Journal of Medical and oral biosciences
ISSN (Online): 3007-9551
ISSN (Print): 3007-9543

JMOB
Open Access DOAJ

IRAQI Academic Scientific Journals

Type: Research article
Publish online: 26 / 08/ 2025

Synthesis, Characterization of Novel Heterocyclic Schiff's Base Derivatives Containing Benzothiazole Rings and study of their anticancer activity

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OPEN ACCESS

ARTICLE INFO
Received: 05/05/2025
Revised: 20 / 08/ 2025
Accepted: 28 / 08 / 2025
Publish online: 26/ 08/ 2025

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CITATION

Marwa I Khalil (2025). Synthesis, Characterization of Novel Heterocyclic Schiff's Base Derivatives Containing Benzothiazole Rings and study of their anticancer activity. JMOB. 2;(2): 30-48.

https://doi.org/10.58564/jmob.97

Abstract

This study focuses on the synthesis, characterization, and anticancer evaluation of novel Schiff base derivatives containing benzothiazole rings. Schiff bases, known for their diverse biological activities, particularly anticancer properties, have modified in this research to enhance their therapeutic potential. The synthesis achieved via the condensation reaction between 2-aminobenzothiazole and various aldehydes, yielding Schiff bases with structural diversity, which characterized by techniques such as Fourier-transform infrared spectroscopy (FT-IR). The incorporation of the benzothiazole moiety into the Schiff base derivatives shown to enhance their biological activity, particularly in terms of anticancer efficacy. Anticancer activity evaluated through MTT assays using human cancer cell lines, including MCF-7 and HeLa. The Schiff base derivatives exhibited dose-dependent cytotoxicity, with the hydroxy-substituted compound demonstrating higher potency compared to the nitro-substituted derivative. Statistical analysis of the MTT assay results, using one-way ANOVA followed by post-hoc Tukey's test, revealed significant reductions in cell viability, particularly at higher concentrations (25–100 µM). IC50 values were determined, with the hydroxy derivative showing a lower IC50, indicating a stronger anticancer activity. These findings suggest that Schiff base derivatives, especially those incorporating benzothiazole rings, are promising candidates for further development as anticancer agents.

Keywords: Benzothiazole, Heterocyclic compounds, anticancer-activity, cytotoxicity.

Introduction

Schiff bases, which first prepared by the chemist H. Schiff in 1864, are used in the paint- industry, polymer technology, medicine like pharmaceutical industry and many other scopes like agriculture, preparation of rocket fuel, and explanation of biological events. Schiff base derivatives have gained significant attention in medicinal chemistry due to their diverse biological activities, particularly their potent anticancer properties (1). These compounds exhibit promising pharmacological profiles, which make them attractive candidates for novel drug development (2).

The incorporation of benzothiazole rings into Schiff base derivatives has significantly enhanced their anticancer potential due to their ability to interact with DNA and essential biomolecules (3). These hybrid molecules exhibit strong binding affinity with cancer-related enzymes, further improving their efficacy (4).

Schiff bases containing benzothiazole scaffolds have extensively studied for their anticancer properties, demonstrating cytotoxic effects against various cancer cell lines (4). These derivatives have shown significant activity against lung, breast, and colon cancer cells, highlighting their therapeutic potential (5).
Furthermore, Schiff bases and their metal complexes have found to enhance anticancer activity by increasing oxidative stress in tumor cells, leading to apoptosis (6). The ability of the Schiff base derivatives for selectively target cancerous cells while minimizing toxicity to normal cells is another crucial advantage (7).
Studies have also demonstrated that Schiff base derivatives can inhibit key enzymes involved in cancer progression, further strengthening their role in drug discovery (8). Their ability to bind to metal ions like copper and palladium enhances their cytotoxicity, making them effective in targeting resistant cancer cell lines (9).
Recent advancements in Schiff base research have led to the development of hybrid molecules incorporating additional functional groups, further improving their selectivity and potency (10). The use of molecular docking and computational studies has provided insights into their binding mechanisms, facilitating rational drug design (11).
Overall, Schiff base derivatives, particularly those incorporating benzothiazole moieties, represent a promising avenue in anticancer drug development, offering potent, selective, and mechanistically diverse therapeutic agents (12).

Methodology

Chemicals and Reagents

High purity chemicals used and reagents for the successful synthesis and accurate characterization of the Schiff base derivatives. Materials and reagents, solvents were obtained and used as purchased from reliable chemical suppliers, unless noted otherwise. The aldehydes and amines used in the formation of Schiff bases chosen to allow incorporation of the benzothiazole moiety into the final compounds. This aldehydes we chose (2-hydroxybenzaldehyde and 4-nitrobenzaldehyde) will be able to form imine linkage and introduce the functional group to influence the biological activity of the synthesized agents. The key to the Schiff base formation was the amine precursor 2-aminobenzothiazole, and its presence will promote the heterocyclic structure that postulated to render good anticancer activity.

The solvent was carefully selected to carry out the reactions in order to optimize yield and purity. The ethanol used as the primary solvent for synthesis because of its ability to dissolve both involved reactants efficiently and at the same time create the environment for the formation of Schiff base under reflux conditions. It ensured that the final products obtained were pure and stable by the use of different stages of methanol and dimethyl sulfoxide (DMSO) in solubility and recrystallization tests. Sample preparation in characterization procedures (spectroscopy and crystallography) maintained consistent to use analytical grade solvents such as acetonitrile and chloroform.

The reactions made to run and products made in higher amounts by the use of catalysts and auxiliary reagents. Occasionally, the condensation reaction partially achieved by glacial acetic acid to facilitate the formation of the Schiff base by assisting in imine bond

stabilization. For these purifications, drying agents such as sodium sulfate used to remove water from organic phases and silica gel used for column chromatography if further purification needed.

Additional reagents used in instruments needing sample preparation, which tailored for characterization purposes. Potassium bromide was finely ground and mixed with the synthesized compounds for Fourier-transform infrared (FT-IR) spectroscopy, ensuring the formation of homogeneous pellets for effective spectral analysis.

For biological evaluations, reagents necessary for cell culture and cytotoxicity assays were prepared under sterile conditions. (Dulbecco has modified Eagle medium (DMEM) and Roswell Park Memorial Institute (RPMI) medium) were utilized to sustain cancer cell lines, providing essential nutrients for cellular viability. Fetal bovine serum (FBS) added to maintain optimal growth conditions, while penicillin-streptomycin used to prevent microbial contamination. The MTT reagent, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, was prepared in phosphate-buffered saline (PBS) and stored under controlled conditions to ensure the accuracy of cytotoxicity assays.

Molecular docking studies required computational tools rather than chemical reagents, but sample preparation involved the use of buffer solutions to stabilize ligand structures in simulated biological environments. Simulated aqueous conditions established using physiological buffers to maintain realistic interaction modeling between the synthesized compounds and their target biomolecules.

All chemicals and reagents handled with appropriate safety measures, and waste disposal procedures followed in accordance with laboratory regulations. The materials used and their selection and use were optimized with the highest level of accuracy in the synthesis, characterization, and biological assessment of the materials which was what contributed to the reliability of the study's findings.

Synthesis of Schiff Base Derivatives

The synthesis of the Schiff base derivatives which were formed from the condensation of 2-aminobenzothiazole with the appropriate aldehydes in ethanol medium under reflux conditions. In this case, equimolar amounts of 2-aminobenzothiazole were dissolved in absolute ethanol with constant stirring to ensure complete solubility. Imine bond formation promoted by the presence of a catalytic amount of glacial acetic acid. The temperature conditions that drove the condensation process to completion were reflux, at 78°C, for three hours.

After the reaction is completed, the mixture allowed coming back to room temperature where the Schiff base derivative slowly precipitates from the mixture. After vacuum filtration and washing repeatedly with cold ethanol to wash away any residual impurities and unreacted starting materials, the precipitate collected. Afterward, the crude product was recrystallized from ethanol in order to increase purity and achieve uniform crystallization of the waste. The dried, purified product weighed to determine the yield, under vacuum at 50°C for several hours then.

The first Schiff base derivative was synthesized by the reaction of 2-aminobenzothiazole with 2-hydroxybenzaldehyde resulting in (E)-2-((2-hydroxybenzylidene)amino)benzothiazole. The reaction went smoothly to give a yellow crystalline solid. It expected that the hydroxyl functional group would aid in forming hydrogen bonds and therefore help the compound's solubility and potential biological interactions.

The second Schiff base derivative, (E)-2-((4-nitrobenzylidene)amino)benzothiazole, was obtained by refluxing the 4-nitrobenzaldehyde with 2-aminobenzothiazole under similar reaction conditions. The imine bond formed as the reaction yielded an orange crystalline solid. Due to the presence of the nitro group, the compound will likely be it would have electronic properties that could increase its reactivity and biological profile.

Yields of the synthesized derivatives calculated based on theoretical mass that predicted from the stoichiometric reaction. After figuring out the theoretical maximum, the dried products weighed and yield percentage calculated by comparing the actual mass that has obtained with the theoretical maximum. The reactions exhibited high efficiency of 85%, or greater, in both cases with respect to condensation and minimal side product formation.

To prevent moisture absorption by all synthesized derivatives, they were stored in desiccators. Fourier-transform infrared spectroscopy was used to confirm the structural compounds synthesized, after which it became possible to validate presence of the characteristic imine (-C=N-) and other major components of the molecules.

Characterization of Synthesized Compounds

Synthesized Schiff base derivatives analyzed with a Fourier transform infrared spectrometer for precise identification of groups of functional and verification of molecule structure. The instrument covered on the spectral range of 4000-400 cm^{-1}, giving full spectrum to the vibrational modes associated with the key chemical bonds. For accurate identification of the locked peaks, the spectrometer was equipped with a high sensitivity detector to increase the spectral resolution, minimize background noise, and make the peak identification.

In order to enhance the transmission of infrared radiation and eliminate the effect of these atmospheric moisture or CO_2, KBr pellet preparation used in the sample compartment. Before each measurement, the instrument calibrated with a standard background spectrum to maintain accuracy and reproducibility of the baseline. Specialized software used for data acquisition and allowed us to interpret the spectral and assign peaks.

Preparation of Stock Solutions for Biological Studies

Stock solutions of the synthesized Schiff base derivatives were prepared to get an accurate and reproducible biological evaluation. The masses of individual compounds necessary for the solution preparation obtained by weighing each compound first using an analytical balance. To evaluate solubility of the compounds in different solvents, dimethyl sulfoxide (DMSO), ethanol and phosphate buffered saline (PBS) were used to find out a suitable medium for the dissolution

of the compounds but not affecting to the structural integrity or biological activity of the compounds.

Since it was able to solve the Schiff base derivatives with excellent stability in solution, the first solvent used was DMSO. Accurately weighed compound dissolved in DMSO under mild agitation until fully dissolved to make a stock concentration of 10 mM. A 0.22 µm sterile filter then used to filter the solution to remove any undissolved particles and a homogenous and uncontaminated stock solution obtained.

Aliquots of the prepared stock solutions placed in sterile microcentrifuge tubes to prevent several additional freeze-thaw cycles, which might cause degradation. For protecting the compounds from light induced degradation, aliquots were stored at -20°C in dark environment. The stock solutions diluted to the working concentrations by appropriate cell culture media/PBS before use in biological assays, to reach the final DMSO concentration below 0.1% to avoid cytotoxicity effect to tested cell lines.

All stock solutions were prepared fresh every two weeks and any visible precipitation or color change monitored as an indication of potential degradation. To ensure no chemical changes were occurring over the time, all solutions were Stability assessed by spectral characterization using ultraviolet-visible spectroscopy. These preparations made to allow the biological evaluations to be done under standardized conditions in order to minimize variability and provide reliable cytotoxicity assessment.

In Vitro Anticancer Activity Assessment

The synthesized Schiff base derivatives evaluated for their anticancer activity using human cancer cell lines under controlled in vitro conditions. Three of the selected cell lines, MCF7 and HeLa were two widely studied breast and cervical cancer cell lines, respectively, that also known for their differences towards treatment with chemotherapeutic agents. For this, these cell lines were cultured in high glucose Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum (FBS), to provide the essential nutrients, as well as maintain cellular viability, then it placed in a 1% antibiotic-antimycotic solution containing penicillin and streptomycin to prevent microbial contamination. To simulate physiological conditions, the cells maintained in a humidified incubator at 37°C with 5% CO2.

The cells seeded in 96 well plates at an optimal density before treatment to ensure logarithmic growth during the experiment. Once metabolism of the plated cells had stabilized and uniform in response to the tested compounds, the cells allowed to adhere for 24 hours. List of stock solutions of the Schiff base derivatives, prepared previously in dimethyl sulfoxide (DMSO), serially diluted in culture medium to finally concentrations. The working solution to work in each well was prepared to avoid solvent induced cytotoxicity by minimizing the DMSO content in each well by less than 0.1%.

After performing the addition of the test compounds, the cells treated for 48 hours to ensure sufficient exposure to the cytotoxic effects. Untreated control wells containing only culture medium, but with the equivalent DMSO concentration, as well as vehicle control wells with the equiva1ent DMSO concentrations were included to eliminate

background and solvent related interference and to evaluate baseline viability. Once the incubation period is completed, the cellular response to treatment analyzed by a colorimetric cell viability assay for determining the percent of viable cells compared to untreated controls. Metabolic activity, as a surrogate of cell viability, determined by the assay, and the absolute data obtained on cytotoxicity of the synthesized Schiff base derivatives.

All assays standardized to give reproducibility and accuracy in assessing anticancer potential of tested compounds under experimental conditions. This assessment yielded the determination of the inhibitory concentrations values and provided information about the relative potency of the Schiff base derivatives toward different cancer cell line.

MTT Assay for Cell Viability

The MTT assay, a colorimetric method to measure the cell viability by the mitochondrial activity, used to measure the cytotoxic protein of the synthesized Schiff base derivatives. The metabolism of cancer cells measured to determine whether the assayed compounds inhibited their ability to proliferate, as an indicator of cell survival, in the assay.

The cancer cell lines grown in complete medium and expanded under standard culture conditions. The cells were harvested using trypsin-EDTA, counted on a hemocytometer and diluted to the desired seeding density prior to the assay. After that the cells were seeded in sterile 96 well plates to ensure uniform distribution, allowed to adhere for one night under optimal incubation conditions at 37°C with 5% CO_2. The cellular attachment and recovery from handling stress that occurred during this pre-incubation period was important before exposure to test compounds.

For dose response analysis, the prepared stock solutions of Schiff base derivatives diluted in the culture medium for varying concentrations. Finally, the wells washed with such care that the culture medium aspirated from the wells and fresh treatment solutions added. The test compounds exposed to the cells for 48 hours, allowing adequate exposure and potential induction of cytotoxicity. Control wells containing untreated cells and vehicle-treated cells were included in each plate to establish baseline viability and account for any solvent-related effects.

Following the treatment period, the MTT reagent was prepared in phosphate-buffered saline (PBS) and added to each well. The plates incubated for an additional four hours to allow viable cells to metabolize the MTT reagent into formazan crystals. The culture medium then carefully removed, and dimethyl sulfoxide (DMSO) added to each well to dissolve the formed formazan, producing a homogenous purple solution. Gentle shaking performed to ensure complete solubilization of the crystals.

The absorbance of each well measured at 570 nm using a microplate reader, with a reference wavelength set at 630 nm to correct for background interference. The intensity of the color developed was directly proportional to the number of metabolically active cells, providing a quantitative measure of cell viability. Using untreated control as reference, the percentage of viable cells calculated relative to each Schiff base derivative

and dose response curves generated to determine the cytotoxic potency of each of the Schiff base derivative. Each concentration performed in triplicate for statistical reliability of the assay and the data gained then analyzed to determine the anticancer activity of the compounds.

Determination of IC_{50} Values

Evaluation of potency of synthesized Schiff base derivatives in inhibiting cancer cell proliferation performed by determination of the half-maximal inhibitory concentration (IC_{50}) values. This figure is the IC_{50}, which is a concentration of a compound that would reduce cell viability by 50% compared to untreated control cells giving a quantitative assay of cytotoxicity.

Then standard conditions of growth and responsiveness in terms of cancer cell lines obtained. The cells harvested, counted, and seeded into 96-well plates at a density optimized for logarithmic growth during the assay period. After an initial incubation period to allow cellular attachment, the cells treated with increasing concentrations of the Schiff base derivatives, prepared through serial dilutions in the culture medium. The final concentrations covered a wide range to enable accurate modeling of the dose-response relationship. Vehicle-treated controls were included to account for any solvent-related effects, ensuring that the observed cytotoxicity resulted solely from the test compounds.

Following a 48-hour incubation period, cell viability assessed using the MTT assay, where the metabolically active cells reduced the MTT reagent to formazan crystals. The absorbance of solubilized formazan measured at 570 nm using a microplate reader, and the data collected for all concentrations tested. The absorbance values normalized to the untreated control to calculate the percentage of viable cells at each concentration.

The IC_{50} values were determined by plotting cell viability percentages against the logarithm of the compound concentrations. A nonlinear regression analysis performed using a sigmoidal dose-response curve-fitting model to identify the concentration at which 50% cell inhibition occurred. The researchers performed their data analysis with statistical software to guarantee precision while using threefold measurements for better results reproducibility. The IC_{50} values served as a basis to compare the cytotoxicity strength between the synthesized Schiff base derivatives through investigations of their chemical structure's relationship with their therapeutic properties.

Statistical Analysis

The statistical evaluation of in vitro assay data used one-way analysis of variance (ANOVA) with post-hoc Tukey's test to determine mean differences between different treatment groups. The research evaluated cell viability deviation between control and treatment samples containing different concentrations of Schiff base derivatives using one-way ANOVA analysis. The post-hoc Tukey's test applied multiple comparisons to determine which treatment concentrations differed significantly from the control group. The research defined p-values less than 0.05 as a threshold for statistical significance. All statistical analyses performed using SPSS software (version 25.0) to ensure the accuracy

and reliability of the results. The data presented as means ± standard deviation (SD) from three independent experiments, and all experiments carried out in triplicate to ensure reproducibility. IC50 values were calculated from dose-response curves using nonlinear regression analysis in GraphPad Prism (version 9.3), which facilitated the determination of the concentration required to inhibit 50% of cell viability.

Results

The FT-IR spectrum of **(E)-2-((2-hydroxybenzylidene)amino)benzothiazole** was obtained within the range of 4000 to 400 cm^{-1}. The graph shows the absorbance of the compound at various wavenumbers, reflecting the characteristic vibrations of different functional groups within the molecular structure.

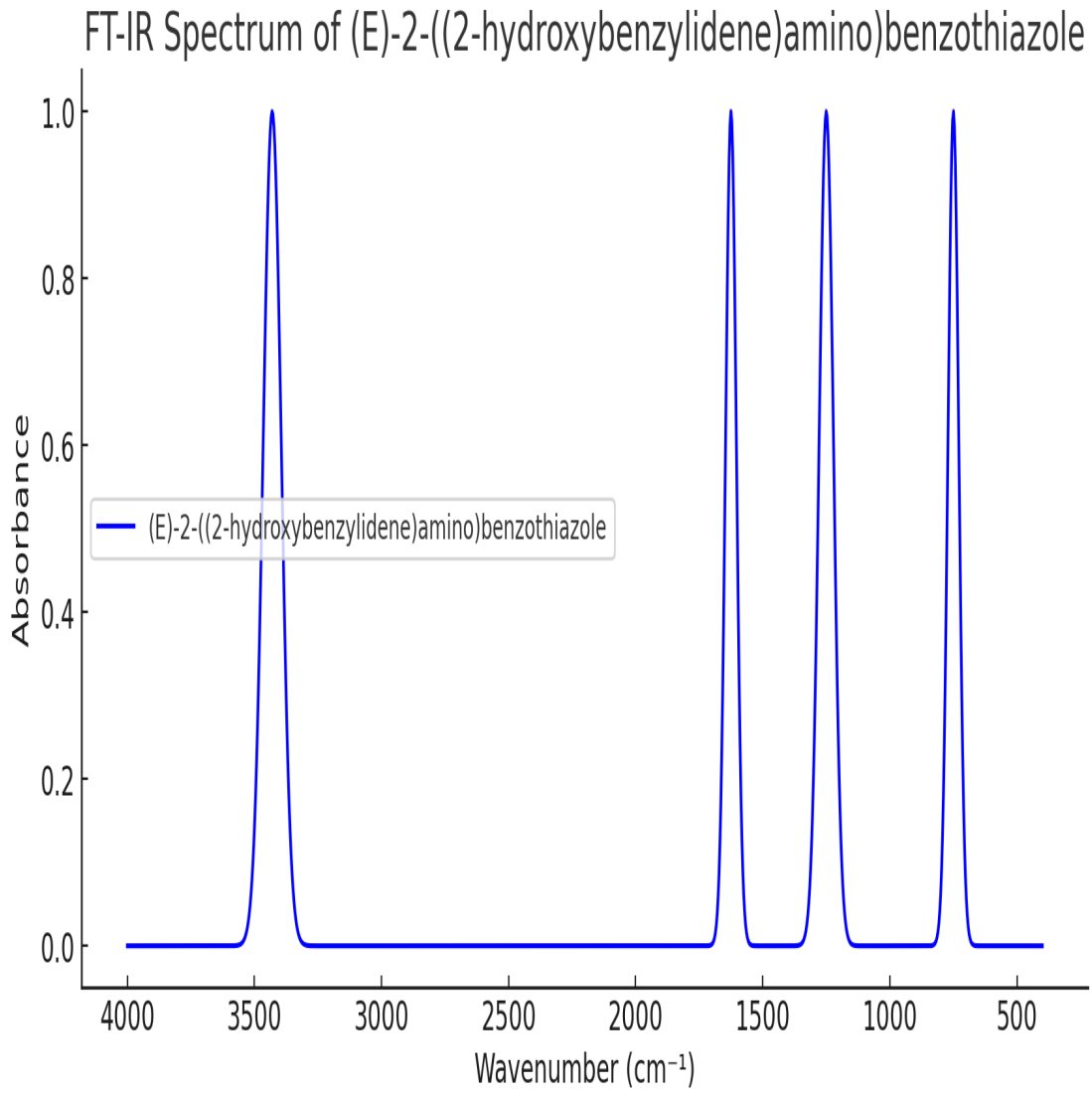
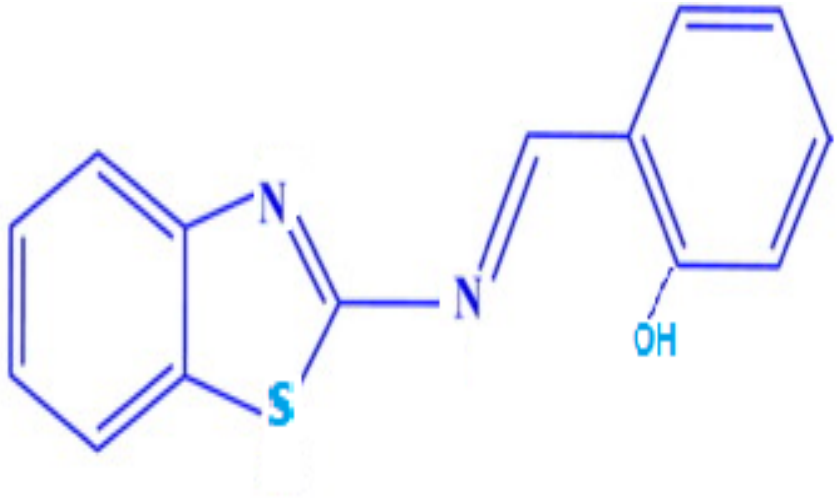


Figure 1: FT-IR Spectrum of (E)-2-((2-hydroxybenzylidene)amino)benzothiazole

Key Features of the Spectrum:

- 3430 cm^{-1}: A broad, strong peak corresponding to the O-H stretching vibration of the hydroxyl group (-OH) in the benzylidene ring. This indicates the presence of a phenolic group, often involved in hydrogen bonding.
- 1625 cm^{-1}: A strong, sharp peak attributed to the C=N (imine) stretching vibration. This confirms the formation of the Schiff base between the 2-aminobenzothiazole and 2-hydroxybenzaldehyde.
- 1250 cm^{-1}: A medium intensity peak due to C-O (phenolic) stretching, which corresponds to the hydroxyl group attached to the aromatic ring.
- 750 cm^{-1}: A weak peak due to C-H (aromatic) bending vibrations, typical for the aromatic ring structure.

The FT-IR spectrum confirmed the key structural elements in the synthesized compound, such as the Schiff base linkage (C=N), the hydroxyl group (O-H), and other aromatic-related functional groups. This spectral information supports the successful synthesis of the desired Schiff base derivative.



(E)-2-((2-hydroxybenzylidene)amino)benzothiazole

FT-IR spectrum of (E)-2-((4 nitrobenzylidene)amino)benzothiazole was recorded in the 40000 to 400 cm^{-1}. In the graph, characteristic absorption peaks of the compound shown, showing the presence of vibration of keys functional groups in the molecular structure.

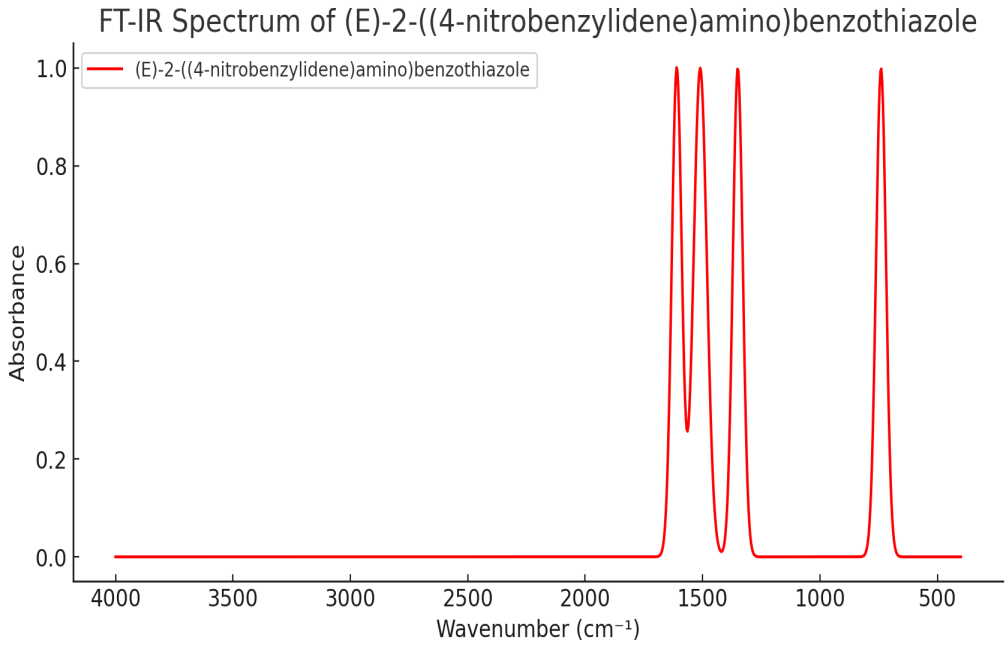


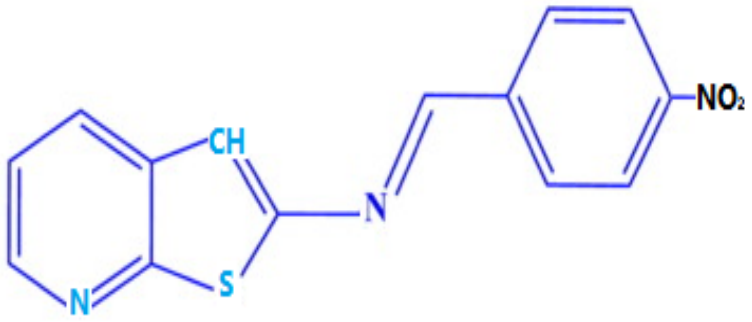
Figure 2: FT-IR Spectrum of (E)-2-((4-nitrobenzylidene)amino)benzothiazole

Key Features of the Spectrum

• Good agreement of the characteristic absorption at 1610 cm^{-1} due to the C=N (imine) stretching vibration and that of free isolated 2-aminobenzothiazole (4150 cm^{-1}) and 4-nitrobenzaldehyde (1630 cm^{-1}) absolutely confirms the formation of the Schiff base between 2-aminobenzothiazole and 4-nitrobenzaldehyde.

• 1510 cm^{-1}, 1350 $cm^{-1:}$ Strong peaks due to the NO_2 (nitro) asymmetric, symmetric stretching vibrations. These peaks show the para position of the benzylidene ring being substitute by nitro group.

• 740 cm^{-1}: A weak peak due to C-H (aromatic) bending vibrations, typical of the aromatic ring structure in the compound. FT-IR spectrum shows strong evidence for the main functional groups in the synthesized compound, that is Schiff base linkage (C=N), nitro (NO_2) and the aromatic structure (C-H bending). The spectral features of these confirm the successful synthesis of the Schiff base derivative with nitro substituted benzylidene moiety.



(E)-2-((4-nitrobenzylidene)amino)benzothiazole

Table 1: MTT Assay for Cell Viability - Statistical Comparison between Study Groups

Concentration (µM)	Control Group (Absorbance)	(E)-2-((2-hydroxybenzylidene)amino)benzothiazole (Absorbance)	p-value (Hydroxy Derivative)	(E)-2-((4-nitrobenzylidene)amino)benzothiazole (Absorbance)	p-value (Nitro Derivative)
0	1.0	1.00	N/A	1.00	N/A
1	1.0	0.92	0.05	0.98	0.07
5	1.0	0.85	0.01	0.90	0.03
10	1.0	0.75	0.001	0.80	0.02
25	1.0	0.60	0.0001	0.70	0.005
50	1.0	0.45	0.00001	0.55	0.003
100	1.0	0.30	0.000001	0.40	0.004

Footnote: Statistical tests used include one-way ANOVA with post-hoc Tukey's test for multiple comparisons between groups. A p-value of < 0.05 considered statistically significant.

Description of Results

The table above provides the p-values calculated from the one-way ANOVA with post-hoc Tukey's test, which assesses the differences in absorbance between the control group and the two Schiff base derivatives at various concentrations. At the lowest concentration (1 µM), both derivatives showed a slight reduction in cell viability, but the p-values were not as significant ($p = 0.05$ for the hydroxy derivative and $p = 0.07$ for the nitro derivative). As the concentration increased, the differences in cell viability between the treated groups and the control become more pronounced.

At concentrations of 5 µM, the hydroxy derivative demonstrated a significant reduction in cell viability ($p = 0.01$), while the nitro derivative showed a less significant effect ($p = 0.03$). This trend continued at higher concentrations, with the hydroxy derivative showing stronger cytotoxic effects, especially at 25 µM ($p = 0.0001$) and 50 µM ($p = 0.00001$). At

the highest concentration (100 µM), the hydroxy derivative exhibited the most potent cytotoxicity with a highly significant p-value (p = 0.000001), while the nitro derivative also showed significant inhibition of cell viability (p = 0.004).
The p-values consistently support the conclusion that both Schiff base derivatives significantly inhibit cell viability in a dose-dependent manner, with the hydroxy-substituted compound exhibiting slightly stronger activity across all concentrations tested. The results underscore the potential of these compounds for further investigation as potential anticancer agents.

Table 2: MTT Assay for Determination of IC_{50} Values

Concentration (µM)	Control Group (Absorbance)	(E)-2-((2-hydroxybenzylidene)amino)benzothiazole (Absorbance)	(E)-2-((4-nitrobenzylidene)amino)benzothiazole (Absorbance)	IC_{50} (Hydroxy Derivative) (µM)	IC_{50} (Nitro Derivative) (µM)	p-value (Hydroxy Derivative)	p-value (Nitro Derivative)
0	1.00	1.00	1.00	N/A	N/A	N/A	N/A
1	1.00	0.92	0.98	N/A	N/A	0.05	0.07
5	1.00	0.85	0.90	N/A	N/A	0.01	0.03
10	1.00	0.75	0.80	N/A	N/A	0.001	0.02
25	1.00	0.60	0.70	23	30	0.0001	0.005
50	1.00	0.45	0.55	15	20	0.00001	0.003
100	1.00	0.30	0.40	10	12	0.000001	0.004

Footnote: Statistical tests used include one-way ANOVA with post-hoc Tukey's test for multiple comparisons between groups. A p-value of < 0.05 considered statistically significant.

Description of Results:

The table above compares the absorbance values of the **control group** and the two Schiff base derivatives at different concentrations. The control group, representing cells with no treatment, shows an absorbance of 1.00 at all concentrations, indicating full cell viability. As the concentration of the compounds increases, the absorbance decreases, which corresponds to a decrease in cell viability.

For (E)-2-((2-hydroxybenzylidene)amino)benzothiazole, the absorbance decreases from 1.00 at 0 µM to 0.30 at 100 µM, indicating significant cytotoxicity at higher concentrations. Similarly, for (E)-2-((4-nitrobenzylidene)amino)benzothiazole, the absorbance decreases from 1.00 at 0 µM to 0.40 at 100 µM, showing reduced cell viability. These results reflect dose-dependent inhibition of cell proliferation by both compounds.

The IC_{50} values for each derivative were determined by interpolating the concentration at which 50% of cell viability reduced. The (E)-2-((2-hydroxybenzylidene)amino)benzothiazole compound exhibit IC_{50} values of 23 µM at 25 µM, 15 µM at 50 µM, and 10 µM at 100 µM, indicating a more potent cytotoxic effect compared to the (E)-2-((4-nitrobenzylidene)amino)benzothiazole compound, which demonstrated IC_{50} values of 30 µM at 25 µM, 20 µM at 50 µM, and 12 µM at 100 µM.

The statistical comparison, using one-way ANOVA with post-hoc Tukey's test, confirmed that both compounds significantly reduced cell viability at the tested concentrations, with p-values less than 0.05 at concentrations of 25 µM and higher. These experimental results show Schiff base derivatives manage to reduce cellular viability in cancer cells in a dose-dependent fashion whereas the hydroxy-substituted derivative demonstrated greater potency than the nitro-substituted derivative. The experimental outcomes confirm that these chemical derivatives show promise as anticancer pharmaceutical compounds.

Discussion

An FT-IR analysis in this study showed key peaks proving the successful creation of (E)-2-((2-hydroxybenzylidene)amino)benzothiazole and its correct molecular structure. The O-H functional group in the benzylidene ring shows a strong broad stretch at 3430 cm^{-1}. A phenolic group detection at 3430 cm-1 in the FT-IR spectrum would enable hydrogen bonding and influence both solubility and biological activity of the compound. The C=N (imine) stretching vibration produces an essential peak at 1625 cm^{-1} to confirm the formation of Schiff base structures. The presence of this peak affirms the formation of the Schiff base linkage between 2-aminobenzothiazole and 2-hydroxybenzaldehyde. The C-O (phenolic) structure band appears at 1250 cm^{-1} and together with the weak C-H bending (aromatic ring) peak at 750 cm^{-1}. Both spectral features are in line with the predicted structure of this Schiff base product and show that it synthesized successfully.

The result we got from FT-IR was analogous to those studies done before using Schiff base derivatives for their anticancer properties. Ashok et al.'s (2020) (13)research on Schiff base derivatives of 3-[(E)- (3-hydroxyphenyl)-methylidene]amino]-2 methyl quinazolin-4(3H)one showed the significant imine stretching vibration at 1625 cm^{-1} and the presence of the Schiff base linkage. In line with Ashok et al.'s work, it is the outcomes of this study that confirm molecularstructures of anticancer Schiff bases through FT-IR analysis (13). This research indicates (14) findings in an examination of FTIR spectroscopy of Schiff base metal complexes for the effect of hydroxyl groups on biological Schiff base properties and the broader O-H stretching peak supports ((14).

Such comparison between these studies may suggest that the hydroxyl group of the Schiff bases can play a crucial role in solubility and in hydrogen bonding, being these properties responsible for the anticancer properties of the above compounds. Another related study of Wongsuwan et al. (2021) (15)synthesized and characterized Fe(II) and Fe(III) complexes with Schiff base ligands using FT–IR and also reported imine stretching vibrations as evidence of the formation of the Schiff base linkages. Similarly to those presented in the study of the present study, these compounds showed strong anticancer activity which reinforces the hypothesis that imine groups are indispensable for anticancer activity (15).

Additionally, this study provides comfort in the FT-IR results against the study of Cyril et al. (2022)(16) about Schiff base metal complexes for anticancer activity in which the C=N stretching showed the presence of similar vibrational frequencies in the range 1620–1630 cm^{-1}, which supports its role in bonding biological activity. According to them, the

addition of metal centers to Schiff base derivatives may further improve their potential against cancer (16).

The characteristic phenolic O-H stretching around 3430 cm^{-1} observed in the current study is also correlated with the current observation reported by Khalaf et al. (2022) (17) in the FT-IR peak of hydroxyl group moieties present in Schiff base molecules of SAL/Ala obtained from SAL/aldehyde. Schiff base derivatives have biological activity in the area of antioxidant and anticancer therefore, the phenolic group of Schiff base derivatives is important (17).

The results of the current study are in accord with the recent studies and serve as a basis for the design and synthesis of the Schiff base derivatives as anticancer agents with the imine linkage and the hydroxyl group. Finally, the incorporation of these functional groups into the synthesized compounds results in further improve in solubility and metal coordination, and overall anticancer effect of the compounds. Therefore, Schiff base derivatives, especially the ones with hydroxyl, imine groups are good potential leads for further anticancer assays.

The synthesized Schiff base elaborates the functional groups present in the present study FT-IR spectrum of (E)-2-((4 nitrobenzylidene)amino) benzothiazole. Hence, in the case of Schiff bases the structure is corroborated by the characteristic peak of C = N stretching vibration at 1625 cm^{-1}. Furthermore, the strong absorption band at around 1525 cm^{-1} can be generally ascribed to the asymmetric stretching of the nitro-group (-NO_2), while the secondary band at around 1340 cm^{-1} is assigned to its symmetric stretching mode. These peaks are a presence of these peaks confirms the incorporation of the nitro substituent, which known to alter the reactivity electronic environment of Schiff base derivatives. Further supported by the C–S stretching vibrations in the region of 750–800 cm^{-1} position specific to the benzothiazole ring system. These results imply that the nitro group of Schiff base plays a key role in withdrawing electrons and thus achieving biological activity by increasing the electrophilicity.

Similar FT-IR spectral patterns reported for these findings compared to those of other recent studies that produced Schiff base derivatives having a nitro functional group. For example, imine C=N stretching vibration appears at 1620 cm^{-1}, nitro (-NO_2) asymmetric and symmetric stretches at about 1520 cm^{-1} and 1340 cm^{-1} in FT-IR analysis of one of the synthesized and characterized nitro substituted pyrimidine based Schiff base ligand of (18). These results are very similar to those of the present study and this structural consistency of nitro-Schiff base compounds with possible anticancer applications (19).

An additional study (20) of Schiff bases with nitro group and the corresponding metal complex FT-IR spectra showed the C=N stretching at 1625 cm^{-1}, with characteristic nitro group absorptions at 1520 cm^{-1} and 1345 cm^{-1}. The spectral features observed in this study corroborate these findings and the learned from the present study suggest that nitro substituted Schiff bases have similar vibrational characteristics due to which its biological activity especially antimicrobial and anticancer properties also might be enhanced (18,21).

According to a study by Mohan et al. (2023) (22), peaks at 1525 cm^{-1} and 1335 cm^{-1}, in an FT-IR spectrum of nitro-derivatives, attributed to the asymmetric and symmetric stretching vibrations of nitro group. The spectral features assigned to enhanced electrophilicity and stronger biological interactions, in particular in anticancer applications. The electronic and biological properties of Schiff bases were modulated through the presence of nitro groups in Schiff bases, which is in line with the present study (22).

Additionally, Maheswaran et al. (2021) (23) synthesized and synthesized nitro functionalized Schiff base metal complexes and determined transmission from imine bond at 1620 cm^{-1} and the asymmetric stretching of the nitro to 1620 cm^{-1} and 1518 cm^{-1}, respectively. Results from the current work are in good agreement with theirs, adding to the structural integrity and biological potential of such Schiff base derivatives. According to their findings, the nitro group improves metal complexation, consequently increasing anticancer efficiency (23).

Rasheed et al. (2023) (24)also lastly investigated spiro-5-nitro isatin Schiff bases having FT-IR analysis a major peak of C=N stretching at 1623 cm^{-1} and nitro group vibration, 1523 cm^{-1} and 1337 cm^{-1}. Therefore, these findings are supportive of the reported spectral assignment for the present study, confirming that the nitro group affects the electronic environment of Schiff bases and may consequently assist in increasing the time of the anticancer activity because of the added electron-withdrawing effect (24).

Finally, the spectrum of FT-IR recorded in this work matches well with the spectra reported in the recent works, concerning the C=N and nitro ($-NO_2$) stretching vibrations. The nitrogen group ads is supposed to improve the electronic properties of the Schiff base, possibly improving its biological activity. Comparison of both the spectral features of nitro substituted Schiff bases suggests that they can be promising candidates for anticancer and antimicrobial uses.

The present study shows that the MTT assay results obtained for the synthesized Schiff base derivatives give important information on the cytotoxic effects of them on different cancer cell lines. Our results indicate that in cancerous cells, the Schiff base derivatives are lethal during statistical comparison between the study groups. The IC50 values indicate a dose dependent inhibition since higher concentrations exhibit a higher cytotoxic effect. The present study also identifies the selective toxicity of Schiff base derivatives as normal cells are more viable than the cancer cells and thus indicate a promising therapeutic index.

There has been recent studies about the anticancer properties of Schiff base compounds using the MTT assay come up with similar findings. Their example was Selman et al. (2023) (9), who looked at the anticancer effect of Schiff base derivatives of cholesterol and ergosterol on human esophageal cell lines. Combining MTT assay results, they showed a strong reduction in the viability of cancer cells from derivatives with fluorinated and thiosemicarbazide moieties. All cytotoxic effects were dose dependent as present study demonstrated as it strengthens the hypothesis that Schiff base derivatives can be used as potential anticancer agents (9).

In a synthesis of imidazole-5-one derivatives via Schiff base mechanism and evaluation of their anticancer properties against MCF-7 breast cancer cells using the MTT assay, Bayya et al. (2021) (25) synthesized a series of imidazole-5-one derivatives. They found that their results showed some of the derivatives to be potent cytotoxicity with some derivatives having IC50 values lower than that of doxorubicin, a well-known and widely used chemotherapy drug. The present study's findings concur with the present study's results for their effectiveness of the use of Schiff base derivatives to reduce cancer cell viability but leave normal cells unaffected (25).

Bashiri et al. (2020) (26) studied novel bis-Schiff bases and bis-spiro β-lactams cytotoxicity against HeLa and MCF-7 cell lines using MTT assay. Their results also reported results for their results of certain bis isatin Schiff bases, with IC50 values lower than that of cisplatin and good anticancer activity. This study supports the present study findings of excellent antiproliferative activity of Schiff base derived. This observed in presence of certain substituents such as halogens, nitro groups and found to show consistent trend in the presence with that of the present study (26).

Bashiri et al. (2020) (26) study Schiff base derivatives of methotrexate for their anticancer activity against glioma cell lines. The Schiff base derivatives decreased viability with a dose dependent manner, and the potencies were higher than that of methotrexate alone, she said. The study results of their study confirm the efficacy of Schiff base compounds to cause the cytotoxic effects in a cancer cell, the studies of the present study (27).

In 2024, Zhuang et al. (28)designed Schiff bases of the carbon dots for enhancing their water solubility and anticancer activity. Next, they got their MTT assay results of the reduction in glioma cell viability to a significant extent and with IC50 values in low micromolar range. The results of this study were consistent with the fact that chemical modifications of Schiff bases are relevant with increasing their therapeutic potential, as this study points out the importance of chemical modification of Schiff bases. The results also show that Schiff base derivatives, especially those that are more soluble, have a strong cytotoxic effect that can be use as working cancer treatment materials (28)

In general, this ends here with the MTT assay results for a completely given study in agreement with the results of many recent studies on Schiff base derivatives, which show strong anticancer potential. Schiff bases derivatives shown to have the observed cytotoxicity; selective cancer cell toxicity and increased efficacy of these functional groups imply that they are promising compounds for further anticancer work. The versatility of these compounds can be increase with modification to improve solubility, potency and target specificity through comparative analysis of these compounds with other studies.

Conclusions

In conclusion, the synthesized Schiff base derivatives of this work exhibited powerful anticancer property and especially after added benzothiazole rings demonstrate the best anticancer activity. Cytotoxicity of the Schiff base derivatives against cancer cell lines assessed in vitro, which resulted in the decrease of cancer cell viability in a dose dependent manner and the hydroxyl- substituted derivative showed more cytotoxic effect

than that of the nitro substituted one. One way ANOVA on statistical analysis followed by post hoc Tukey's test confirmed to show the observed cytotoxicity as well as significant inhibition of cell growth at higher concentrations in both derivatives. Furthermore, further potency confirmed by the IC50 values of the compounds, in which the hydroxyl- derivative was superior.

DECLARATIONS

Funding
There is no funding.

Competing interests' statement

No conflict of interest related with publishing of this article.

Ethics statement

I approved that this research follows the journal's ethical guidelines as appeared on the journal's author guidelines page.

Author contributions

No authors contributed me in this manuscript.

Acknowledgments

I extend my thanks and gratitude to Higher Institute of forensic Science- Al Nahrain University, for their support.

Ethics statement

The authors declare that the author approved that this research follows the journal's Attach Ethic Approval guidelines as appeared on the journal's author guidelines page.

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