7.41 (d, J = 8 Hz, 2H, Ar-H), 7.58-7.60 (m, 4H, Ar-H), 8.46 (s, 1H, -CH=N), 8.57 (m, 1H, Ar-H), 9.1 (s, 1H, pyridine-N=CH), 11.89 (s, 1H, -CO-NH). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ_C 111.2 ($C_{13,}C_{15}$), 124.7 (C_1), 129.4 (C_{11}), 130.4 (C_5), 131.3 (C_{12} C_{16}), 131.8 (C_6), 146.4 (C_2), 148.5 (C_4), 149.9 (C_{10}), 160.6 (C_7), 164.8 (C_{14}). MS *m/z*: 243.18 (M+H); Anal. Calcd. for $C_{13}H_{10}FN_3O$: C: 64.19, H, 4.14; F, 7.81; N, 17.28; O, 6.58; found: C, 64.11; H, 4.05; N, 17.23.

N'-(4-Chlorobenzylidene)nicotinohydrazide (3e). Brown solid, Yield 89%, Mp 227-229 ^oC. IR (ν_{max}, cm^{-1}): 3343 (-NH, str), 3059 (=C-H, str), 1643 (-C=O, str), 1584 (-C=N, str), 1437 (-C-N, str), 1066 (-C-Cl, str). ^1H NMR (400 MHz, DMSO-d_6): δ_H 7.56 (d, J = 8.4 Hz, 3H, Ar-H), 7.77 (d, J= 8.0, 2H, Ar-H), 8.25 (d, J = 7.2 Hz, 1H, Ar-H), 8.44 (s, 1H, Ar-H), 8.77 (s, 1H, -CH=N), 9.07 (s, 1H, pyridine-N=CH), 12.11 (s, 1H, -CO-NH). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ_C 123.6 (C_1), 128.8 ($C_{13,}C_{15}$), 128.9 ($C_{12,}C_{16}$), 129.1 (C_5), 133.1 (C_{11}), 134.69 (C_6), 135.4 (C_{14}), 147.0 (C_{10}), 148.6 (C_2), 152.3 (C_4), 161.7 (C_7). MS *m/z*: 259.54 (M+H); Anal. Calcd. for $C_{13}H_{10}ClN_3O$: C, 60.12; H, 3.88; Cl, 13.65; N, 16.18; O, 6.16; found: C, 60.04; H, 3.78; N, 16.15.

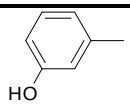
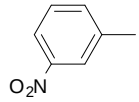
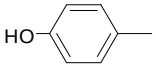
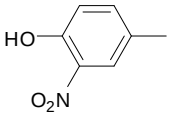
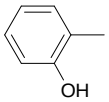
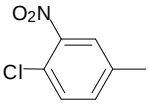
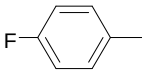
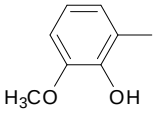
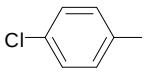
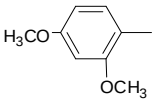
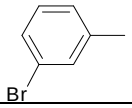
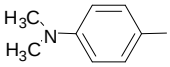
N'-(3-Bromobenzylidene)nicotinohydrazide (3f). Light brown solid, Yield 88%, Mp 105-106 ^oC. IR (ν_{max}, cm^{-1}): 3346 (-NH, str), 3061 (=C-H, str), 1647 (-C=O, str), 1587 (-C=N, str), 1439 (-C-N, str), 680 (-C-Br, str). ^1H NMR (400 MHz, DMSO-d_6): δ_H 7.54-7.58 (m, 3H, Ar-H), 7.59 (d, J= 4.8, 2H, Ar-H), 7.77 (d, J = 8.4 Hz, 1H, Ar-H), 8.25 (d, J = 8.0, 1H, Ar-H), 8.44 (s, 1H, -CH=N), 8.77 (d, J = 5.2 Hz, 1H, Ar-H), 9.06 (s, 1H, pyridine-N=CH), 12.11 (s, 1H, -CO-NH). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ_C 123.6 (C_{15}), 124.3 (C_1), 128.8 (C_{12}), 129.0 (C_{13}), 131.5 (C_5, C_{16}), 133.2 (C_{14}), 135.5 (C_{11}, C_6), 147.0 (C_{10}), 148.6 (C_2,C_4), 164.8 (C_7). MS *m/z*: 304.08 (M+H); Anal. Calcd. for $C_{13}H_{10}BrN_3O$: C, 51.34; H, 3.31; Br, 26.27; N, 13.82; O, 5.26; found: C, 51.14; H, 3.25; N, 13.69.

N'-(3-Nitrobenzylidene)nicotinohydrazide (3g). Yellow solid, Yield 92%, Mp 195-197 ^oC. IR (ν_{max}, cm^{-1}): 3353 (-NH, str), 3055 (=C-H, str), 1659 (-C=O, str), 1594 (-C=N, str), 1534 (Ar-N=O, str), 1433 (-C-N, str). ^1H NMR (400 MHz, DMSO-d_6): δ_H 7.60 (s, 1H, Ar-H), 7.79 (t, J= 7.2, 1H, Ar-H), 8.19-8.29 (m, 3H, Ar-H), 8.57 (d, J = 8.4, 2H, Ar-H), 8.79 (s, 1H, -CH=N), 9.09 (s, 1H, pyridine-N=CH), 12.31 (s, 1H, -CO-NH). ^{13}C-NMR (100.6 MHz, DMSO-d_6): δ_C 121.1 (C_{16}), 123.7 (C_1), 124.4 (C_{14}), 128.9 (C_{13}), 130.5 (C_5), 133.4 (C_{12}), 135.6 (C_{11}), 135.9 (C_6), 146.0 (C_{10}), 148.2 (C_2), 148.6 (C_4), 152.4 (C_{15}) 162.0 (C_7). MS *m/z*: 271.04 (M+H); Anal. Calcd. for $C_{13}H_{10}N_4O$: C, 57.78; H, 3.73; N, 20.73; O, 17.76; found: C, 57.58; H, 3.70; N, 20.52.

N'-(4-Hydroxy-3-nitrobenzylidene)nicotinohydrazide (3h). Yellow solid, Yield 91%, Mp 243-245 ^oC. IR (ν_{max}, cm^{-1}): 3479 (-OH, str), 3358 (-NH, str), 3053 (=C-H, str), 1658 (-C=O, str), 1586 (-C=N, str), 1521 (Ar-N=O, str), 1437 (-C-N, str). ^1H NMR (400 MHz, DMSO-d_6): δ_H 6.99 (d, J = 5.6 Hz, 1H, Ar-H), 7.24-7.26 (m, 1H, Ar-H), 7.81 (d, J= 6.0 Hz, 1H, Ar-H), 8.23 (s, 1H, Ar-H), 8.54 (s, 1H, -CH=N), 8.80-8.82 (m, 2H, Ar-H), 9.24 (s, 1H, pyridine-

N=CH), 9.83 (s, 1H, Ar-OH), 12.38 (s, 1H, -CO-NH). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ_C 117.2 (C_{13}), 121.6 (C_{16}), 124.7 (C_1), 126.2 (C_{11}), 131.8 (C_5), 135.9 ($C_{6,}C_{12}$), 138.1 (C_{15}), 147.9 (C_{10}), 149.7 (C_2), 151.1 (C_4), 154.3 (C_{14}), 162.9 (C_7). MS *m/z*: 287.12 (M+H); Anal. Calcd. for $C_{13}H_{10}N_4O_4$: C, 54.55; H, 3.52; N, 19.57; O, 22.36; found: C, 54.47; H, 3.48; N, 19.51.

Table. 1. Synthesis of N'-(substituted-benzylidene)nicotinohydrazides 3(a-l) and their physical properties

Compd	R	Time a(h)/ b(min)	Yield (a/b) %	Melting points (°C)	Compd	R	Time a(h)/ b(min)	Yield (a/b) %	Melting points (°C)
3a		3/15	82/90	202-204	**3g**		3/14	82//92	195-197
3b		4/16	70/85	231-232	**3h**		3/14	80/92	243-245
3c		4/17	72/87	172-174	**3i**		3/15	83/90	220-222
3d		3.5/15	81/92	182-183	**3j**		4.5/20	69/82	198-199
3e		3.5/14	71/89	227-229	**3k**		3.5/23	73/80	206-208
3f		3/17	76/88	105-106	**3l**		3.5/24	73/82	145-147

N'-(4-Chloro-3-nitrobenzylidene)nicotinohydrazide (3i). Light yellow solid, Yield 90%, Mp 220-222 °C. IR (ν_{max}, cm^{-1}): 3352 (-NH, str), 3052 (=C-H, str), 1655 (-C=O, str), 1586 (-C=N, str), 1524 (Ar-N=O, str), 1436 (-C-N, str), 1055 (-C-Cl, str). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 7.01 (d, J = 7.2 Hz, 1H, Ar-H), 7.21-7.22 (m, 1H, Ar-H), 7.64 (d, J= 6.8 Hz, 1H, Ar-H), 8.15 (s, 1H, Ar-H), 8.55 (s, 1H, -CH=N), 8.65-8.67 (m, 2H, Ar-H), 9.22 (s, 1H, pyridine-N=CH), 12.11 (s, 1H, -CO-NH). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ_C 117.4 (C_{13}), 121.2 (C_{16}), 123.4 (C_1), 126.1 (C_{11}), 130.3 ($C_{5,}$ C_{14}), 134.7 ($C_{6,}C_{12}$), 138.0 (C_{15}), 146.9 (C_{10}), 149.4 (C_2), 149.9 (C_4), 161.5 (C_7). MS *m/z*: 305.18 (M+H); Anal. Calcd. for $C_{13}H_9ClN_4O_3$: C, 51.25; H, 2.98; Cl, 11.64; N, 18.39; O, 15.75; found: C, 51.12; H, 2.89; N, 18.51.

N'-(2-Hydroxy-3-methoxybenzylidene)nicotinohydrazide (3j). Brown solid, Yield 82%, Mp 198-199 °C. IR (ν_{max}, cm^{-1}): 3488 (-OH, str), 3343 (-NH, str), 3063 (=C-H, str), 1664 (-C=O, str), 1581 (-C=N, str), 1445 (-C-N, str). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 3.63 (s, 3H, -OCH_3), 6.87 (m, 1H, Ar-H), 7.12-7.16 (m, 2H, Ar-H), 7.49 (m, 1H, Ar-H), 8.35 (d, J = 8.4 Hz, 1H, Ar-H), 8.61 (s, 1H, -CH=N), 8.74-8.75 (m, 1H, Ar-H), 9.08 (s, 1H, pyridine-N=CH), 11.35 (s, 1H, Ar-OH), 12.20 (s, 1H, -CO-NH). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ_C 63.4 (-O-$\underline{C}H_3$), 111.8 (C_{14}), 114.6 (C_{15}), 121.1 (C_{11}), 124.3 (C_{16}), 125.2 (C_1), 131.1 (C_5), 134.8 (C_6), 145.6 (C_{10}), 147.7 (C_2), 148.1 (C_4), 151.3 (C_{13}), 151.9 (C_{12}), 163.1 (C_7). MS *m/z*: 272.09 (M+H); Anal. Calcd. for $C_{14}H_{13}N_3O_3$: C, 61.99; H, 4.83; N, 15.49; O, 17.69; found: C, 61.90; H, 4.75; N, 15.41.

N'-(2,4-Dimethoxybenzylidene)nicotinohydrazide (3k). Pale brown solid, Yield 80%, Mp 206-208 °C. IR (ν_{max}, cm^{-1}): 3333 (-NH, str), 3023 (=C-H, str), 1653 (-C=O, str), 1580 (-C=N, str), 1449 (-C-N, str). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 3.59 (s, 3H, -OCH_3), 3.81 (s, 3H, -OCH3), 6.63 (m, 1H, Ar-H), 7.11-7.12 (m, 1H, Ar-H), 7.40 (m, 1H, Ar-H), 8.05 (d, J = 8.4 Hz, 1H, Ar-H), 8.25 (s, 1H, Ar-H), 8.53 (s, 1H, -CH=N), 8.70-8.71 (m, 1H, Ar-H), 9.02 (s, 1H, pyridine-N=CH), 12.09 (s, 1H, -CO-NH). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ_C 63.1 (-O-$\underline{C}H_3$), 62.9 (-O-

$\underline{C}H_3$), 114.8 (C_{14}), 115.2 (C_{15}), 121.0 (C_{11}), 122.1 (C_{16}), 124.8 (C_1), 130.4 (C_5), 134.3 (C_6), 144.8 (C_{10}), 147.2 (C_2), 148.6 (C_4), 150.8 (C_{13}), 151.5 (C_{12}), 162.1 (C_7). MS *m/z*: 286.23 (M+H); Anal. Calcd. for $C_{15}H_{15}N_3O_3$: C, 63.15; H, 5.30; N, 14.73; O, 16.82; found: C, 63.06; H, 5.41; N, 14.61.

N'-(4-N,N-Dimethylaminebenzylidene)nicotinohydrazide (3l). White solid, Yield 82%, Mp 145-147 ^oC. IR (ν_{max}, cm^{-1}): 3389 (-NH, str), 3092 (=C-H, str), 1678 (-C=O, str), 1594 (-C=N, str), 1434 (-C-N, str). ^1H NMR (400 MHz, DMSO-d_6): δ_H 3.12 (s, 6H, -N(CH_3)$_2$), 6.72 (d, J = 8 Hz, 2 Hz, 2H, Ar-H), 7.15-7.16 (m, 1H, Ar-H), 7.43 (d, J= 7.6 Hz, 2H, Ar-H), 8.2 (m, 2H, Ar-H), 8.28 (s, 1H, -CH=N), 9.08 (s, 1H, pyridine-N=CH), 9.47 (s, 1H, Ar-OH), 12.21 (s, 1H, -CO-NH). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ_C 45.3 (-N(CH_3)$_2$), 111.6 (C_{13},C_{15}), 122.5 (C_1), 125.3 (C_{11}), 130.1 (C_{12} C_{16}), 132.1 (C_5), 133.7 (C_6), 147.8 (C_2), 149.2 (C_4), 150.8 (C_{10}), 152.9 (C_{14}), 161.8 (C_7). MS *m/z*: 270.00 (M+H); Anal. Calcd. for $C_{15}H_{16}N_4O$: C, 67.15; H, 6.01; N, 20.88; O, 5.96; found: C, 67.07; H, 5.92; N, 20.84.

Antibacterial Activity:

The significant antibacterial potency of Schiff's base derivatives **3(a-l)** of nicotinohydrazide **(1)** were performed against bacterial strains such as *Escherichia Coli*, *Bacillus Subtilis* and *Streptococcus aureus* by employing Disc diffusion method [41, 42]. A standard inoculum (1-2 x 10^{-7} c.f.u/mL 0.5 McFarland standards) was introduced onto the surface of sterile agar plates and a sterile glass spreader was used for even distribution of the inoculums. The discs measuring 6 mm in diameter were prepared from Whatman No.1 filter paper and sterilized by dry heat at 40 ^oC for an hour. The dry sterilized discs previously soaked in a known concentrations (100, 250 µg/mL) of the test compounds were placed in nutrient agar medium. Blank test showed that, DMF used in the preparation of the test solutions does not affect the test organisms; the plates were inverted and incubated for 24 h at 37 ^oC. The zone of inhibition around the disc was calculated edge to edge zone of the confluent growth which is usually corresponds to the sharpest edge of the zone and was measured in millimeters. All tests were repeated three times and average data has taken as final result. Ciprofloxacin was used as a standard drug for bacterial study. The inhibition zones of the tested compounds were compared with controls (**Table. 2**). Minimum inhibitory concentrations (MICs) were determined by broth dilution technique [43]. Different concentrations like 2.5, 5, 7.5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80 µg/mL of test solutions were evaluated. Specifically 0.1 mL of standardized inoculum (1-2 x 10^7 c.f.u/mL) was added to test tubes and incubated for 24 h at 37 ^oC and two controls were maintained for each test sample. The growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC) and the MICs of the test solutions are presented in **Table 2**.

Table. 2 Antibacterial zone of inhibition (mm) and Minimum inhibition concentrations (MICs) of the synthesized compounds 3(a-l).

Compd	Bacterial culture								
	E. coli		MICs (µg/mL)	*S. aureus*		MIC (µg/mL)	*P. Aeruginosa*		MIC (µg/mL)
	100	250		100	250		100	250	
3a	5.2	13.6	80	4.6	13.6	80	4.3	11.9	80
3b	10.0	23.7	40	10.1	20.7	40	10.6	19.4	40
3c	11.4	25.5	30	10.7	21.7	30	11.1	19.6	30
3d	14.2	24.3	30	11.2	19.9	25	11.6	19.9	25
3e	8.2	18.7	50	7.2	14.1	60	6.6	13.9	60
3f	5.6	13.6	70	4.8	11.9	80	4.5	11.5	80
3g	6.1	13.9	70	5.1	10.9	80	5.4	11.0	80
3h	8.8	15.6	60	8.8	14.0	60	7.0	13.6	50
3i	7.5	15.1	60	6.9	13.0	70	6.9	14.5	50
3j	9.7	20.4	50	9.9	18.9	40	10.9	19.7	30
3k	6.9	15.2	60	5.9	16.0	70	6.8	15.6	60
3l	6.8	14.9	70	5.1	15.5	80	3.9	15.0	80
Ciprofloxacin	27			25			25		

Antifungal Activity:

The antifungal potency of Schiff's base derivatives **3(a-l)** of nicotinohydrazide **(1)** were performed against fungal strains such as *Fusarium oxysporum*, *Aspegillus flavus* and *Aspergillus niger* by using agar Disc-diffusion method [39]. The fungal strains were maintained on Potato Dextrose Agar (PDA) medium (Hi-Media). A loopful of culture from the slant was inoculated into the Potato Dextrose broth and incubated at 37˚C for 48-72 h. 0.1 mL of this culture was spread on the potato dextrose agar plate and a sterile glass spreader was used for even distribution of the inoculum. All the compounds were dissolved in dimethylformamide (DMF, Merck). Sterile discs of Whatmann No.1 filter paper of about 6 mm diameter were impregnated on the surface of the media. Blank test showed that,

DMF used in the preparations of the test solutions does not affect the test organisms, different concentrations (100, 250 µg/mL) of various test compounds were prepared and applied on the discs and incubated for 48-72 h at 37 ˚C. The zone of inhibition around the disc was calculated edge to edge zone of the confluent growth which is usually corresponds to the sharpest edge of the zone and was measured in millimeters. All tests were repeated three times and average data has taken as final result. Flucanozole was used as a standard drug for antifungal study and the inhibition zones of the test compounds were compared with controls (**Table 3**). Minimum Inhibitory Concentrations (MICs) were determined by micro-broth-dilution method [39]. The minimum concentration, at which there was no visually detectable bacterial growth, was taken as MIC. Concentration of 0.1-2 mg /mL in steps of 80 µg/mL were evaluated. Test compounds concentrations of 0.1-2 µg /mL in steps of 50 µg/mL were evaluated. Specifically 0.1 mL of standardized inoculum (1-2 x 10^7 c. f.u/mL) was added to each test tube. The tubes were incubated aerobically at 37 ^oC for 48-72 h. Control was maintained for each test sample. The lowest concentration (highest dilution) of test compound that produced no visible signs of bacterial growth (no turbidity) when compared with the control tubes were regarded as MICs (**Table 3**).

Table. 3 Antifungal zone of inhibition (mm) and Minimum inhibition concentrations (MICs) of the synthesized compounds 3(a-l)

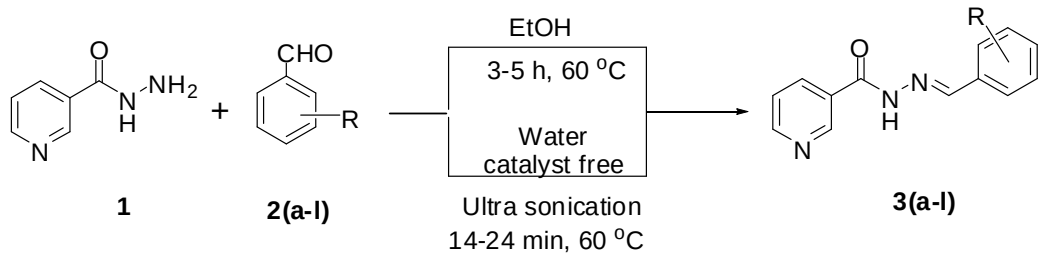
Compd	Fungal culture								
	A. flavus		MIC (µg/mL)	*A. niger*		MIC (µg/mL)	*C. albicans*		MIC (µg/mL)
	100	250		100	250		100	250	
3a	4.1	13.6	80	4.3	12.0	80	4.0	11.8	80
3b	9.6	20.7	40	11.8	20.0	30	9.6	18.9	30
3c	9.9	21.7	30	12.0	21.1	30	9.1	17.9	40
3d	10.2	19.9	30	10.6	19.4	30	10.5	19.7	30
3e	8.1	17.1	40	7.3	14.4	60	6.4	17.2	60
3f	4.6	11.0	80	4.9	13.3	80	4.1	10.6	80
3g	5.0	11.7	80	5.4	14.2	70	5.3	12.0	70
3h	7.2	13.6	50	7.0	14.2	50	5.9	11.6	60
3i	6.3	14.8	60	6.8	14.5	50	6.0	12.9	60
3j	10.0	18.6	40	11.0	19.6	30	10.0	18.0	30
3k	6.0	15.9	60	6.1	16.7	60	6.1	14.0	60
3l	3.8	12.5	80	5.1	15.8	80	3.7	11.6	80
Flucanozole	22			24			21		

RESULTS AND DISCUSSION

A facile and catalyst-free green synthetic protocol for the synthesis of N'-(substituted-benzylidene)nicotinohydrazide Schiff's base derivatives **3(a-l)** of nicotinohydrazide **(1)** by reacting with various substituted aldehydes **2(a-l)** in aqueous medium without using any catalyst under ultrasonic irradiation at 60 ^oC, schematic representation is shown in **Scheme. 1**.

At first attempt to optimize the reaction conditions, we carried out Schiff's base reaction between nicotinohydrazide **(1)** and *m*-nitro benzaldehyde **(2g)** in ethanol without using any catalyst at 60 ^oC, low yields were obtained after long time (3-4.5 h). The same reaction was carried out under ultrasonic irradiation in aqueous medium at 60 ^oC without using any catalyst. Amazingly, the excellent yields were obtained within 14-24 min in aqueous medium at 60 ^oC using simple work-up procedure such as filtration, recrystalization without using further column chromatography technique. After optimization of the reaction conditions, this reaction was employed for various substituted aldehydes **2(a-l)** and nicotinohydrazide **(1)** to synthesize title compounds under aqueous conditions, results are presented in **Table. 1**. The progress of the reaction was examined by TLC and the structures of the title compounds were confirmed by IR, NMR (^1H, ^{13}C), mass spectral and elemental analysis. IR absorption bands at 3290-3330, 3000-3100, 1640-1680 and 1550-1600 cm^{-1} in their spectra confirmed the functional identity –NH, =CH, -C=O and –C=N respectively in the compounds. The δ values of ^1H NMR spectrum at 11.5-12.5, 8.5-9.4 and 7.5-8.7 confirm –N-H, =C-H and aromatic protons respectively. Presence of molecular ions in mass spectra and elemental analysis of the title compounds provide further evidence for the structural confirmation of the title compounds **3(a-l).**

Scheme. 1. Schematic representation for synthesis of N'-(substituted-benzylidene) nicotinohydrazide 3(a-l) derivatives in aqueous medium under ultrasonic conditions.



R =			
m-hydroxy	(**3a**)	*m*-nitro	(**3g**)
p-hydroxy	(**3b**)	*p*-hydroxy, *m*-nitro	(**3h**)
o-hydroxy	(**3c**)	*p*-chloro, *m*-nitro	(**3i**)
p-fluoro	(**3d**)	*o*-hydroxy, *m*-methoxy	(**3j**)
p-chloro	(**3e**)	*o,p*-dimethoxy	(**3k**)
m-bromo	(**3f**)	*p-N,N* dimethyl	(**3l**)

Antibacterial and antifungal activities of the synthesized compounds were screened against the growth of bacterial strains such as *Escherichia Coli*, *Bacillus subtilis* and *Streptococcus aureus* by employing Disc diffusion method [37, 38] and fungi such as *Fusarium oxysporum*, *Aspegillus flavus* and *Aspergillus niger* by using agar Disc-diffusion method [39]. Ciprofloxacin and Fluconozole was used as standard drugs for antibacterial and antifungal study. In the investigation of the microbial activity of the tested compounds the results disclosed that all the compounds exhibited moderate to potential activity towards tested microorganisms and the changes in the functional groups of the molecule were affected the effectiveness of the activity in killing mechanism of tested pathogens. Whereas, compounds **3b**, **3c**, **3d** and **3j** showed potential antibacterial and antifungal activities against the tested strains, which might be the functional groups like as hydroxyl functionality in **3b**, **3c**, fluorine atom in **3d** and hydroxyl/methoxy groups in **3j** compounds.

CONCLUSION

In Summary, we developed an efficient and environmentally benign approach for synthesis of Schiff's base derivatives of nicotinohydrazide such as N'-(substituted- benzylidene)nicotinohydrazides **3(a-l)** to afford optimum yields in aqueous medium employing ultrasonic irradiation conditions without using any catalyst. Antibacterial and antifungal activities of the title compounds were evaluated against the growth of different bacterial strains, fungi and also minimum inhibitory concentrations of the title compounds against tested pathogens were examined. Compounds **3b**, **3c**, **3d** and **3j** exhibited potential antimicrobial activity.

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