Synthesis, spectroscopic characterization, antibacterial and antioxidant activity of benzylidene-aniline derivatives

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Abstract

A benzylidene-aniline derivative was prepared through condensation of substituted aniline with different salicylaldehyde. These Benzylidene-aniline derivatives were characterized by elemental and spectroscopic method including IR, UV–vis.,\textsuperscript{1}H NMR and ESI-MS. furthermore explored for their antibacterial activity against \textit{Staphylococcus griseus} as gram positive bacteria, \textit{Salmonella typhi} as Gram negative bacteria by disc diffusion method and the antioxidant activities of these compounds were determined by hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) scavenging activity. The biological activity data of benzylidene-aniline derivatives show promising antibacterial activity.

Key words: benzylidene-aniline derivatives, antibacterial and antioxidant activity.

1. Introduction

Benzylidene-aniline and their derivatives are well known biologically active compounds which containing H-C=N- linkage [1]. These compounds synthesize by condensation of substituent aldehydes with aromatic amines and first reported by Hugo Schiff in 1864. The benzylidene-aniline also referred as Anils, Azomethines, Imines, Schiff base and ligand for complex formation ability etc. due to -H-C=N- linkage present in their structure. it is most important class in medicinal and pharmaceutical field.[2] They exhibit diverse biological properties such as antibacterial, antifungal, antitubercular, anti-inflammatory antioxidant, antiparasitic activities. The benzylidene-aniline is active ligand coordinated with transition metal ions to form complex which is play importance role in large number of widely different biologically activity.[3-4]

Keeping in view the biological and pharmaceutical importance of benzylidene-aniline derivative we synthesized and discus their antibacterial and antioxidant activity.
2. Experimental

2.1. Materials and physical measurements

All the chemicals used were of analytical reagent grade (AR) or chemically pure grade and purchased from SD-Fine Ltd and Sigma-Aldrich Chemicals Ltd. Melting points determined on electrical melting point apparatus using one end seal capillary. Reactions were monitored by TLC on silica gel G plates with iodine vapors in UV chamber for visualizing spots. Rf value measured using solvent system chloroform: Ethyl acetate (7:3). FT-IR spectra were recorded on Bruker spectrometer (500-4000 cm⁻¹). UV-Vis spectra were performed on a Shimadzu UV-1800 spectrophotometer. The elemental analysis was carried out with Thermo finnigan FLASH EA CHN analyser. The mass spectrum was obtaining on JEOL GCMATE II GC-MS. ¹H NMR spectra were run on Bruker Advance II, 400MHz NMR spectrometer in DMSO.

2.2. Synthesis

The benzylidene-aniline derivatives were synthesis according to the reported method.[5]

2.2.1. Synthesis of 2-(2,5-dinitrobenzylideneamino)-4,6-dichlorophenol (DNCL):

A solution of 2,5-dinitro aniline (0.1 mol) is dissolved in absolute ethanol (20ml) and added slowly to a solution of 3,5-dichlorosalicyldehyde (0.1 mol) in absolute ethanol (20ml) after addition the mixture was continuously stirred for additional 2-3 hr. at 40-50°C. Cool reaction mixture at room temperature and poured on to cooled water. The yellow solid separated by filtered, washed with little cold water and recrystallized from ethanol.

Yield 78%; yellow solid, M.P:172°C, Rf 0.54. IR (cm⁻¹): 3318(-OH phenolic), 1607 (C=N str), 1508 (C=C Aromatic str), 1125 (C=Nstr), 1039(C-Ostr), 662 (C-C Aromatic ring str). λ max (nm): 243 (π–π*), 373 (n–π*). Anal. for C₁₃H₇N₃O₅Cl₂ (Calcd.): C, 43.84; H, 1.98; N, 11.80 %. (Found): C, 43.30; H, 2.02; N, 11.72 %.

2.2.2. Synthesis of 2-(2,5-dinitrobenzylideneamino)-4-chlorophenol (NCL):

A solution of 2,5-dinitro aniline (0.1 mol) is dissolved in absolute ethanol (20ml) and added slowly to a solution of 3-chlorosalicyldehyde (0.1 mol) in absolute ethanol (20ml) after addition the mixture was continuously stirred for additional 2 hr. at 40-50°C. Cool reaction mixture at room temperature and poured on to cooled water. The yellow solid separated by filtered, washed with little cold water and recrystallized from ethanol.

Yield 68 %; yellow solid, M.P:165°C, Rf: 0.68, IR (cm⁻¹): 3460(-OH phenolic), 1624 (C=Nstr), 1508 (C=C Aromatic str), 1466 (C=C Aromatic str), 1124 (C-Nstr), 1071(C-Ostr), 714 (C-C Aromatic ring str). λ max (nm): 238 (π–π*), 367 (n–π*). ¹H NMR:(400MHZ DMSO-d6, δ/ppm):- δ 12.05 (1H,-OH-), δ 10.3 (1H,-CH=N-). δ 7.1-8.82 (6H, Ar).
2.2.3. Synthesis of 2-((2-methyl-4-nitrobenzylideneamino)-5-methoxyphenol (MNM):-

A solution of 2,5-dinitro aniline (0.1 mol) is dissolved in absolute ethanol (20ml) and added slowly to a solution of 4-methoxy salicylaldehyde (0.1 mol) in absolute ethanol (20ml) after addition the mixture was continuously stirred for additional 2 hr. at 40-50°C. Cool reaction mixture at room temperature and poured on to cooled water. The light yellow orange solid separated by filtered, washed with little cold water and recrystallized from ethanol.

Yield 58 %; yellow orange solid, M.P:158°C, Rf : 0.58. IR (cm⁻¹): 3466(-OH phenolic), 1611 (C=N̅), 1509 (C=C̅) 1466 (C=C̅ Aromatic str), 1127 (C-Nstr), 1043(C-Ostr), 719 (C-C Aromatic ring str).


3. Antibacterial and Antioxidant activity

3.1 Antibacterial activities

All the synthesized compounds were screened for their antibacterial activities by disc diffusion method. The antibacterial were done at 100 μg/ml concentrations in DMF solvent.[6] The antibacterial activity of these compounds tested against Gram-positive (Streptomyces griseus), Gram-negative (Salmonella typhi).The bacterial stains were incubated at 37°C for 24 hrs. Streptomycin was used as standard drugs under the similar conditions.[7] Following fig.1 show the antibacterial activity of synthesis benzylidene-aniline derivatives.

![Antibacterial Activity Graph](image)

**Fig 1:** Antibacterial Activities of Benzylidene-aniline derivatives.

3.2 Antioxidant activity

**Hydrogen peroxide (H₂O₂) scavenging activity**

The hydrogen peroxide scavenging activity of the synthesized compounds was determined according to the method described by Y. Harinath, et al. In this assay the reaction mixtures consists 2.5 ml of phosphate buffer (0.15 M, pH= 7.4), 1 ml 945 μM EDTA-Fe(II), 0.5 ml 114 μM safranin, 1 ml 3% H₂O₂ and diluted to various concentrations (25, 50 and 100 μg/ml) were prepared in DMF.[8] This reaction mixture was vigorously shaken to appear a clear
homogeneous solution. The reaction mixture without test compound was used as the control. The reaction mixtures were incubated at 37°C for 1 hr. in a water bath. The absorbance of the resulting mixture was read at 520 nm. BHT was used as standard. The scavenging ratio (%) was calculated from the following formula:

$$Scavenging\ ratio\ (%) = \frac{A_i - A_o}{A_c - A_o} \times 100$$

Where, ‘Ai’ is the absorbance of the tested compound present in reaction mixture; ‘Ao’ is the absorbance of absence tested compound; and ‘Ac’ is the absorbance of the absence the tested compound, EDTA-Fe (II) and H$_2$O$_2$. Following fig.2 show the Hydrogen peroxide (H$_2$O$_2$) scavenging activity of synthesis benzylidene-aniline derivatives.

![Antioxidant Activity Chart](image)

**Fig 2:** Antioxidant activities of Benzylidene-aniline derivatives.

![IR Spectra Chart](image)

**Fig 3:** IR spectra of 2-(2,5-dinitrobenzylideneamino)-4-chlorophenol (NCL)
4. Results and Discussion

4.1 Characterization

The FT-IR spectrum of these benzylidene-aniline derivatives exhibited strong absorption peak at 3460 -3439 cm\(^{-1}\) is due to stretching vibrations of \(\nu\) (-OH). The characteristic absorption band at 1605-1624 cm\(^{-1}\) corresponding to \(\nu\)(C=N) while \(\nu\) (C=C) stretching modes observe at 1466-1508 cm\(^{-1}\). The \(\nu\)(C-O) Phenolic band exhibits strong bands at 1032-1071 cm\(^{-1}\) and the corresponding aromatic ring \(\nu\) (C-C) stretching was assigned at 662-720 cm\(^{-1}\).[10] Fig 3: show IR spectra of NCL compound.

The UV-Visible absorption spectra take 200-800 nm in methanol at room temperature and in the absorbance spectra \(\pi-\pi^*\) transitions of the aromatic rings are observed in the 238-243 nm region whereas the absorption band at 364-373nm may be assigned to the n–\(\pi^*\) transitions of the C=N bond.[10-13]The \(^1\)H NMR spectra of compound NCL show signal at 12.05 ppm is assigned to \(\delta\)(-OH), the signal at 10.3 ppm is assigned to \(\delta\)(-CH=N) and the aromatic ring protons are observed in the range 7.1-8.82 ppm. In ESI-MS spectra of compound MNM show molecular ions with their relative abundance at 287.

Thus all the spectrum data include FT-IR, UV-Visible, \(^1\)H NMR and Mass are in full agreement with the proposed structures. In FT-IR spectra for derivatives shows the assigned to azomethene (HC=N) linkage peak at 1605-1624cm\(^{-1}\) and UV-Visible absorption at 364-373 nm is assigned n–\(\pi^*\) transitions of (-C=N).This also support by\(^1\)H NMR spectrum of compound show peak at 10.3 ppm assigned to (-CH=N).

4.2. Antibacterial activities

The synthesized compounds were evaluated for their anti-bacterial activity against *Streptomyces griseus* and *Slamonella typhi* which showed that compound DNCL is more active than other synthesized compounds. The compound NCL and MNM showed anti-bacterial activity against the tested microorganisms. The compounds DNCL show Maximum anti-bacterial activity due to substitution –Cl and –NO\(_2\) group present in it. The results of biological testing indicate that Gram-positive bacteria are most active as compared Gram-negative bacteria. The *Nitro and Chloro* group on phenolic moiety in the structure exhibits antibacterial activity and highly effective against bacteria.

4.3. Antioxidant activity

All the compounds were tested for their in vitro antioxidant activity by H\(_2\)O\(_2\) free radicals scavenging activities. The investigation of antioxidant screening revealed that all the synthesized compounds showed potent to moderate in hydrogen peroxide scavenging activity when compared to the standard butylated hydroxyl toluene (BHT). Amongst the test compounds MNM have shown strong inhibitory effect as compared with other. In compound MNM the methoxy (-OCH\(_3\)) group present on phenolic moiety in their structure which is strongly influenced the antioxidative activity.[13-15]The results showed that the hydrogen peroxide (H\(_2\)O\(_2\)) scavenging activity is in the order of BHT >MNM > DNCL > NCL.
References:


