

Microwave-Assisted and Eco-Friendly Synthesis of Benzylidene Aniline Schiff's Base & Its Anticancer Activity

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Abstract—Cancer is a deadly disease many treatment strategies are available to treat cancer. Amongst the different chemical compounds, Schiff's bases are considered ubiquitous owing to their ease of microwave-assisted and eco-friendly synthesis of benzylidene aniline and substituted benzylidene aniline. Schiff's base has varied applications. In this work, we synthesized Schiff's bases from substituted benzylidene anilines and characterized all the molecules by spectral analysis using IR, ¹H NMR, ¹³C NMR, and mass spectrometry. The compounds were further studied for their anti-cancer activity. These Schiff's base ligands exhibit specific bioactivity and catalytic properties; some bioactive Schiff's bases exhibit anticancer activity against various cell lines. The discovery of antitumor activity of Schiff's base against various cell lines fascinates researchers to develop an anticancer drug without any side effects. Thus, the present study focuses on the anticancer activity of enamine linkage-containing compounds generally known as Schiff's base.

Index Terms—Microwave Synthesis, Schiff's base, Spectral analysis, anticancer activity.

I. INTRODUCTION:

Compounds containing enamine linkage (-CH=N-) are generally called as Schiff's bases. Hugo Schiff's was the first who was prepared such compounds in 1900 Century.^[1] In organic chemistry enamines and heterocycles having azomethine linkage plays an important role in life sciences along with pharmaceutical and medicinal sciences.^[2] Green synthesis method is an important tool for the organic preparation and drug designing.^[3] A microwave assisted or non-conventional method for the synthesis of Schiff' base or any other compound is not hazardous for environment as that of conventional

method. Therefore it is called as green synthetic method as well as eco-friendly synthesis.^[4-5] Cancer is a multistage progressive illness in which a group of cells are replicate in an uncontrolled pattern after a major gene alteration occur.^[6] Genes which are responsible for cellular growth, reproduction, and cell cycle get change and turn normal cells into cancerous cells.^[7] Unlike other diseases, it can affected all important organs of the body like bones, muscles, brain, blood, lungs, liver and other organs.^[8] breast cancer is characterized by extra growth of cervical cancer cells and is the third most common cancer among women. ^[9] Chemotherapy is one of the most vital tools of cancer treatment, although no ideal chemotherapeutic drug has yet to be developed.^[10] Schiff's bases are the condensation reaction product of aldehyde/ketone with primary amines. These Schiff's bases show good anticancer, anti-tumor and antibacterial properties. ^[11-15] The present investigation is based upon our interest in the area of therapeutic drug containing an enamine linkage is driven by the search for new effective drugs for human health

II. EXPERIMENTAL SECTION

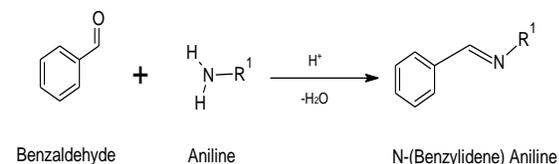
Material and method:

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

Synthesis of Schiff's base:

All chemicals were used of A.R grade. An equimolar quantity of benzaldehyde 10.61 ml [0.1 mol and aniline 9.30 ml [0.1 mol] were mixed by dropwise addition in 50 ml quantity of dimethyl sulphoxide (DMSO) 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 degrees Celsius and 160 – 400 W power depending upon nature of solvent. Reaction was completed within 10 minute and synthesized yellow coloured solid product were obtained. It was recrystallized with hot ethanol and its purity was confirmed by TLC techniques.

General Reaction



III. RESULT AND DISCUSSION:

Synthesis and Spectral analysis

Schiff's base was synthesized by the condensation reaction of aniline (1.02 mmol) with benzaldehyde (1.14 mmol) 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 degree celsius for 12 hours. A yellow-coloured solid compound were obtained.

IR Spectral analysis

IR spectral analysis was taken with an instrument called an Infrared spectrometer of Shimadzu. The IR Spectra of N-Benzylidene Aniline shows stretching and out of plane bending for $SP^2 = CH$ at 685.9–700 cm^{-1} respectively. indicating 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 1600-1640 cm^{-1} (Ar-N) in the spectrum.

NMR Spectra

The Nuclear Magnetic Resonance NMR Spectra were recorded in deuterated dimethyl sulphoxide (DMSO-d₆) on Bruker Fourier 300 MHz instrument operating at 300 MHz for H^1 -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 were 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 (CH=N-) and aromatic carbon atom, with quaternary carbon atom showing higher peak values and characteristics property of two aryl rings.

Mass Spectra

The mass spectrum was recorded using instrument generally called as mass spectrometer these instruments consist of an ion source which separates ion based upon their mass to charge ratio. The mass spectrum of N-Benzylidene Aniline $C_{13}H_{11}N$ molecular weight 181.24 is characterized by $[M^+]$ 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-CH=N-) m/z 91 (for benzyl) and m/z 77 (for Phenyl) were observed.

Anticancer Activity

NRU Assay

Cytotoxicity of the provided samples on MCF-7 cell line was determined by NRU Assay. The cells (5000-8000 cells/well) were cultured in 96 well plates for 24 h in DMEM medium (AT149-1L) supplemented with 10% FBS (HIMEDIA-RM 10432) and 1% antibiotic solution at 37°C with 5% CO_2 . Next day, medium was removed and fresh culture medium was added to each well of the plate. 5 μ l of Treatment dilutions (of different concentrations) were added to the defined wells and treated plates were incubated for 24 h. 100 μ l of NRU (40 μ g/ml in PBS) was added to the defined

wells and incubated (Heal Force-Smart cell CO₂ Incubator-Hf-90) for 1 h. After that medium was removed, NRU was dissolved in 100 µl of NRU Distain solution. Finally, plates were read at 550/660 nm.

Cytotoxic efficacy of the compounds benzylidene aniline against HeLa, MCF-7, and cell lines at 100 mM.

The result of in vitro anticancer activity of the tested Schiff's base ligand was evaluated for Cytotoxicity against MCF-7 and Hela cells of humans in comparison with Cis-platin as positive control. SRS – II has higher cytotoxic activity towards HeLa cells. MCF- IC₅₀ value for SRS -I and SRS – II are 31.1% and 29.7% respectively which is less than 50% therefore it indicates that it has minimum side effect. Cis platin is used as a control drug which shows HeLa 6.6 ± 0.31 and MCF-7 IC₅₀ 89.3%.

IV. CONCLUSION:

The main aim of our research work is to prepare Schiff's bases, generally called the azomethine group, containing compounds by green synthesis or microwave-assisted methods. It is possible to easily synthesize these compounds by the condensation reaction between an aldehyde and an amine with the application of green chemistry. Another area of ongoing research is the discovery of an anticancer agent. Biological screening has shown positive anticancer activity. These have been reported to be the most potent inhibitors towards MCF-7 breast cancer cell lines as evidenced by the cytotoxic activity observed at a lower IC₅₀ value of 20.7 ± 0.45 & 19.5 ± 0.3 in the compounds I and II, respectively, when samples were exposed to the MCF-07 breast cell line.

V. CONFLICT OF INTEREST:

The authors have no conflict of interest.

VI. IMPLICATION.

Beyond the medicinal property, they are important for synthesis of complexes, as well as it can also show catalytic properties for many organic reactions. It can also be used in advanced materials like nanomaterials.

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