



(RESEARCH ARTICLE)



Green synthesis, spectral analysis and antifungal activity of n-(benzylidene) aniline & 4-substituted-n-(2-substituted-benzylidene) aniline

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Abstract

Green synthesis means “The design of chemical products and processes that reduce or eliminate the use and generation of hazardous substances”. There are some environmental issues and problems which constantly facing by society such as soil and water pollution, air pollution, ozone depletion, acid rain, the depletion of natural resources global climate change. Schiff's Bases are well known for their important biological applications due to the presence of azomethine linkage ($-\text{CH}=\text{N}-$) In recent years compounds from Schiff's base group have been extensively studied for their biological applications. Accordingly, a series of Schiff's bases were synthesized from substituted aromatic aldehydes and differently substituted aromatic amines and their antifungal activities were evaluated. The synthesis of Schiff's bases was confirmed by spectral characterization using Infra Red IR spectroscopy, ^1H & ^{13}C NMR spectroscopy & Mass spectroscopy studies. All of these titled synthesized compounds have been screened for antifungal study and are found to possess Excellent antifungal activity against Pathogenic strains of *Penicillium sp*, *Candida albicans* and *Asperigillus Nigerfungi*. *Candida albican* is a pathogenic fungus that causes mucosal and systematic infection in human being while *Sacchromyces cerevisiae* is a commonly used non pathogenic model organism in research, baking and brewing. The synthesized compounds N-(Benzylidene) Aniline, N-(2-Hydroxy benzylidene) Aniline, 4-Chloro-(N-2-Hydroxy-benzylidene) Aniline, & 4-Nitro-N-(2-Chloro-benzylidene) Aniline were coded as SRS I, II, III & IV.

Keywords: Schiff's Base; Green Synthesis; Physical Data; Spectral Analysis; Antifungal Activity

1. Introduction

Microwave-assisted synthesis is considered one of the extremely important part of green chemistry.¹ Microwave synthesis under free or less solvent conditions are eye-catching and important tool to reduced pollution, cheap price, and high yield together with ease in processing and handling. Green approaches improve selectivity, reduce reaction time, and simplify product isolation when compared to traditional methods.²⁻⁵ goal of green synthesis is to minimize the release and usage of dangerous toxic materials during the synthesis process.⁶ Green chemistry is a recent path for carrying out the organic synthesis and the drug design methodology by eco-friendly method.⁶⁻⁷ The goal of green synthesis is to minimize the release and usage of dangerous toxic materials during the synthesis process⁸ Environmental impact can be minimized by enhancing chemical processes through these methods.⁹ Compounds containing azomethine linkage ($-\text{CH}=\text{N}-$) are generally called as Schiff's bases¹⁰ Such types of compounds was first prepared by German Scientist Hugo Schiff's in the year 1864 therefore called as Schiff's base.¹¹ These Schiff's bases can be prepared by the condensation reaction between aromatic aldehyde or ketone with aromatic primary amine.¹² These compounds possesses enamine unit ($\text{HC}=\text{N}-$) represented by the general formula $\text{RN} = \text{CR}'\text{R}''$ (azomethine group), where R, R', and R'' denote various substituted alkyl, aryl, heteroaryl or cycloalkyl groups.¹³ Schiff bases play a crucial role in synthesizing various industrially and physiologically beneficial chemicals through closure, cycloaddition, and replacement reactions.¹⁴⁻¹⁵ Fungal diseases are directly influence on crop yield and quality which results in a huge

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economic loss for farmers worldwide these diseases can be minimized by the applications of Schiff's bases.¹⁶⁻¹⁷ Literature reviews indicate that both natural and non-natural Schiff bases, along with their metal complexes, exhibit a wide spectrum of biological activities, including antibacterial, antifungal, anti-tubercular, anti-parasitic, antiviral, antioxidant, anticancer, analgesic, and anti-inflammatory properties.¹⁸⁻²⁵

2. Methods and material

All reagents and solvents used for the preparations/synthesis were of analytical grade, with purity ranging from 98 - 99.8 %. They were obtained from Sigma-Aldrich and were used without further purification

2.1. Synthesis of Schiff's base: A Green Synthesis Method

All chemicals were used of A.R grade. Aromatic amines and aromatic aldehydes are a very important class of organic compounds from which Schiff bases derived. An equimolar quantity of benzaldehyde 10.14 ml [0.1 mol.] and aniline 9.10 ml [0.1 mol.] (same procedure was follows to synthesized substituted benzylidene aniline) were mixed by drop wise addition in 50 ml quantity of dimethyl sulphoxide (DMSO) or N-N-Dimethyl Formamide (DMF) as a solvent with continuous and constant stirring on magnetic stirrer 400-500 rpm by the addition of 1-2 drop of acid catalyst. Then whole reaction mixture was transferred to 250 ml microwave reaction vessel. Microwave synthesis of Schiff's base typically required rapid heating between 70 to 100 degree Celsius and 160 – 400 W power depending upon nature of solvent. (The same procedure was followed to synthesize substituted benzylidene aniline) reactions was completed within 10 minute and synthesized yellow coloured solid product were obtained. It was recrystallized with hot ethanol and it's purity was confirmed by TLC techniques.

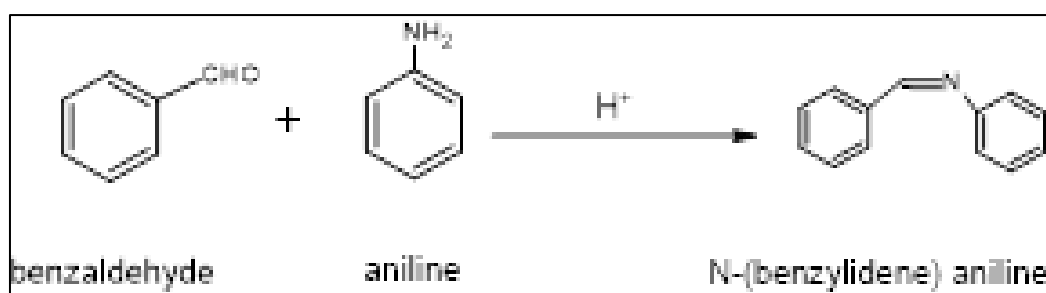


Figure 1 Preparation of N-(Benzylidene) Aniline

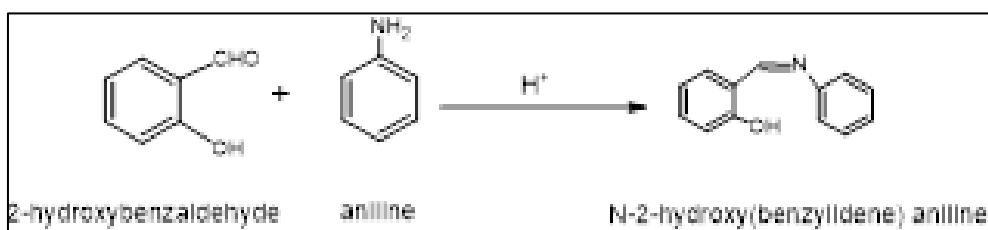


Figure 2 Preparation of N-(2-hydroxy-benzylidene) aniline

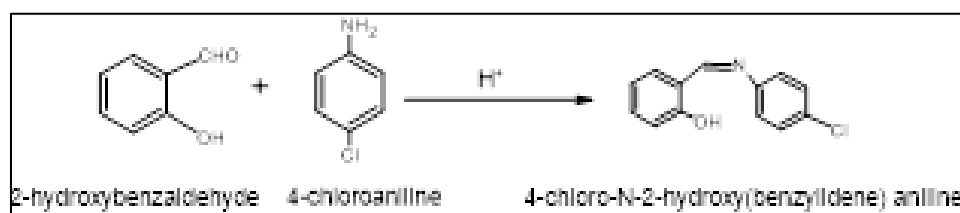


Figure 3 Preparation of 4-Chloro-N-(2-Hydroxy-benzylidene) Aniline

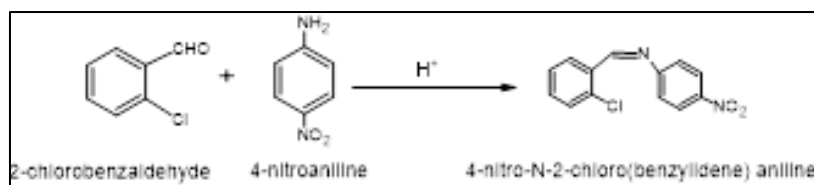


Figure 4 Preparation of 4-Nitro-N-(2-Chloro-benzylidene) Aniline

Table 1 Physical Data of synthesized Schiff's base compounds

Sr. No.	Compound	M.W. gmol ⁻¹	Colour	M.P in °C
1	N-(benzylidene) aniline	181.23	yellow	52
2	N-(2-hydroxy benzylidene) aniline	197.24	orange	130
3	4-chloro-(N-2-hydroxy-benzylidene) aniline,	231.71	yellow	51
4	4-nitro-N-(2-chloro-benzylidene) aniline	260.50	white	89

2.2. Antifungal Activity

The antifungal activity of the synthesized azomethine compounds were studied using *Penicillium sp*, *Candida albicans* and *Aspergillus niger* fungi. Schiff's bases were dried at room temperature and dissolved 20 mg/ml Dimethyl Sulphoxide (DMSO). Antifungal Activity of compounds were measured by using agar disc diffusion method. Potatoes dextrose agar was prepared and sterilized. After a complete solidification of media, holes were bored by sterilized cork borer and the fungal spore suspension was completely transferred to the Petri-plates by taking two loopful of the standard inoculums which was evenly marked on the plates in duplicates. The bored holes were gently filled with 05 mg, 10 mg and 15 mg of the N-(benzylidene) Aniline & 4-substituted-N-(2-substituted benzylidene) aniline solutions and allowed to diffuse into the media. It was incubated at room temperature (25 – 30 C) for 72 hours. Each of the plates was examined for clear zones of inhibition. Inhibition zone diameter was recorded in millimeters by measuring the total area of inhibited growth.

3. Result and discussion

3.1. Synthesis and Spectral analysis

Schiff's bases were synthesized by the condensation reaction of substituted aniline (0.1mol.) with substituted benzaldehyde (0.1 mol.) (Figure 1 to Figure 4) in 50 ml dimethyl sulphoxide by conventional method (microwave assisted) The precipitated was filtered, recrystallized with ethanol/ methanol and dried in hot air oven at 70 – 90 C⁰ for 12 hours. Yellow, orange and white coloured solid compounds were obtained.



Figure 5 Synthesized Schiff's bases N-(Benzylidene) Aniline & 4-Substituted-N-(2-substituted benzylidene) Aniline

3.2. IR Spectral analysis

The FT-IR spectrum (250 - 4000 cm^{-1}) for compound was recorded as KBr disc using Perkin Elmer-Spectrum RX-FTIR instrument. The IR Spectra of N-Benzylidene Aniline shows stretching and out of plane bending for $\text{SP}^2 = \text{CH}$ at 3059.5 and 689.9 cm^{-1} respectively. Indicating that the presence of aromatic ring in the synthesized compound. The N-Benzylidene Aniline free ligand also showing stretching vibration of azomethine nitrogen atom with the aromatic ring at 1600 cm^{-1} - 1622 cm^{-1} attributed to $\nu(\text{C}=\text{N})$ in the spectrum.

In 4-substituted N-(2-substituted benzylidene) aniline the IR stretching frequency for -OH group was found to be 3427.62 cm^{-1} , for Cl group it was 1089-1096 cm^{-1} and for nitro group it was observed in the range of 1530 cm^{-1} (asymmetric) and 1350 cm^{-1} (symmetric) ν stretching.

3.2.1. NMR Spectra

The Nuclear Magnetic Resonance NMR Spectra were recorded in deuterated dimethyl sulphoxide (DMSO-d_6) on Bruker Fourier 300 MHz instrument operating at 300 MHz for ^1H -NMR and 75 MHz for ^{13}C -NMR. N-Benzylidene Aniline shows highly de-shielded singlet absorption at δ (10.0183 ppm 1H) which can be attributed to the enamine hydrogen atom. The region of δ (6.4941 – 8.6123 ppm, 10 H) with multiple signal absorption was assigned to chemically non-equivalent aromatic hydrogen atom (Ar-H). The strong signal appeared at δ (3.3389 ppm) can be attributed to excess droplet of aniline during the synthesis.

^{13}C NMR shows signal around 150-162 and 110-140 ppm indicating that the presence of azomethine linkage ($-\text{CH}=\text{N}-$) and aromatic carbon atom, with quaternary carbon atom showing higher peak values and characteristics property of two aryl rings.

3.2.2. Mass Spectra

The mass spectrum were recorded using instrument generally called as mass spectrometer these instrument consist of an ion source which separates ion based upon their mass to charge ratio. The mass spectrum of N-Benzylidene Aniline $\text{C}_{13}\text{H}_{11}\text{N}$ molecular weight 181.24 is characterized by $[\text{M}^+]$ at m/z 181 appearing as base peak. Loss of hydrogen atom shows m/z 180 further loss of phenyl radical shows m/z 103 and on the loss of benzyl radical shows m/z 77. Fragments such as 103 (enamine- $\text{CH}=\text{N}-$) m/z 91 (for benzyl) and m/z 77 (for Phenyl) were observed.

3.2.3. Antifungal Activity

Table 2 *Penicillium sp*,

Sr. No	Compounds	Zone of Inhibition(in mm)		
		05 mg.	10 mg.	15 mg.
1	SRS-I	06	10	13
2	SRS-II	14	27	35
3	SRS-III	12	19	30
4	SRS-IV	10	18	25

Table 3 *Candida Albican*

Sr. No	Compounds	Zone of Inhibition		
		05 mg.	10 mg.	15 mg.
1	SRS-I	07	11	15
2	SRS-II	14	26	40
3	SRS-III	11	23	35
4	SRS-IV	07	15	23

Table 4 *Asperigillus Niger*

Sr. No	Compounds	Zone of Inhibition		
		05 mg.	10 mg.	15 mg.
1	SRS-I	02	05	09
2	SRS-II	16	24	33
3	SRS-III	12	25	35
4	SRS-IV	10	19	28

Table 2, 3 and 4 Showing results against antifungal activity.

Each of the plates was examined for clear zones of inhibition. Inhibition zone diameter were recorded in millimeter by measuring the total area of inhibited growth. From the result obtained by the antifungal activity it is found that the Schiff base is more active against all tested fungi. Compound SRS-II and SRS-III are most potent candidate against all type of tested fungi. SRS-I & SRS-IV are showing moderate activity. The greater activity of this compound is probably due to the presence of azomethine group.

4. Conclusion

On the basis of result obtained from spectral analysis such as IR., ^1H NMR, ^{13}C NMR and Mass spectroscopy, It has been conclude that a series of Schiff base were successfully synthesized from substituted aromatic aldehydes and differently substituted aromatic amines. Viz. N-(benzylidene aniline) and 4-substituted-N-(2-substituted benzylidene) aniline. Similarly, result obtained by the evaluation of antifungal activity it has been found that the Schiff base is more active against all tested fungi viz. *Penicillium sp.* *Candida albicans* and *Asperigillus niger* fungi. Compound is the most potent candidate against all type of tested fungi. The greater activity of this compound is probably due to the presence of (-CH = N-) azomethine group.

Compliance with ethical standards

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Disclosure of conflict of interest

The author hereby declared that there is no conflict of interest.

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