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AN INNOVATIVE GREEN SYNTHESIS OF SOME SCHIFF BASES AND THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT

An efficient, simple clean synthesis of Schiff bases of some aminobenzoic acid/ aminopyridine with ketone (acetyl acetone / cyclohexanone) and benzaldehyde were done by an ultrasound irradiation method as well as conventional method. The major advantages of ultrasonication are short reaction time, operational simplicity, high yield, easy workup and environment friendly procedure. The isolated compounds obtained from both the methods were compared and characterized by IR and NMR spectral data. These were screened against certain strains of bacteria and fungi and showed significant anti microbial activity.

KEYWORDS: **amino benzoic acid/ pyridine, Schiff bases, ketone, benzaldehyde, ultrasonication, antimicrobial activity.**

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INTRODUCTION

Compounds containing azomethine group (–C=N–) usually known as Schiff bases have been synthesized by the condensation of primary amines with active carbonyls compounds since several decades. The chemistry of Schiff bases is very diverse. Schiff bases form a significant class of compounds in medicinal and pharmaceutical chemistry with several biological applications which includes antibacterial,[1-3] antifungal[4] and antitumor activities[5]. They have been also studied extensively in coordination chemistry as ligands[6,7] and are known to coordinate with metal ions through the azomethine nitrogen atom to form metal complexes. They play a vital role in designing metal complexes. Metal complexes make these compounds effective as stereospecific catalysts towards oxidation, reduction, hydrolysis, biological activity and other transformations. Conventionally, Schiff's bases can be prepared by refluxing the primary amines and aldehyde/ketone in an organic solvents and presence of traces of acid or base. The combination of solvents, long reaction time and workup procedure makes this method environmentally hazardous. In recent years, environmentally benign synthetic green methods have received considerable attention and some solvent-free protocols have been developed. Schmeyers et al[8] reported the solid-state synthesis of various kinds of benzylideneaniline derivatives by grinding together solid anilines and solid benzaldehydes. Varma et al[9] reported the clay catalyzed synthesis of imines and enamines under solvent-free conditions using microwave irradiation. Synthesis of Schiff's bases in aqueous medium was done by V.K. Rao et al[10]. The use of water as solvent allows for rapid reactions and also the products are often insoluble in water, facilitating their readily isolation. Sonochemistry is one of the green chemistry research area in which molecules undergo a reaction due to the application of powerful ultrasonic radiation. The ultrasound irradiation is a powerful technique[11] for establishing unique chemical and physical conditions, such as a local increase in temperature of several thousands of Kelvins and pressure by several bars by which reaction proceeds. This method has several advantages such as higher atom economy, energy efficiency, environmental friendly, waste and hazards minimization etc. The above findings by various group of scientist stimulated us to synthesize new Schiff bases using classical as well as green technique.

MATERIALS AND METHODS

All the chemicals 2-/4-aminobenzoic acid(2-ABA/4-ABA), 2-/4-aminopyridine(2-APy/4-APy), benzaldehyde(Bz), acetyl acetone (AcAc), cyclohexanone(Ch) were purchased from Coastal Enterprises and used as it is. All the solvents were of analytical grade and were distilled before use. Melting points of the synthesized compounds were determined in open capillaries on melting point apparatus and are uncorrected. IR spectra were recorded on Thermo Nicolet FTIR spectrophotometer at Laila Impex Research Centre, Vijayawada. ^{1}H NMR spectra were taken on JEOL Model AL 90 MHz NMR at Andhra University and 400 MHz NMR at E Sai Pharma, Visakhapatnam in DMSO-d_6 and $CDCl_3$ using TMS as internal reference. Ultrasonication was done at 3.5L 100 at GITAM University.

(i) General procedure of synthesis of Schiff base of amino benzoic acid/ aminopyridine and acetyl acetone/ cyclohexanone/ benzaldehyde:

Method a: In conventional synthesis 0.20 mol of 2-/4-amino benzoic acid,2-/4-aminopyridine (in separate reactions) in methanol (~ 10 mL) was charged in a round bottom flask. Then 0.10 mol of acetyl acetone or 0.20 mol of cyclohexanone / benzaldehyde in 10 mL of methanol was added drop wise while stirring followed by two drops of glacial acetic acid. The reaction mixture was refluxed for about 7-8 h (Scheme 1 & 2). After cooling, the reaction content was kept in the refrigerator for overnight. The excess solvent was removed under reduced pressure. The solid product thus obtained was recrystallized with ethanol.

Method b: In the sonochemical green synthesis, 0.20 mol 2-/4-amino benzoic acid, 2-/4-aminopyridine (in separate reactions) in

methanol (~ 10 mL) and 0.10 mol of acetyl acetone or 0.20 mol of cyclohexanone / benzaldehyde alongwith 10 mL of distilled water were taken in the separate conical flasks. The conical flasks were kept in ultrasonic bath.The reaction was allowed to proceed for 5-15 mins (Scheme 2). The flask containing solid product was kept in a refrigerator for overnight. The crystalline product thus obtained were filtered and recrystallized with ethanol.

Spectroscopic data of synthesized compounds

1. $C_{14}H_{11}O_2N$: White crystals; IR(KBr)cm^{-1}: 3010(Ar-CH); 2482 COOH; 1681(C=O),1622(C=N of azomethine); 1594,1572(C=C); NMR ($DMSOd_6$) δ ppm: 10.2(COOH), 8.3 CH of azomethine; 8.23,7.5(H of 2-ABA); 7.3,6.6 (H of Bz).
2. $C_{14}H_{11}O_2N$:White;IR(KBr)cm^{-1}: 3008(Ar-CH); 2485 COOH;1681(C=O);1618(C=N of azomethine); 1594,1575(C=C); NMR ($DMSOd_6$) δ ppm: 10.1(COOH); 8.5 CH of azomethine ; 8.12, 7.45(H of 4-ABA); 7.3,6.6 (H of Bz).
3. $C_{19}H_{18}O_4N_2$ **:**Yellow ,IR (KBr) cm^{-1} : 3012(Ar-CH); 2988,2891 CH_3; 2480 COOH; 1697 (C=O of COOH); 1599 (C=N of azomethine); NMR ($DMSOd_6$) δ ppm: 12.6 (COOH); 7.9, 7.22 (H of 4-ABA), 3.2 (CH_2 AcAc), 1.93(CH_3 of AcAc).
4. $C_{19}H_{18}O_4N_2$ **:** Light yellow, IR(KBr) cm^{-1}: 3010(Ar-CH); 2990,2868 CH_3 ;2491 for COOH; 1699 (C=O of COOH); 1598 (C=N of azomethine); NMR($DMSOd_6$) δ ppm:12.5 (COOH); 7.6, 7.2,6.7,6.54(H of 2-ABA), 3.2 (CH_2 AcAc), 1.89(CH_3 of AcAc).
5. $C_{13}H_{15}O_2N$: Pale white; IR(KBr) cm^{-1} : 3010(Ar-CH); 2966 CH_2 ; 2480 for COOH; 1667 (C=O of COOH); 1620 (C=N of azomethine); 1600,1520 for C=C; NMR($CDCl_3$) δ ppm: 11.8 (COOH); 8.1, 7.92, 6.67(H of 2-ABA);1.4 (CH_2 of Ch closer to C=N), 1.2(other CH_2 of Ch).
6. $C_{13}H_{15}O_2N$: Pale white; IR(KBr)cm^{-1}:3014(Ar-CH); 2988,2892 CH_3 ; 2485 for COOH;1676 (C=O of COOH); 1616 (C=N of azomethine);1587,1561 (C=C); NMR($CDCl_3$) δ ppm: 11.5 (COOH); 7.9,7.4,7.3(H of 2-ABA);2.2 (CH_2 closer to C=N); 1.8 ,1.6 (other CH_2 of Ch).
7. $C_{12}H_{10}N_2$**:** White; IR(KBr)cm^{-1}:3171(Ar-CH); 1596 (C=N of azomethine); 1556,1540(C=C); NMR($DMSOd_6$) δ ppm: 7.9 (azomethine proton); 7.8,7.52 (H of Bz);7.49 &7.30 (H of 2-Apy).
8. $C_{12}H_{10}N_2$: Pale white; IR(KBr) cm^{-1}: 3014(Ar-CH); 1610 (C=N of azomethine);1565,1538 (C=C); NMR($CDCl_3$) δ ppm: 8.1(azomethine proton); 7.9,7.7, 7.4(H of Bz); 7.4 &7.23 (H of 4-Apy).
9. $C_{15}H_{16}N_4$: Pale white; IR(KBr) cm^{-1}:3014(Ar-CH); 2988,2892 CH_3;1603(C=N of azomethine); NMR($CDCl_3$) δ ppm: 11.5 (COOH); 7.7, 7.4(H of 4-Apy); 3.1 (CH_2 AcAc), 1.95(CH_3 of AcAc).
10. $C_{15}H_{16}N_4$: Pale white; IR(KBr) cm^{-1}: 3014(Ar-CH); 2988,2892 CH_3 ; 1603 (C= of azomethine); NMR($CDCl_3$) δ ppm: 11.5 (COOH); 7.8, 7.25(H of 2-Apy); 3.1 (CH_2 AcAc), 1.95(CH_3 of AcAc).
11. $C_{11}H_{14}N_2$: Pale white; IR(KBr) cm^{-1}: 3014(Ar-CH); 2988,2892 CH_3 ; 1603 (C=N of azomethine); NMR($CDCl_3$) δ ppm: 11.5 (COOH); 8.0, 7.7, 7.4(H of 2-Apy);3.1 (CH_2 of Ch), 2.3(CH_2 of Ch).
12. $C_{11}H_{14}N_2$: Pale white; IR(KBr) cm^{-1}: : 3014(Ar-CH); 2988,2892 CH_3 ; 1603 (C=N of azomethine); NMR($CDCl_3$) δ ppm: 11.5 (COOH); 7.8,7.4(H of 4-Apy);3.1 (CH_2 of Ch), 2.3(CH_2 of Ch).

(ii) Antimicrobial Assay

The synthesized compounds (1-12) were examined for antimicrobial assay against six bacteria [*BS-Bacillus subtilis, PV-Pseudomonas vulgaris, EC-Escherichia coli, SF-Streptococcus faecalis, KP-Klebsilia pneuomonia, ML-Micrococcus luteus*] and three fungi [*AF-Aspergillus flavus, PE-Pencillian expasom, RS-Rhizoctonia solani*] using the well diffusion method. 200 mL of nutrient agar growth medium was dispensed into sterile conical flasks, these were then inoculated with 20 µL of cultures mixed gently and poured into a sterile petridish. After setting a borer with 6 mm diameter was properly sterilized by flaming and used to make three uniform wells in each petridish. The wells were loaded with 50 µL of different investigated compounds. The solvent DMSO, used for reconstituting solvent for diluting the compounds were similarly analyzed for control. The plates were incubated at 37 ^{0}C for

24 h. The above procedure is adopted for fungal assays also and the medium is potato dextrose agar (instead of nutrient agar) and incubated at 27 ^{0}C for 48 h. The zone of inhibition was measured with a Hi Anti Biotic Zone Scale in mm and the experiment was carried out in duplicate. The results are shown in Table 2.

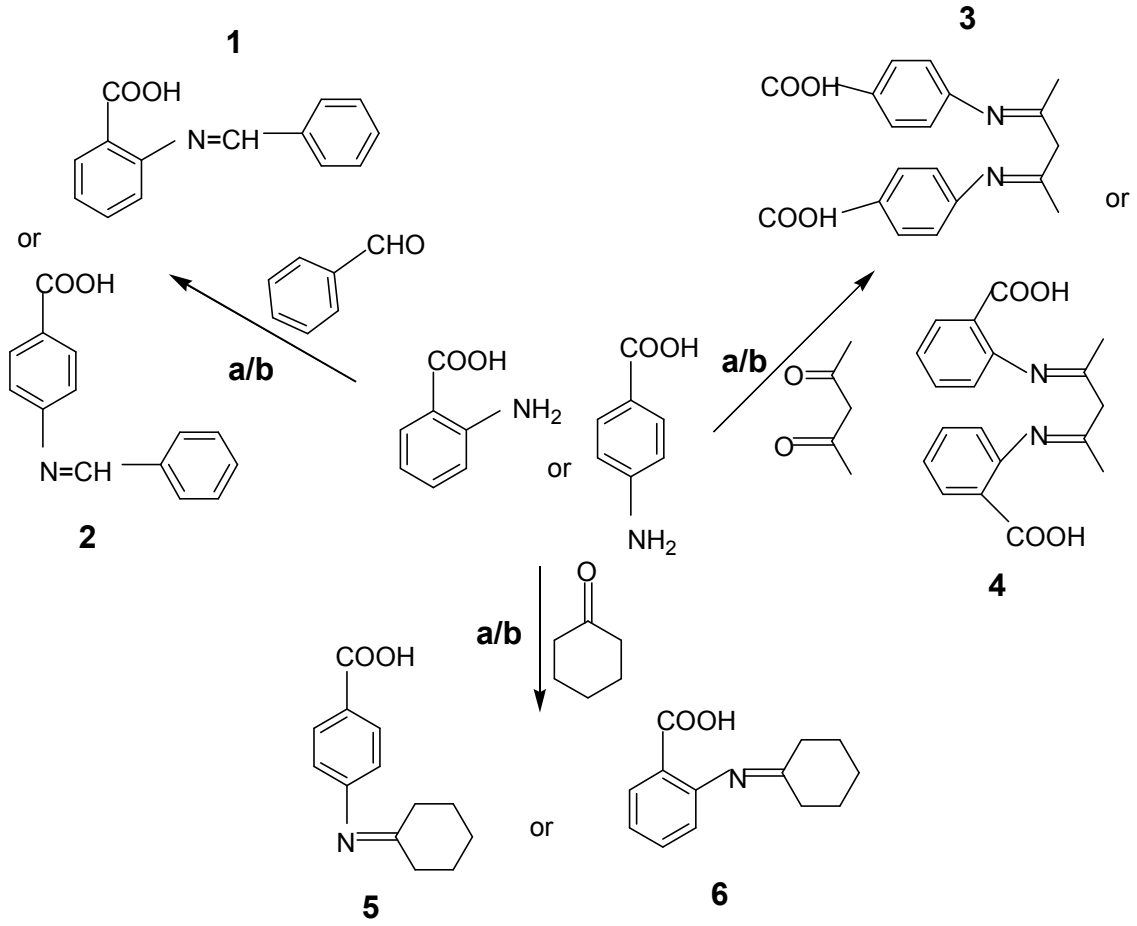
RESULTS AND DISCUSSIONS

The formation of carbon–nitrogen double bond plays important role in organic synthesis and can be achieved by the reaction of aldehydes/ketones and amines in acidic medium which leads to synthesis of Schiff bases (imines). Schiff bases have attracted considerable attention of organic chemists due to their significant biological[12,13] activities. In the classical synthetic route involves condensation of primary amines with carbonyl compounds under azeotropic distillation with the simultaneous removal of water. To overcome these difficulties, an alternative method were also used by taking Lewis acid as a catalyst which accelerates nucleophilic attack of amines on carbonyl carbon as well as serving as dehydrating agent for removal of the water. Several modified methods for synthesis of Schiff bases including environmental-friendly methods were also mentioned in literature. The use of ultrasonic waves in organic synthesis came in light during the last few years[14,15]. Most of the observed effects are due to cavitation, the formation, growth and collapse of bubbles in an irradiated liquid, which makes sonochemistry unique. Cavitation induces very high local temperatures and pressures or strong electric fields inside the bubbles (cavities) and enhances mass transfer and turbulent flow in the liquid[16]. Many homogeneous and heterogeneous reactions can be conducted smoothly by sonication[17] to improve yields with increased selectivity. Compared with traditional methods, this technique is more convenient and easily controllable. Considering these facts and our interest to develop a new synthetic route, we have decided to synthesize Schiff bases of aminobenzoic acids/aminopyridine with aldehyde/ketone by green approach using ultrasonic waves and by conventional method as well. The synthetic details of both the methods are given in the Table 1. The completion of reaction and purity of the synthesized compounds was checked by performing TLC. The structures of the synthesized compounds (1-12) were established on the basis of their FTIR and 1HNMR data. Identical TLC and melting points were observed for the product obtained from both the methods. The IR spectra of the synthesized compounds showed a stretching band at 1620-1585 cm^{-1} corresponding to azomethine[18] group. The disappearances of NH_2 and CO (carbonyl) peaks in all the IR spectra were further supportive of condensation of amino group of ABA/APy with carbonyl group of Bz/AcAc/Ch. This was further confirmed by their ^{1}HNMR spectra. The azomethine proton peak was clearly observed[19] in the compound 1, 2, 7, 8 at δ 8.5-7.9 ppm. Other protons peaks were found to be well consistent with the expected structures.

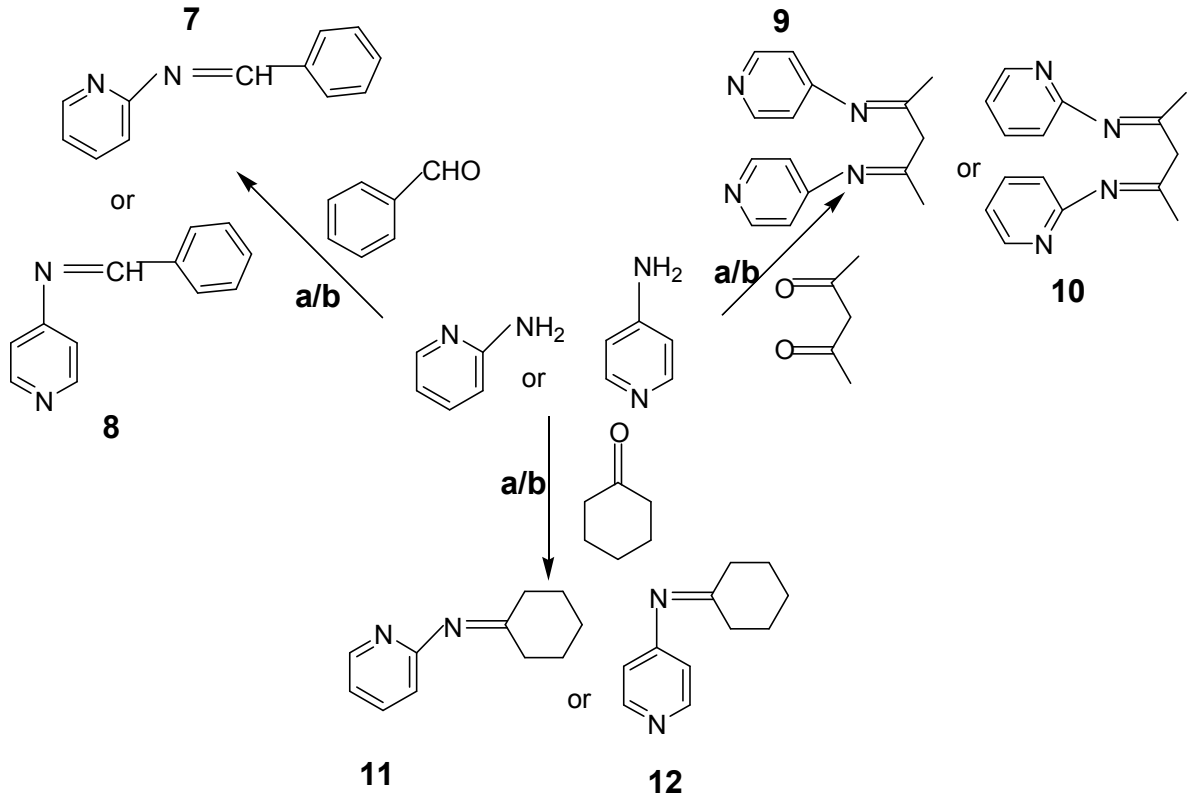
Table 1
Details of the synthetic method

Sl.No.	Compound	Reaction time (mins) US*	Conven.*	% Yield US*	Conven.*	M.P. (^{0}C)
1	$C_{14}H_{11}O_2N$	12	360	87	65	7 0
2	$C_{14}H_{11}O_2N$	10	320	84	67	7 1-74
3	$C_{19}H_{18}O_4N_2$	8	290	85	74	97-98
4	$C_{19}H_{18}O_4N_2$	10	310	79	70	87-89
5	$C_{13}H_{15}O_2N$	14	300	74	69	136-140
6	$C_{13}H_{15}O_2N$	12	310	85	66	66-70
7	$C_{12}H_{10}N_2$	15	290	88	68	110-112
8	$C_{12}H_{10}N_2$	10	270	78	65	116-118
9	$C_{15}H_{16}N_4$	12	280	80	64	122-124
10	$C_{15}H_{16}N_4$	14	260	82	65	135-137
11	$C_{11}H_{14}N_2$	10	300	80	59	144-146
12	$C_{11}H_{14}N_2$	10	280	84	62	152-155

US* =Ultrasonication , Conven*.=Conventional method



Scheme 1- a = conventional / b = ultrasonication



Scheme 2- a = conventional / b = ultrasonication

As described in literature[20], benzoic acid is the simplest aromatic acid is being used in food and pharmaceutical preparations as preservatives due to its antimicrobial activity. It is also an important precursor for the synthesis of many other important organic compounds. Diverse biological activities of pyridines derivatives as antitumor[21] and antimicrobial[22] were reported. Considering these observations all synthesized compounds (1-12) containing benzoic acid /pyridine moiety were screened for antibacterial and antifungal activity against six bacterial and three fungal strains, results are shown in Table 2. Antimicrobial evaluations of some of the compound against bacteria and fungi are encouraging since compound 2, 7, 8, 9, 12 showed promising antimicrobial activities against all the test microorganisms. It is observed that the compounds having pyridine moiety have shown better activity. However, compounds having 4-ABA group also showed significant activity, may be due to the possibility of more hydrogen bonding, by which it could be able to penetrate to the cell walls of microorganisms easily. Designing more polar compounds may overcome solubility problems of synthesized compounds, and might achieve more active compounds. Further studies with these types of compounds are under investigation in our laboratory.

Table 2
Antimicrobial activities of synthesized compounds(10µg/ml)

Entry*	*Bacteria*						*Fungi*		
	BS	*PV*	*EC*	*SF*	*KP*	*ML*	*AF*	*PE*	*RS*
1	18	14	19	10	10	11	12	14	11
2	21	17	22	9	10	13	14	11	12
3	10	11	15	13	12	12	11	10	10
4	9	10	12	10	NA	10	9	9	8
5	16	11	16	15	14	18	22	18	8
6	12	10	11	12	11	13	17	12	NA
7	20	15	18	17	15	14	10	11	11
8	24	18	21	20	18	16	12	13	15
9	22	19	16	12	13	11	13	11	9
10	18	15	14	10	10	9	NA	8	7
11	16	17	18	18	11	8	8	9	NA
12	19	21	24	25	14	10	11	12	8
DMSO	NA	NA	NA	NA	NA	NA	NA	NA	NA
STD	30	32	33	29	30	31	29	28	25

Entry*=same as Table1; BS-Bacillus subtilis, PV-Pseudomonas vulgaris, EC-Escherichia coli, SF-Streptococcus faecalis, KP-Klebsilia pneuomonia, ML-Micrococcus luteus, AF-Aspergillus flavus, PE-Pencillian expasom, RS-Rhizoctonia solani; STD Ampicillin, NA-Not active

CONCLUSION

We have developed a clean, green, concise and efficient alternative method for synthesis of Schiff bases. The present procedure has the advantage of reduced reaction times, mild reaction conditions, high yields, and greener aspects such as avoiding hazardous organic solvent, ease of recovery, and reuse of reaction medium, thus making it a worthwhile addition to the existing methods. These compounds were also shown the considerable antimicrobial effect against 6 bacteria and 3 fungi. The biological activity of these compounds will trigger more interest in the synthesis of such compounds from the easily available starting materials.

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