



Preparation and Cytotoxicity Evaluation of Some Amino Acid Methyl Ester Schiff Bases

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Abstract: In this study, we prepared nine Schiff bases by condensation of amino acid methyl esters (isoleucine, phenylalanine, and methionine) with salicylaldehyde derivatives (2,4-dihydroxybenzaldehyde, 2-hydroxy-3-methoxybenzaldehyde, and 5-bromo-2-hydroxybenzaldehyde) and characterized by various spectroscopic methods (FT-IR, UV-Vis, and NMR techniques). FT-IR and UV-Vis spectra exhibited characteristic peaks for all imine compounds. NMR spectra pointed out the imine bond which is the indicator of the formation of Schiff bases. Besides, antiproliferative and cytotoxic features of the Schiff bases were examined by using MTT cell proliferation and LDH cytotoxicity assays, respectively. Amongst the synthesized Schiff bases, compound **3d** exhibited a very strong antiproliferative effect against all cells except A549. The experimental studies revealed that the Schiff bases synthesized in this study, especially **3d**, have an important potential to enter drug developmental studies.

Keywords: Amino acid Schiff bases, salicylaldehyde, antiproliferative activity, cytotoxicity, NMR spectra.

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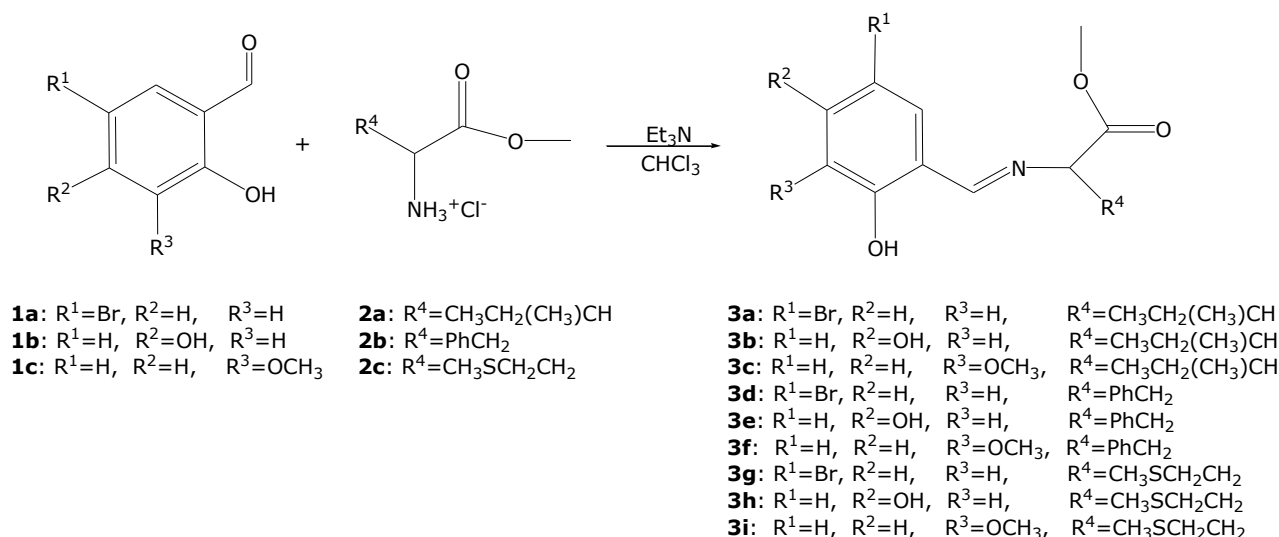
INTRODUCTION

The nitrogen atom in an amine compound can attack to an electrophilic carbon atom of a carbonyl compound under certain conditions like acidic or basic media or with heating. This nucleophilic attack results in a compound known as Schiff base named after Hugo Schiff, who synthesized first this compound group in 1864 (1).

Schiff bases are regarded as very significant compounds because of their potential uses ranging from pharmacology and industry to biology and chemistry. According to the literature survey, Schiff bases have wide applications containing antibacterial (2-6), antifungal (7-11), anticancer (12-17), antimalarial (18-22), and antioxidant (23-28). Besides, they are convenient pioneers of a great number of compounds, such as α -branched amines (29,30), tetramic acids (31), α - and β -amino acids (32,33), bicyclic lactams (34,35), and oxaziridines (36,37).

Amino acid-derived Schiff bases have attracted more attention because of incorporating amino acid component to the structure, taking important parts in chemical processes in living organisms. There are so many studies about the synthesis and the biological activities of amino acid Schiff bases and metal complexes. One of them focused on the effect of two amino acid Schiff bases on certain enzyme activities (38). The results showed that the Schiff bases acted as the activator or inhibitor on total, prostatic and non prostatic acid phosphatase enzymes depending upon their concentration. In another study, Zhang *et al.* synthesized chiral gossypol derivative Schiff bases (39). Some of the Schiff bases exhibited high anticancer activity, even higher than cisplatin anticancer drug. A series of benzaldehyde Schiff bases were prepared and their structure-microbicidal activity correlation was studied by Xia *et al.* (40). They expressed that the Schiff bases exhibited unique antibacterial activity and might be used for therapeutic purposes for bacterial infections. Another Schiff base series with aloin, a bioactive compound obtained from Aloe Vera, and amino acids was examined for anticancer and antioxidant properties (41). The results emphasized the enhanced antioxidant activity of aloin by the Schiff bases. The amino acid Schiff bases with a pyrazole derivative synthesized by Joksovic *et al.* were studied for anticancer activity (42). According to the results, the phenylalanine Schiff base was the most active compound against leukemic cell lines.

The purpose of this work was to synthesize nine Schiff bases (**3a-i**), from which the reduced form of **3a** was known (43), from amino acid methyl esters and salicylaldehyde derivatives (Scheme 1). Structures of the obtained Schiff base compounds were elucidated by FT-IR and UV-Vis spectrometry, elemental analysis, $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ techniques. Besides, cytotoxic activities of aforesaid compounds were investigated.



Scheme 1: The Schiff bases synthesized in this study.

MATERIALS AND METHODS

Chemistry

¹H-NMR and ¹³C-NMR spectra of all imine compounds were measured using AC Bruker 400 MHz NMR spectrometer in DMSO-d₆ at ambient temperature. FT-IR spectra were recorded on a Jasco FT-IR 430 spectrometer in the range of 400-4000 cm⁻¹ by using KBr pellets. UV-Vis absorption spectra were measured using a Perkin-Elmer Lambda 35 Spectrophotometer in a 10 mm quartz cell. The concentration of the Schiff base solutions prepared in methanol was about 10⁻⁴ M. Elemental analyses were recorded on a Elementar Vario Micro Cube elemental analyzer. Isoleucine, phenylalanine and methionine were used without purification and these amino acids were converted to corresponding methyl esters according to the literature (44). Solvents were used as received from commercial sources.

Experimental Procedure For the Preparation of the Amino Acid Schiff Bases

A round-bottomed flask containing amino acid methyl ester (**2a-c**) (1 mmol), N(Et)₃ (0.7 mmol) and CHCl₃ (20 mL) was fitted with a reflux condenser. This mixture was refluxed until all amino acid was dissolved. When all content was dissolved, salicylaldehyde derivative (**1a-c**) (0.7 mmol) was added to the clear solution. The reaction mixture was refluxed a couple of days long. During this time the color of the solution has changed to yellow. The completion of the reaction was followed by TLC. After completion of the reaction, the reaction mixture was extracted with CHCl₃ and 1 M HCl. The extract was dried using Na₂SO₄ and evaporated under vacuum.

3a: Yield 65%; FT-IR (KBr, cm⁻¹): 3458 (O-H), 2963,2935,2876 (aliphatic C-H), 1737 (C=O), 1630 (azomethine, C=N), 1256 (C-O phenolic); ¹H-NMR (DMSO-d₆, δ, ppm): 0.84 (3H, d, ³J_{HH}=1.5, CH₃), 0.86 (3H, t, ³J_{HH}=7.3, CH₃), 1.14 (H₁, m, CH₂), 1.43 (H₂, m, CH₂), 2.00 (1H, m, CH), 3.67 (3H, s, OCH₃), 4.02 (1H, d, ³J_{HH}=5.5, CH), 6.86 (1H, d, ³J_{HH}=8.8, Ph), 7.48 (1H, dd,

$^3J_{HH}=8.8$, $^4J_{HH}=2.5$, Ph), 7.68 (1H, d, $^4J_{HH}=2.5$, Ph), 8.53 (1H, s, azomethine), 13.39 (1H, s, OH). $^{13}\text{C-NMR}$ (DMSO- d_6 , δ , ppm): 11.16, 15.56, 24.54, 37.98, 51.94 (OCH₃), 74.84, 109.38 (PhBr), 119.01, 120.19, 133.63, 135.17, 159.68 (PhOH), 166.38 (azomethine), 170.99 (C=O). Anal. calcd. for C₁₄H₁₈BrNO₃ (328.2 g/mol): C, 51.23; H, 5.53; N, 4.27. Found: C, 51.68; H, 6.27; N, 4.96%.

3b: Yield 94%; FT-IR (KBr, cm^{-1}): 3371 (O-H), 2960,2932,2876 (aliphatic C-H), 1737 (C=O), 1638 (azomethine, C=N), 1227 (C-O phenolic); $^1\text{H-NMR}$ (DMSO- d_6 , δ , ppm): 0.83 (3H, d, $^3J_{HH}=6.7$, CH₃), 0.86 (3H, t, $^3J_{HH}=7.7$, CH₃), 1.12 (H₁, m, CH₂), 1.42 (H₂, m, CH₂), 1.96 (1H, m, CH), 3.65 (3H, s, OCH₃), 3.91 (1H, d, $^3J_{HH}=5.6$, CH), 6.20 (1H, d, $^4J_{HH}=2.5$, Ph), 6.30 (1H, dd, $^3J_{HH}=8.5$, $^4J_{HH}=2.5$, Ph), 7.21 (1H, d, $^3J_{HH}=8.5$, Ph), 8.37 (1H, s, azomethine), 13.64 (1H, s, OH). $^{13}\text{C-NMR}$ (DMSO- d_6 , δ , ppm): 11.16, 15.63, 24.54, 37.91, 51.83 (OCH₃), 74.43, 102.40, 107.22, 111.19, 133.57, 161.97 (azomethine), 163.44 (PhOH), 166.77 (PhOH), 171.48 (C=O). Anal. calcd. for C₁₄H₁₉NO₄ (265.3 g/mol): C, 63.38; H, 7.22; N, 5.28. Found: C, 63.51; H, 7.26; N, 4.87%.

3c: Yield 73%; FT-IR (KBr, cm^{-1}): 3424 (O-H), 2963,2935,2876 (aliphatic C-H), 1739 (C=O), 1630 (azomethine, C=N), 1226 (C-O phenolic); $^1\text{H-NMR}$ (DMSO- d_6 , δ , ppm): 0.84 (3H, d, $^3J_{HH}=7.7$, CH₃), 0.87 (3H, t, $^3J_{HH}=6.8$, CH₃), 1.15 (H₁, m, CH₂), 1.43 (H₂, m, CH₂), 2.03 (1H, m, CH), 3.67 (3H, s, OCH₃), 3.77 (3H, s, OCH₃), 4.03 (1H, d, $^3J_{HH}=5.4$, CH), 6.82 (1H, t, $^3J_{HH}=7.9$, Ph), 7.03 (1H, d, $^3J_{HH}=7.9$, Ph), 7.05 (1H, d, $^3J_{HH}=7.9$, Ph), 8.53 (1H, s, azomethine), 13.51 (1H, s, OH). $^{13}\text{C-NMR}$ (DMSO- d_6 , δ , ppm): 11.20, 15.62, 24.56, 37.94, 51.91 (OCH₃), 55.81 (OCH₃), 74.65, 115.25, 118.11, 118.28, 123.34, 147.95 (Ph-OCH₃), 151.04 (PhOH), 167.93 (azomethine), 171.19 (C=O). Anal. calcd. for C₁₅H₂₁NO₄ (279.3 g/mol): C, 64.50; H, 7.58; N, 5.01. Found: C, 64.86; H, 8.01; N, 5.52%.

3d: Yield 78%; FT-IR (KBr, cm^{-1}): 3382 (O-H), 3087,3061,3028 (aromatic C-H), 2950,2901 (aliphatic C-H), 1744 (C=O), 1630 (azomethine, C=N), 1276 (C-O phenolic); $^1\text{H-NMR}$ (DMSO- d_6 , δ , ppm): 3.09 (1H, dd, $^2J_{HH}=13.6$, $^3J_{HH}=8.2$, CH₂), 3.25 (1H, dd, $^2J_{HH}=13.6$, $^4J_{HH}=5.3$, CH₂), 3.65 (3H, s, OCH₃), 4.43 (1H, dd, $^3J_{HH}=8.2$, $^4J_{HH}=5.3$, CH), 6.85 (1H, d, $^3J_{HH}=8.8$, Ph), 7.18 (3H, m, Ph), 7.26 (2H, m, Ph), 7.45 (1H, dd, $^3J_{HH}=8.8$, $^4J_{HH}=2.5$, Ph), 7.57 (1H, d, $^4J_{HH}=2.5$, Ph), 8.32 (1H, s, azomethine), 13.01 (1H, s, OH). $^{13}\text{C-NMR}$ (DMSO- d_6 , δ , ppm): 51.39, 52.15 (OCH₃), 71.13, 118.98 (PhBr), 120.24, 126.31, 126.60, 128.27, 129.35, 133.39, 135.19, 136.68, 159.35 (PhOH), 166.17 (azomethine), 170.82 (C=O). Anal. calcd. for C₁₇H₁₆BrNO₃ (362.2 g/mol): C, 56.37; H, 4.45; N, 3.87. Found: C, 56.97; H, 4.63; N, 3.51%.

3e: Yield 63%; FT-IR (KBr, cm^{-1}): 3390 (O-H), 3059,3028 (aromatic C-H), 2950,2926 (aliphatic C-H), 1741 (C=O), 1626 (azomethine, C=N), 1221 (C-O phenolic); $^1\text{H-NMR}$ (DMSO- d_6 , δ , ppm): 3.04 (1H, dd, $^2J_{HH}=13.6$, $^3J_{HH}=8.2$, CH₂), 3.21 (1H, dd, $^3J_{HH}=13.6$, $^4J_{HH}=5.4$, CH₂), 3.64 (3H, s,

OCH₃), 4.33 (1H, dd, ³J_{HH}=8.2, ⁴J_{HH}=5.4, CH), 6.19 (1H, d, ⁴J_{HH}=2.2, Ph), 6.27 (1H, dd, ³J_{HH}=8.4, ⁴J_{HH}=2.2, Ph), 7.09 (1H, d, ³J_{HH}=8.4, Ph), 7.18 (3H, m, Ph), 7.25 (2H, m, Ph), 8.15 (1H, s, azomethine), 13.29 (1H, s, OH). ¹³C-NMR (DMSO-d₆, δ, ppm): 52.03, 70.74 (OCH₃), 79.16, 102.34, 107.26, 111.15, 126.50, 128.23, 129.35, 133.53, 136.97, 161.90 (azomethine), 162.96 (PhOH), 166.88 (PhOH), 171.27 (C=O). Anal. calcd. for C₁₇H₁₇NO₄ (299.3 g/mol): C, 68.21; H, 5.72; N, 4.68. Found: C, 68.16; H, 5.91; N, 4.44%.

3f: Yield 88%; FT-IR (KBr, cm⁻¹): 3412 (O-H), 3061,3028,3003 (aromatic C-H), 2950,2938 (aliphatic C-H), 1741 (C=O), 1632 (azomethine, C=N), 1224 (C-O phenolic); ¹H-NMR (DMSO-d₆, δ, ppm): 3.08 (1H, dd, ²J_{HH}=13.5, ³J_{HH}=8.3, CH₂), 3.25 (1H, dd, ²J_{HH}=13.5, ⁴J_{HH}=5.3, CH₂), 3.66 (3H, s, OCH₃), 3.76 (3H, s, OCH₃), 4.44 (1H, dd, ³J_{HH}=8.3, ⁴J_{HH}=5.3, CH), 6.78 (1H, t, ³J_{HH}=7.9, Ph), 6.90 (1H, d, ³J_{HH}=7.9, Ph), 7.03 (1H, d, ³J_{HH}=7.9, Ph), 7.19 (3H, m, Ph), 7.26 (2H, m, Ph), 8.32 (1H, s, azomethine). ¹³C-NMR (DMSO-d₆, δ, ppm): 51.29, 52.11 (OCH₃), 55.73 (OCH₃), 70.97, 115.16, 118.19, 123.20, 126.24, 126.56, 128.08, 128.26, 129.16, 129.37, 137.91, 147.83 (Ph-OCH₃), 150.58 (PhOH), 167.94 (azomethine), 170.99 (C=O). Anal. calcd. for C₁₈H₁₉NO₄ (313.4 g/mol): C, 68.99; H, 6.11; N, 4.47. Found: C, 69.71; H, 6.82 N, 4.64%.

3g: Yield 86%; FT-IR (KBr, cm⁻¹): 3436 (O-H), 2953,2916,2840 (aliphatic C-H), 1741 (C=O), 1632 (azomethine, C=N), 1276 (C-O phenolic); ¹H-NMR (DMSO-d₆, δ, ppm): 2.03 (3H, s, SCH₃), 2.08 (H₁, m, CH₂), 2.18 (H₂, m, CH₂), 2.42 (H₁, m, CH₂), 2.49 (H₂, m, CH₂), 3.68 (3H, s, OCH₃), 4.30 (1H, dd, ³J_{HH}=7.9, ⁴J_{HH}=5.0, CH), 6.87 (1H, d, ³J_{HH}=8.8, Ph), 7.48 (1H, dd, ³J_{HH}=8.8, ⁴J_{HH}=2.5, Ph), 7.71 (1H, d, ⁴J_{HH}=2.5, Ph), 8.57 (1H, s, azomethine). ¹³C-NMR (DMSO-d₆, δ, ppm): 14.46 (SCH₃), 29.20 (SCH₂), 32.15, 52.25 (OCH₃), 68.52, 109.55 (PhBr), 118.97, 120.40, 133.55, 135.23, 159.36 (PhOH), 166.55 (azomethine), 171.05 (C=O). Anal. calcd. for C₁₃H₁₆BrNO₃S (346.2 g/mol): C, 45.10; H, 4.66; N, 4.05. Found: C, 45.38; H, 5.54; N, 4.73%.

3h: Yield 69%; FT-IR (KBr, cm⁻¹): 3412 (O-H), 2953,2916,2837 (aliphatic C-H), 1740 (C=O), 1630 (azomethine, C=N), 1226 (C-O phenolic); ¹H-NMR (DMSO-d₆, δ, ppm): 2.02 (3H, s, SCH₃), 2.06 (2H, m, CH₂), 2.47 (2H, m, CH₂), 3.66 (3H, s, OCH₃), 4.19 (1H, s, CH), 6.21 (1H, s, Ph), 6.32 (1H, d, ³J_{HH}=8.0, Ph), 7.24 (1H, d, ³J_{HH}=8.0, Ph), 8.40 (1H, s, azomethine), 13.20 (1H, s, OH). ¹³C-NMR (DMSO-d₆, δ, ppm): 14.50 (SCH₃), 29.30 (SCH₂), 32.33, 52.14 (OCH₃), 68.08, 102.35, 107.37, 111.20, 133.66, 162.04 (azomethine), 162.96 (PhOH), 167.28 (PhOH), 171.49 (C=O). Anal. calcd. for C₁₃H₁₇NO₄S (283.3 g/mol): C, 55.11; H, 6.05; N, 4.94. Found: C, 55.80; H, 6.63; N, 5.71%.

3i: Yield 78%; FT-IR (KBr, cm⁻¹): 3435 (O-H), 2950,2913,2837 (aliphatic C-H), 1740 (C=O), 1630 (azomethine, C=N), 1227 (C-O phenolic); ¹H-NMR (DMSO-d₆, δ, ppm): 2.02 (3H, s, SCH₃), 2.10 (H₁, m, CH₂), 2.18 (H₂, m, CH₂), 2.43 (H₁, m, CH₂), 2.50 (H₂, m, CH₂), 3.67 (3H, s, OCH₃), 3.78 (3H, s, OCH₃), 4.30 (1H, dd, ³J_{HH}=7.9, ⁴J_{HH}=5.0, CH), 6.83 (1H, t, ³J_{HH}=7.9, Ph), 7.05 (1H,

d, $^3J_{HH}=7.9$, Ph), 7.06 (1H, d, $^3J_{HH}=7.9$, Ph), 8.58 (1H, s, azomethine), 13.12 (1H, s, OH). ^{13}C -NMR (DMSO- d_6 , δ , ppm): 14.48 (SCH₃), 29.25 (SCH₂), 32.23, 52.21 (OCH₃), 55.82 (OCH₃), 68.36, 115.33, 118.27, 118.41, 123.36, 147.89 (Ph-OCH₃), 150.63 (Ph-OH), 168.31 (azomethine), 171.19 (C=O). Anal. calcd. for C₁₄H₁₉NO₄S (297.4 g/mol): C, 56.55; H, 6.44; N, 4.71. Found: C, 57.28; H, 6.89; N, 5.00%.

BIOLOGICAL ACTIVITY

MTT Cell Proliferation Assay

HT29 (Human colorectal adenocarcinoma, ATCC[®] HTB-38[™]), HeLa (Human cervix adenocarcinoma, ATCC[®] CCL-2[™]), MCF7 (Human breast adenocarcinoma, ATCC[®] HTB22[™]), A549 (Human lung carcinoma, ATCC[®] CCL185[™]), C6 (Rat brain glioma, ATCC[®] CCL-107[™]), and Hep3B (Human hepatocellular carcinoma, ATCC[®] HB8064[™]) cancer cells and, FL (Human amnion cells, ATCC[®] CCL62[™]) and Vero (African green monkey kidney normal epithelial, ATCC[®] CCL-81[™]) normal cells were kept in a suitable medium involving fetal bovine serum and antibiotic solution. The cell suspension was adjusted to 1×10^6 cells in 10 mL and transferred 100 μL into the each well in culture plates. The compounds dissolved in sterile DMSO at 10-200 $\mu\text{g}/\text{mL}$ final concentration were transferred to the cells and incubated at 37 °C under 5% CO₂ atmosphere overnight. The cytotoxic activity of the Schiff bases was examined using MTT cell proliferation assay noted as percent inhibition. The percent inhibition calculated as shown below:

$$\% \text{inhibition} = [1 - (\text{Absorbance of Treatments} / \text{Absorbance of DMSO}) \times 100].$$

The half maximal inhibitory concentration (IC₅₀) of the compounds was noted in $\mu\text{g}/\text{mL}$ at 95 % confidence intervals. GI₅₀ values were calculated by following:

$$[(\text{Ti}-\text{Tz})/(\text{C}-\text{Tz})] \times 100 = 50,$$

which is the drug concentration bringing about a 50% reduction in the net growth in control cells in the course of the drug incubation.

Total growth inhibition (TGI) was calculated by $\text{Ti} = \text{Tz}$. The LC₅₀ indicating a net loss of cells following treatment was calculated according to the formula below:

$$[(\text{Ti}-\text{Tz})/\text{Tz}] \times 100 = -50.$$

Cytotoxicity Assay

The cytotoxicity of the Schiff bases was determined by using the cytosolic LDH measurement kit. Into each well, 5×10^3 cells were conveyed as triplicates in order to expose to IC₅₀

concentrations of the test compounds and incubated at 37 °C under 5% CO₂ atmosphere overnight. The percentage cytotoxicity was obtained by the following equation:

$$(\text{experimental value} - \text{low control} / \text{high control} - \text{low control}) \times 100,$$

in which experimental value is the test-compound treated cells, high control (maximum LDH release) is Triton X-100 treated cells, low control (spontaneous LDH release) is the untreated cells.

Cell imaging

HeLa and C6 cells were plated in 96-well plates at a density of 5.000 cells per well and awaited for 24 h. The compound **3d** in a concentration dependent manner was added and morphological changes of the cells were screened by phase contrast microscopy in every 6 h for 24 h. Images of the treated and untreated cells were photographed after the completion of the process by an inverted microscope equipped with a digital camera.

Statistical Analysis

The statistical significance of the variations was determined by the one-way analyses of variance (one-way ANOVA) followed by Tukey's test. SPSS for Windows was used for statistical analyses.

RESULTS AND DISCUSSION

The Schiff bases were obtained by refluxing amino acid esters with salicylaldehyde derivatives in a chloroform medium under basic conditions. The condensation products were obtained in a couple of days with moderate to high yields. Azomethines were yellow oily compounds which did not need further purification. The compounds were soluble in common organic solvents (methanol, ethanol, CHCl₃, CH₂Cl₂, etc.) Their structures were proved by elemental analysis and, UV-Vis, FT-IR, ¹H- and ¹³C-NMR spectroscopic methods.

FT-IR and UV-Vis Spectra

The positions of the important IR bands were shown in the experimental part and the FT-IR spectra of **3a** was depicted in Figure 1. Sharp absorption bands between 1638-1626 cm⁻¹ were ascribed to the CH=N stretching bands for all of the Schiff bases. Aromatic hydroxy substituent participating in intramolecular hydrogen bonding with azomethine nitrogen atom was observed as a broad band about 3400 cm⁻¹. The sharp absorption bands near 1740 cm⁻¹ were related to the asymmetric carboxylate stretches (COO). The Ph-O vibrations were observed around 1276-1221 cm⁻¹. Symmetric and asymmetric aliphatic C-H stretching bands were assigned to 2953-2837 cm⁻¹ range. As it comes to aromatic C-H protons, they could not be observed in **3a-c** and **3g-i** Schiff bases, however the FT-IR spectra of **3d-f** Schiff bases exhibited aromatic proton

stretching vibration peaks because of existence of aromatic protons of phenylalanine in addition to those of aldehyde part.

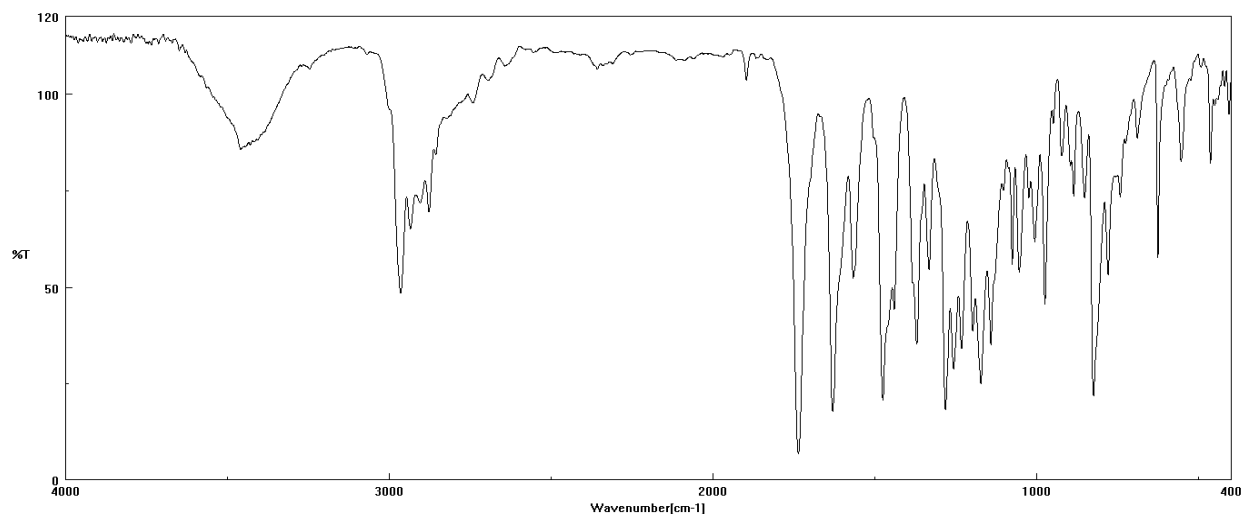


Figure 1: FT-IR spectrum of **3a**.

UV-Vis studies were also performed for all of the Schiff bases by dissolving in methanol. UV-Vis spectra exhibited three bands for all compounds as listed in Table 1.

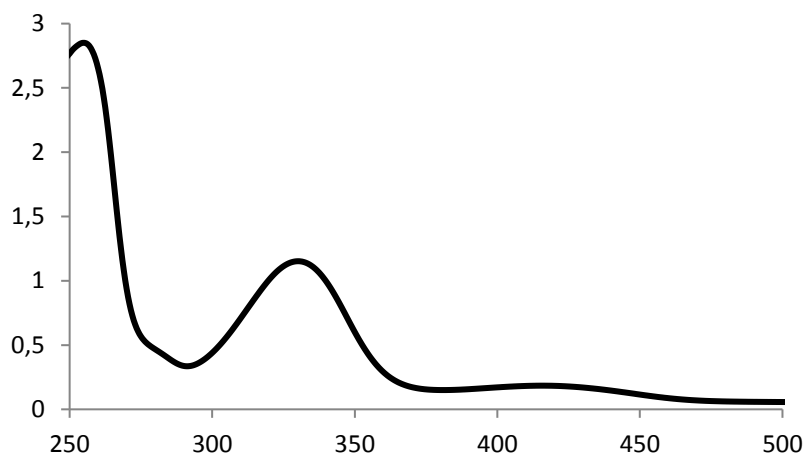


Figure 2: UV-Vis spectrum of **3a**.

A high energy band observed below 300 nm can be assigned to $\pi \rightarrow \pi^*$ transitions carried out in aromatic rings. The band at 376 and 379 nm in phenylalanine Schiff bases **3b**, **3e** and **3h** can be sprung from charge transfer within the whole Schiff base molecule. That kind of band is caused by the strong intramolecular hydrogen bond between the hydroxyl and the azomethine group of o-hydroxyl Schiff bases (45). As it comes to the bands observed in the range of 327-424 nm, they can be assigned to the $n \rightarrow \pi^*$ transitions for the isoleucine and methionine Schiff bases.

¹H- and ¹³C-NMR Spectra

The ¹H- and ¹³C-NMR spectra of all of the Schiff bases were consistent with the suggested structures. The ¹H- and ¹³C-NMR spectra for **3a** were demonstrated in Figure 3. A singlet peak about 13.5 ppm was attributed to the hydroxyl group of all imine compounds. The azomethine peak, an evidence for the Schiff base formation, was observed as a singlet in the range of 8.15-8.58 ppm. Methoxy group belonging to the amino acid ester induced to a singlet about 3.65 ppm. Aromatic ring protons were observed at 6.20-7.71 ppm as doublets and triplets for isoleucine (**3a-c**) and methionine (**3g-i**) Schiff bases. Same protons appeared in the similar region, but as overlapped with aromatic protons of phenylalanine for the Schiff bases **3d-f**. As it comes to aliphatic protons, they were observed in characteristic methyl, methylene and methyne region as shown in the experimental.

BIOLOGICAL ACTIVITY

Antiproliferative Actions of the Compounds

The antiproliferative activities of the Schiff bases and positive control against cell lines were screened according to a cell proliferation assay performed by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The antiproliferative actions and selectivity of the Schiff bases were evaluated through tumor cells and normal cells (Vero and FL). Also, statistically significant differences ($P < 0.05$) attracted the attention among cancer cell lines compared to normal cell lines for the different concentrations. According to TGI and IC₅₀ values in Table 2, **3d** displayed very strong cytotoxic activity than the control drugs, 5-fluorouracil and cisplatin, on all cancer cell lines (TGI 5.81-52.36 µg/mL, IC₅₀ 5.72-46.35), except for A549. Compounds **3c**, **3e**, **3h** and **3i** were effective on HeLa (TGI 44.24 µg/mL, IC₅₀ 41.99±2.9 µg/mL), HT29 (TGI 28.19 µg/mL, IC₅₀ 18.58±3.4 µg/mL), and C6 (TGI 38.70 µg/mL, IC₅₀ 36.33±3.8 for **3h** and TGI 3.75 µg/mL, IC₅₀ 3.69±2.7 µg/mL for **3i**) cell lines, respectively (Table 2-4) (Figure 4). For HeLa and HT29 cell lines, compounds **3a**, **3b**, **3f**, and **3g** had remarkable TGI values (Table 3-4). The IC₅₀ values of the **3a**, **3b**, **3f**, and **3g** (2.79-23.31, 12.49-64.17, 21.98-26.59, and 6.27-89.89 µg/mL, respectively) for these two cancer lines were similar to the TGI parameters. **3a** and **3b** also had potent cytotoxic activity on C6 cells (TGI 5.63, IC₅₀ 5.42 and TGI 7.59, IC₅₀ 7.49 µg/mL, respectively) (Table 3-4) (Figure 4). For C6 cells, compounds **3b**, **3d** and **3i** had remarkable LC₅₀ values (37.21, 26.76 and 17.39 µg/mL, respectively) (Figure 4). However, the LC₅₀ values of compounds **3a** and **3g** were only meaningful for HeLa cell line (11.09 and 29.73 µg/mL, respectively) (Tables 2-4) (Figure 4). Compound **3a** had the best GI₅₀ values (1.55, 1.93, and 1.78 µg/mL, respectively) when it came to the HeLa, MCF7, and C6 cell lines. In addition, compound **3d** and **3e** had remarkable GI₅₀ values (1.68 and 1.84 µg/mL, respectively) for MCF7 cell line. Compound **3i** exhibited significant GI₅₀ value (1.84 µg/mL) for C6 cell line.

Table 1: UV-Vis spectral data for the Schiff bases in methanol.

Compound	λ (nm)
3a	255
	330
3b	282
	307
3c	265
	327
3d	255
	330
3e	282
	310
3f	264
	328
3g	255
	330
3h	282
	310
3i	265
	330

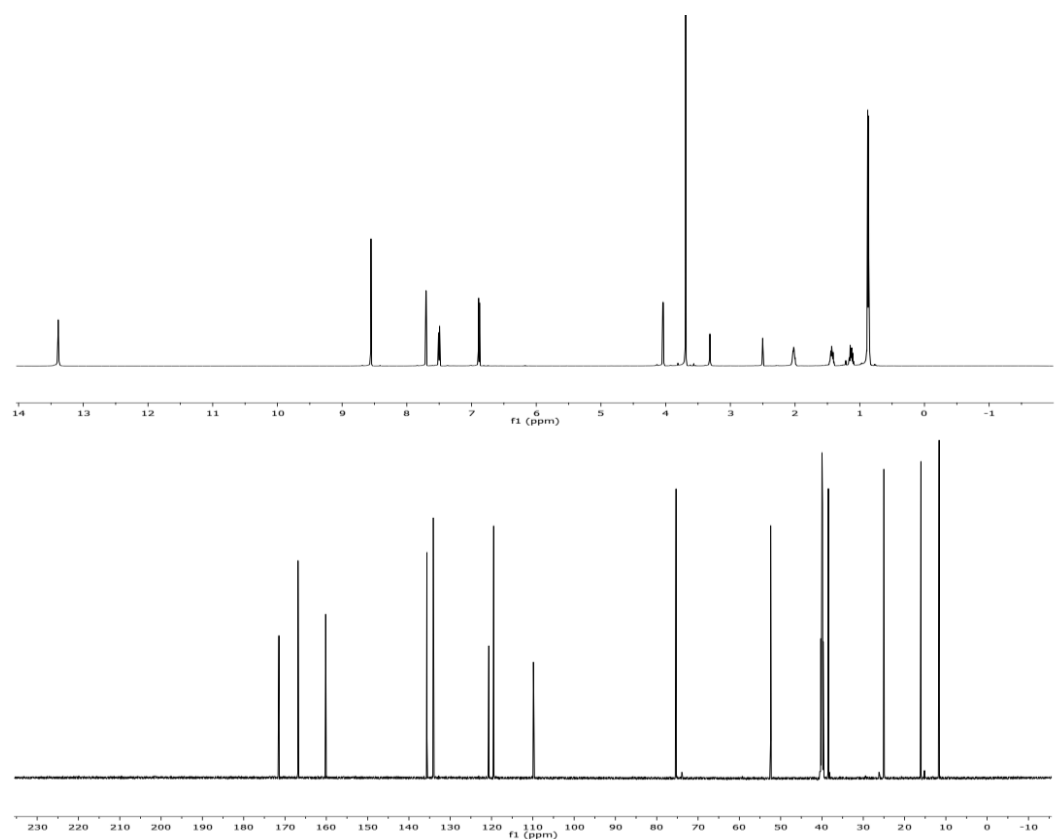
**Figure 3:** ^1H - and ^{13}C -NMR spectra of **3a**.

Table 2: GI50, TGI, LC50 and IC50 values for **3a**, **3b**, and **3c**.

$\mu\text{g/mL}$	3a				3b				3c			
	GI50	TGI	LC50	IC50	GI50	TGI	LC50	IC50	GI50	TGI	LC50	IC50
A549	68.49 ± 8.34	>10000	>10000	>10000	104.53 ± 8.4	>10000	>10000	>10000	18.89 ± 1.0	>10000	>10000	>10000
HeLa	1.55 \pm 0.08	2.80 \pm 0. 08	11.09 \pm 0 .77	2.79 \pm 0. 07	2.92 \pm 0 .21	12.70 \pm 0 .64	416.42 \pm 15.3	12.49 \pm 0 .71	2.64 \pm 0.12	44.24 \pm 3 .7	>10000	41.99 \pm 3 .1
HT29	4.18 \pm 0.17	28.39 \pm 1 .3	2504.05 ± 51.9	23.31 \pm 0 .89	3.79 \pm 0 .25	115.02 \pm 7.9	>10000	64.17 \pm 4 .3	3.60 \pm 0.17	3613.74 ± 54.6	>10000	702.60 \pm 28.3
Hep3B	3.81 \pm 0.11	1316.12 ± 47.5	>10000	1133.57 ± 33.9	6.53 \pm 0 .37	>10000	>10000	>10000	5.11 \pm 0.43	>10000	>10000	>10000
MCF7	1.93 \pm 0.09	1296.89 ± 42.8	>10000	904.30 \pm 28.4	2.68 \pm 0 .29	4169.61 ± 61.7	>10000	2833.66 ± 59.2	1.68 \pm 0.08	2099.80 ± 72.3	>10000	1347.86 ± 54.8
C6	1.78 \pm 0.09	5.63 \pm 0. 46	176.18 \pm 14.5	5.42 \pm 0. 18	3.11 \pm 0 .20	7.59 \pm 0. 76	37.21 \pm 2 .1	7.49 \pm 0. 33	5.32 \pm 0.51	5675.32 ± 71.4	>10000	3796,80 ± 65.4
Vero	3.50 \pm 0.21	10.55 \pm 0 .98	83.99 \pm 1 1.0	10.26 \pm 0 .34	2.43 \pm 0 .19	115.33 \pm 5.6	>10000	92.31 \pm 5 .32	4.03 \pm 0.36	248.67 \pm 17.9	>10000	195.66 \pm 15.8
FL	4.21 \pm 0.27	31.57 \pm 1 .4	3977.09 ± 71.4	30.91 \pm 1 .5	3.63 \pm 0 .27	19.90 \pm 2 .7	1020.77 ± 22.6	19.54 \pm 1 .4	4.14 \pm 0.37	2736.46 ± 65.1	>10000	2394.84 ± 69.1

Values are given as the mean \pm SD of three experiments and $r^2=0.86$ to 0.97 . Significant at $P < 0.05$

Table 3: GI50, TGI, LC50 and IC50 values for **3d**, **3e**, and **3f**.

$\mu\text{g/mL}$	3d				3e				3f			
	GI50	TGI	LC50	IC50	GI50	TGI	LC50	IC50	GI50	TGI	LC50	IC50
A549	74.49 ± 4.3	>10000	>10000	>10000	152.22 ± 9.7	>10000	>10000	>10000	32.98 ± 1.2	>10000	>10000	>10000
HeLa	3.06 \pm 0.41	13.63 \pm 0.49	442.07 \pm 21.0	13.39 \pm 0.77	3.59 \pm 0 .63	108.90 ± 9.1	>10000	102.28 \pm 5.7	3.28 \pm 0.54	22.56 \pm 0.87	3513.62 ± 71.6	21.98 \pm 0 .88
HT29	2.63 \pm 0.22	14.97 \pm 0.41	1933.71 ± 59.7	12.01 \pm 0.63	2.48 \pm 0 .57	28.19 \pm 0.93	>10000	18.58 \pm 1 .1	3.29 \pm 0.21	36.87 \pm 0.93	>10000	26.59 \pm 0 .87
Hep3B	3.07 \pm 0.37	30.62 \pm 0.97	>10000	29.55 \pm 1.5	4.67 \pm 0 .45	>10000	>10000	>10000	7.13 \pm 0.48	>10000	>10000	>10000
MCF7	2.19 \pm 0.33	52.36 \pm 1.4	>10000	46.35 \pm 1.7	1.84 \pm 0 .24	814.18 ± 19.2	>10000	581.23 \pm 17.8	3.96 \pm 0.27	>10000	>10000	>10000
C6	2.56 \pm 0.31	5.81 \pm 0. 37	26.76 \pm 1 .0	5.72 \pm 0. 19	9.61 \pm 0 .61	>10000	>10000	7380.55 ± 51.0	4.13 \pm 0.18	2897.4 ± 62.7	>10000	1962.94 ± 21.3
Vero	2.34 \pm 0.34	12.43 \pm 0.42	1740.98 ± 44.0	11.61 \pm 0.23	4.64 \pm 0 .42	100.54 ± 8.7	>10000	86.56 \pm 5 .9	3.76 \pm 0.19	36.31 \pm 0.99	>10000	32.89 \pm 1 .1
FL	3.58 \pm 0.33	20.70 \pm 0.88	1327.85 ± 42.5	20.31 \pm 0.41	4.20 \pm 0 .39	66.21 \pm 4.3	>10000	63.61 \pm 5 .3	5.53 \pm 0.34	265.96 ± 8.6	>10000	250.97 \pm 7.4

Values are given as the mean \pm SD of three experiments and $r^2=0.86$ to 0.97 . Significant at $P < 0.05$

Table 4: GI50, TGI, LC50 and IC50 values for **3g**, **3h**, and **3i**.

<i>µg/mL</i>	3g				3h				3i			
	GI50	TGI	LC50	IC50	GI50	TGI	LC50	IC50	GI50	TGI	LC50	IC50
A549	145.66± 14.7	>10000	>10000	>10000	27.42± 1.2	>10000	>10000	>10000	26.43 ±0.97	>10000	>10000	>10000
HeLa	2.72± 0.09	6.31±0 .30	29.73 ±1.7	6.27± 0.43	5.36± 0.52	3123.2 ±57.8	>10000	2708.12 ±59.2	3.75± 0.44	116.36 ±5.4	>10000	108.75± 9.8
HT29	3.71± 0.13	160.88± 15.2	>10000	89.89± 11.4	2.56± 0.43	>10000	>10000	>10000	2.86± 0.11	1193.61 ±14.9	>10000	268.47± 10.1
Hep3B	4.42± 0.22	996.31± 37.8	>10000	874.91 ±32.1	2.74± 0.37	>10000	>10000	>10000	7.23± 0.28	>10000	>10000	>10000
MCF7	2.15± 0.14	374.11± 24.7	>10000	293.37 ±17.4	3.09± 0.39	2657.17 ±54.6	>10000	1903.43 ±51.3	2.43± 0.14	>10000	>10000	>10000
C6	4.79± 0.25	>10000	>10000	>10000	4.98± 0.40	38.70± 1.6	4110.5 ±67.4	36.33± 1.1	1.84± 0.07	3.75± 0.19	17.39± 2.2	3.69± 0.55
Vero	4.06± 0.31	17.57± 1.7	349.06± 27.6	16.84± 0.49	3.00± 0.35	>10000	>10000	>10000	3.35± 0.34	647.63± 13.5	>10000	452.61± 12.7
FL	3.43± 0.27	26.20± 1.8	5663.91 ±77.3	25.60± 1.0	5.06± 0.46	>10000	>10000	9447.3 ±71.4	6.82± 0.95	1625.41 ±37.2	>10000	1475.11 ±47.6

Values are given as the mean ± SD of three experiments and $r^2=0.86$ to 0.97 . Significant at $P < 0.05$

Some cancer cells were more sensitive to the tested Schiff bases than FL and Vero normal cells. A comparison of the activities of these compounds in regard to the IC₅₀ values as against normal cells (FL and Vero) revealed the following: **3d**, **3e**, **3h** for HT29, **3a**, **3b**, **3c**, **3d**, **3e**, **3f**, **3g** for HeLa and **3a**, **3b**, **3d**, **3h**, **3i** for C6 were more selective than others. Hence, the findings significantly highlight some compounds as possible selective antiproliferative agents for some cancer types. The pharmacological activities of these compounds are probably due to the major bioactive substituents such as hydroxy, methoxy, phenyl, methylthio, and bromine. Especially, bromine group may be responsible for cytotoxic activities of **3a**, **3d** and **3g** having the highest pharmacological activity against cancer cells. The higher antiproliferative activity of compound **3d** than **3a** points out the importance of the phenyl substituent on cytotoxic activity. Overall, the high cytotoxic effects of these compounds may be related to their unique three-dimensional structures arising from the substituents. It was noted that three parameters (GI₅₀, TGI, LC₅₀) of these compounds met NCI-60 criteria enough to pass into further pharmacological investigations.

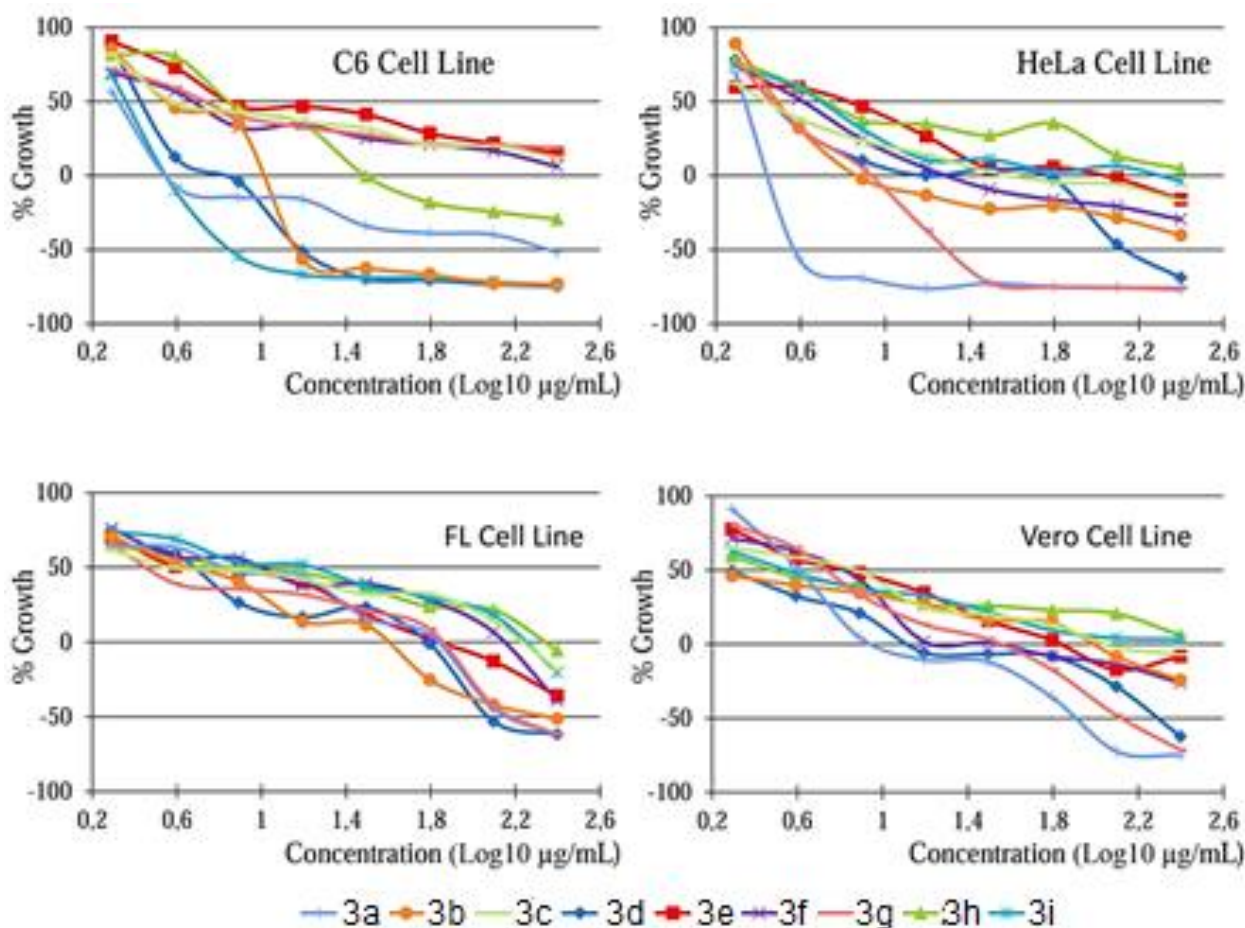


Figure 4: Effects of the compounds on the proliferation of C6, HeLa, FL, and Vero cells. Exponentially growing cells were incubated with the Schiff bases for 24 hours.

Table 5: IC₅₀ (µg/mL) of positive controls in cell lines.

	HeLa	HT29	A549	MCF7	C6	Hep3B	FL	Vero
Cisplatin	50.29±	40.39±	60.49±	63.79±	33.08±	48.69±	52.79±	56.20±
	8.3	5.6	8.5	7.4	4.8	5.3	6.0	5.3
5FU	61.59±	65.19±	69.79±	74.19±	54.30±	62.89±	59.09±	65.35±
	7.1	5.3	8.1	8.0	7.3	6.8	6.4	5.9

Values are given as the mean ± SD of three experiments and $r^2=0.94$ to 0.99 . Significant at $P < 0.05$

Cytotoxic Mode of the Compounds

Cytotoxic activities of the Schiff bases (25, 50, 75, and 100 µg/mL) on cell lines were monitored by the LDH cytotoxicity kit. The %cytotoxicity values of these compounds at concentration of 25 µg/mL were in the range of 6.21%-34.74% against all cell lines. Compound **3b** caused the most powerful cytotoxic effect (34.71%, $p<0.05$) against C6 cell when compared to the other compounds and cell lines (Table 7). Compound **3e** had the lowest percentage cytotoxicity with the values of 10.45% (A549) and 23.31% (C6) (Tables 6 and 7) (Figure 5). Compound **3c** also exhibited the smallest percentage cytotoxicity for HeLa (8.11%) and FL (14.88%) cells. Compound **3i**, the condensation product of methionine methyl ester and o-vanilin, was interestingly highly toxic for A549 and HT29 cells (22.80% and 32.68%, respectively), whereas less toxic for Hep3B and Vero cells (8.36% and 11.91%, respectively) (Tables 6-8) (Figure 5). Similarly, **3b**, composed of isoleucine methyl ester and 2,4-dihydroxybenzaldehyde, was very toxic for C6 (34.71%) and less toxic for HT29 (16.34%). However, **3i** and **3b** may cause cytotoxic effect via the same mechanisms of action. It is noteworthy that the compound **3f**, containing phenylalanine methyl ester and o-vanilin, had slightly high toxicity against MCF7, Vero, and FL cells (11.91%, 23.69%, and 29.32%, respectively) than the other compounds which had similar potent cytotoxic activity. Compound **3h** exhibited the lowest percentage cytotoxicity for MCF7 cell lines. The rest of the compounds (**3a** and **3d**) had the highest cytotoxic effect towards HeLa and Hep3B cells (31.22% and 19.57, respectively). One of the most important conditions for being an anticancer agent is the minimal toxicity against normal cells. Therefore, cytotoxic features were compared in order to find out the advanced pharmacological capacities of these compounds. Compounds **3c** (for HeLa, 8.11%), **3e** (for A549, 10.45%), **3h** (for MCF7, 6.21%), and **3i** (for Hep3B and Vero, 8.36% and 11.91%, respectively) exhibited equal cytotoxicity with controls, indicating their significant antiproliferative potential as well as low cytotoxicity (Tables 6-9).

Table 6: % Cytotoxicity of the compounds against A549, HeLa, and HT29.

%Cytotoxicity	A549				HeLa				HT29			
	25µg/mL	50µg/mL	75µg/mL	100µg/mL	25µg/mL	50µg/mL	75µg/mL	100µg/mL	25µg/mL	50µg/mL	75µg/mL	100µg/mL
3a	17,73± 1.3	26,85± 1.9	50,73± 3.7	60,67± 4.3	31,22± 1.6	68,90± 1.7	77,71± 2.7	103,86± 5.1	23,12± 1.4	52,44± 2.9	70,17± 3.5	89,74± 3.9
3b	17,54± 1.4	24,07± 2.1	43,64± 3.1	53,45± 3.9	18,94± 1.4	34,96± 1.5	53,07± 2.1	58,45± 2.4	16,34± 1.1	54,34± 2.5	80,24± 3.7	100,63± 4.9
3c	15,64± 1.4	27,61± 1.8	47,44± 2.9	71,56± 4.4	8,11± 0.8	18,43± 1.2	36,10± 1.8	44,52± 2.4	22,55± 1.1	28,37± 1.8	54,97± 2.8	79,16± 4.0
3d	10,64± 1.2	20,27± 1.5	27,99± 1.8	46,87± 3.1	18,62± 1.4	31,92± 1.8	52,06± 2.2	81,06± 3.1	25,21± 1.6	53,70± 2.3	80,11± 4.0	102,47± 5.0
3e	10,45± 1.1	20,01± 1.5	42,37± 2.7	51,11± 3.8	17,16± 1.4	26,60± 1.5	35,02± 1.7	50,28± 1.9	25,97± 1.5	48,01± 1.9	72,70± 3.8	85,18± 4.1
3f	18,43± 1.5	39,01± 2.1	60,73± 3.6	66,56± 4.1	21,72± 1.5	36,16± 1.6	49,40± 2.0	93,79± 4.7	32,17± 1.5	34,71± 1.8	60,67± 3.1	76,12± 3.7
3g	14,12± 1.4	34,77± 2.0	60,92± 3.4	76,50± 4.5	22,23± 1.5	72,70± 2.3	82,77± 3.4	93,98± 5.0	20,39± 1.4	49,27± 2.7	72,70± 3.5	100,13± 4.3
3h	21,15± 1.7	28,56± 1.7	50,03± 3.3	58,71± 4.0	14,00± 1.1	22,42± 1.3	31,86± 1.9	50,73± 2.4	24,89± 1.4	43,57± 2.0	69,54± 3.4	85,81± 3.9
3i	22,80± 1.5	48,20± 3.7	54,53± 3.5	58,96± 4.0	18,05± 1.1	24,70± 1.4	43,64± 1.9	45,60± 2.3	32,68± 1.6	44,84± 1.9	72,70± 3.4	90,63± 4.6

Values are given as the mean ± SD of three experiments and $r^2=0.88$ to 0.99 . Significant at $P < 0.05$

Table 7: % Cytotoxicity of the compounds against Hep3B, MCF7, and C6.

%Cytotoxicity	<i>Hep3B</i>				<i>MCF7</i>				<i>C6</i>			
	25µg/mL	50µg/mL	75µg/mL	100µg/mL	25µg/mL	50µg/mL	75µg/mL	100µg/mL	25µg/mL	50µg/mL	75µg/mL	100µg/mL
3a	10,01± 0.8	25,46± 2.2	38,38± 2.7	51,04± 3.0	10,64± 0.8	18,49± 1.5	29,51± 1.9	43,89± 3.0	30,27± 1.5	66,37± 3.4	78,97± 4.0	99,94± 5.1
3b	14,00± 1.0	23,81± 2.1	41,61± 2.8	55,29± 3.0	6,84± 0.7	14,31± 1.0	32,74± 2.1	50,66± 3.6	34,71± 1.4	64,47± 3.5	82,08± 4.5	96,20± 5.2
3c	11,91± 1.1	21,22± 1.7	32,55± 2.3	49,91± 3.1	9,37± 0.8	18,49± 1.5	26,47± 1.9	54,21± 3.7	30,91± 1.8	67,00± 3.9	82,96± 4.7	99,11± 5.3
3d	19,57± 1.5	28,88± 2.0	48,01± 2.5	60,99± 3.8	10,01± 0.9	20,14± 1.1	27,04± 1.8	45,35± 2.1	27,68± 1.5	63,84± 2.9	79,42± 4.0	104,75± 5.4
3e	18,56± 1.4	26,35± 1.6	45,41± 2.3	51,23± 3.4	9,37± 0.9	19,19± 1.3	30,53± 1.9	40,28± 2.0	23,31± 1.4	62,57± 3.1	78,28± 4.2	85,18± 4.9
3f	14,63± 1.2	32,68± 2.5	57,82± 3.4	70,80± 4.1	11,91± 0.9	15,96± 1.0	29,26± 1.5	47,31± 2.2	31,54± 1.8	55,60± 3.1	80,56± 4.9	96,45± 5.0
3g	14,31± 1.1	26,98± 2.0	35,66± 2.7	48,51± 2.9	11,27± 0.9	19,13± 1.4	27,68± 1.8	57,88± 3.4	25,84± 1.1	63,84± 3.5	82,46± 4.8	97,21± 5.4
3h	14,25± 1.4	25,40± 2.1	39,65± 2.9	51,68± 3.2	6,21± 0.8	16,02± 1.2	32,11± 1.9	47,18± 2.3	29,64± 1.4	56,87± 3.0	78,72± 4.6	93,60± 5.0
3i	8,36± 0.78	25,90± 2.0	33,82± 2.4	51,99± 3.1	8,11± 0.7	23,75± 1.1	38,51± 2.3	50,35± 3.4	33,44± 1.9	63,20± 3.7	81,13± 4.2	101,01± 5.3

Values are given as the mean ± SD of three experiments and $r^2=0.88$ to 0.99 . Significant at $P < 0.05$

Table 8: % Cytotoxicity of the compounds against Vero and FL.

%Cytotoxicity	Vero				FL			
	25µg/mL	50µg/mL	75µg/mL	100µg/mL	25µg/mL	50µg/mL	75µg/mL	100µg/mL
3a	19,76± 1.5	32,93± 2.1	44,97± 2.8	73,46± 3.9	20,08± 1.5	29,39± 1.9	57,76± 3.5	72,96± 4.0
3b	16,85± 1.5	29,32± 1.9	56,87± 3.2	81,63± 4.2	15,64± 1.1	29,83± 1.7	48,77± 3.0	70,87± 4.1
3c	17,23± 1.3	36,67± 2.2	51,61± 2.9	60,99± 3.7	14,88± 1.2	32,74± 2.1	44,59± 2.9	68,14± 3.7
3d	17,99± 1.2	33,38± 1.9	52,56± 3.1	74,48± 3.9	25,40± 1.6	65,10± 2.9	70,93± 4.1	77,33± 4.4
3e	14,95± 1.0	30,91± 1.8	63,77± 3.4	79,16± 4.0	18,94± 1.4	33,38± 1.7	46,55± 2.4	66,75± 2.9
3f	23,69± 1.5	34,33± 1.8	54,65± 2.7	87,71± 5.0	29,32± 1.4	54,34± 2.0	82,08± 4.3	93,35± 5.1
3g	18,11± 1.4	30,40± 2.0	59,66± 3.5	83,09± 4.5	16,21± 1.2	37,75± 2.4	46,61± 2.8	70,74± 4.0
3h	19,25± 1.4	32,74± 1.7	64,28± 3.0	80,30± 4.4	19,19± 1.5	46,42± 1.8	59,91± 3.6	72,32± 4.0
3i	11,91± 0.9	24,13± 1.5	43,19± 2.9	54,02± 3.5	28,37± 1.6	43,57± 1.9	63,84± 3.8	73,46± 4.2

Values are given as the mean ± SD of three experiments and r²=0.88 to 0.99. Significant at P < 0.05

Table 9: % Cytotoxicity of positive controls at IC50 concentrations.

	HeLa	HT29	A549	MCF7	C6	ep3B	FL	Vero
Cisplatin	9.85± 0.8	11.23± 0.9	8.63± 0.8	10.71± 0.9	9.04± 0.8	8.46± 0.8	8.33± 0.7	9.41± 0.9
5FU	8.83± 0.7	7.91± 0.7	9.19± 0.8	7.69± 0.7	10.01± 0.9	9.67± 0.8	8.44± 0.8	8.81± 0.9

Values are given as the mean ± SD of three experiments and r²=0.95 to 0.98. Significant at P < 0.05

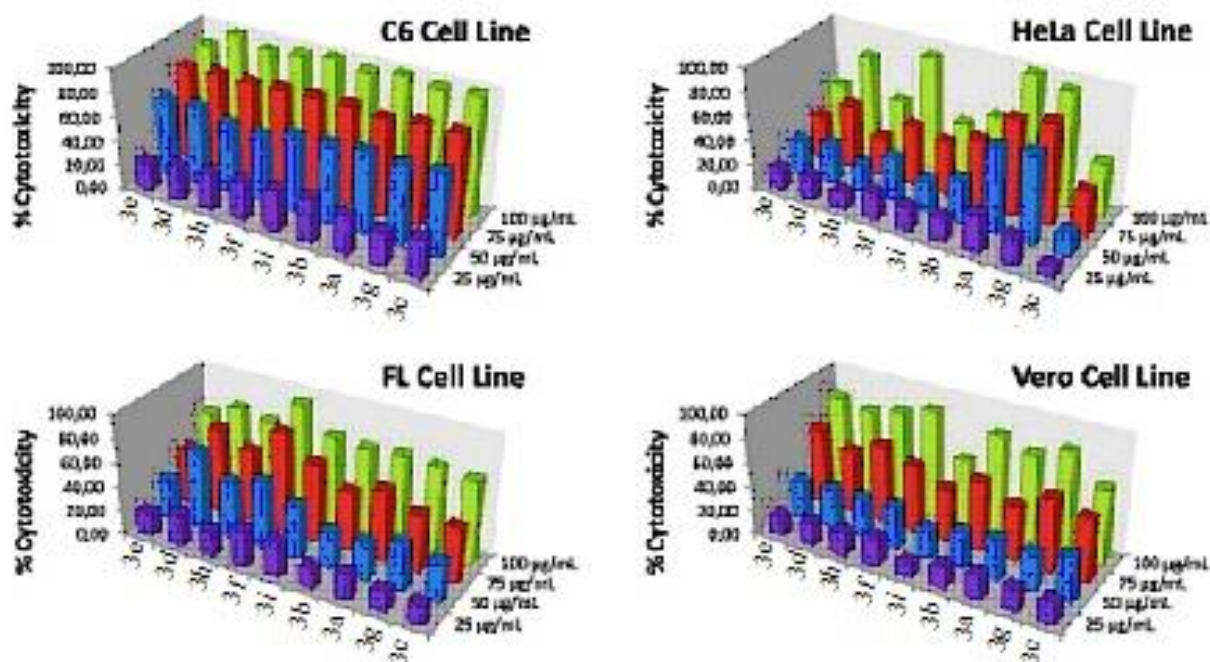


Figure 5. The cytotoxic activity of the compounds on C6, HeLa, FL, and Vero cells. Exponentially growing cells were incubated with various concentrations of the Schiff bases and cytotoxicity was measured by LDH Cytotoxicity Assay.

The Effect of the 3d on the Morphology of the Cells

The morphology alters of treated and untreated (control) cells were monitored by using the inverted phase-contrast microscopy techniques. As shown in Figures 6 and 7, visible morphology alters such as cytoplasmic blebs, anomalous globular structure and reduction in cell quality and cell count in the flask monolayer were recorded.

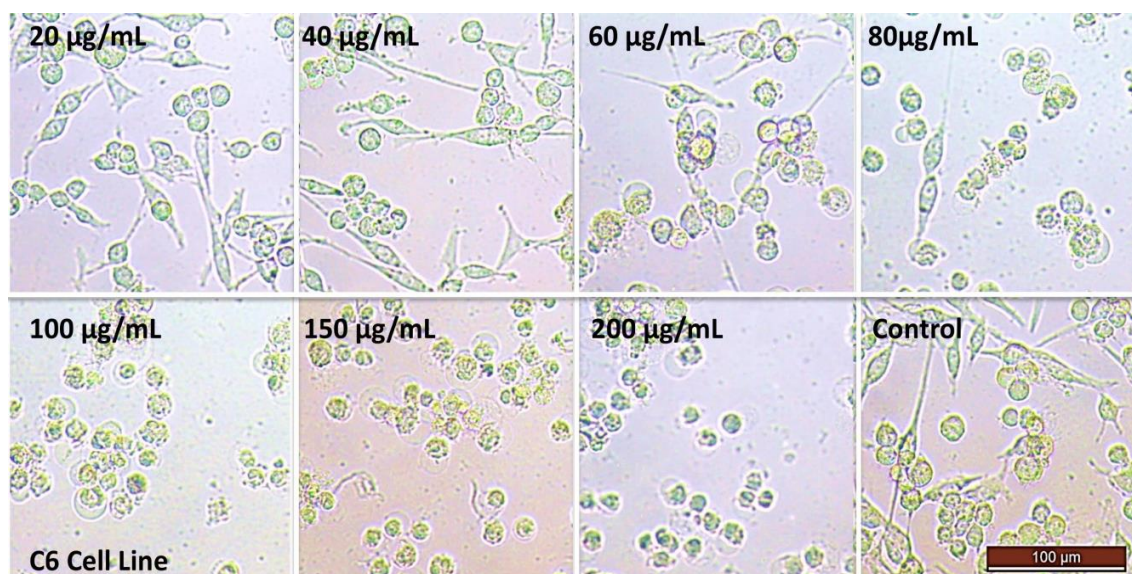


Figure 6: The effect of **3d** on the morphology of C6 cell line. Exponentially growing cells were incubated by adding various concentrations of **3d** at 37 °C overnight. Control cells were treated with only DMSO.

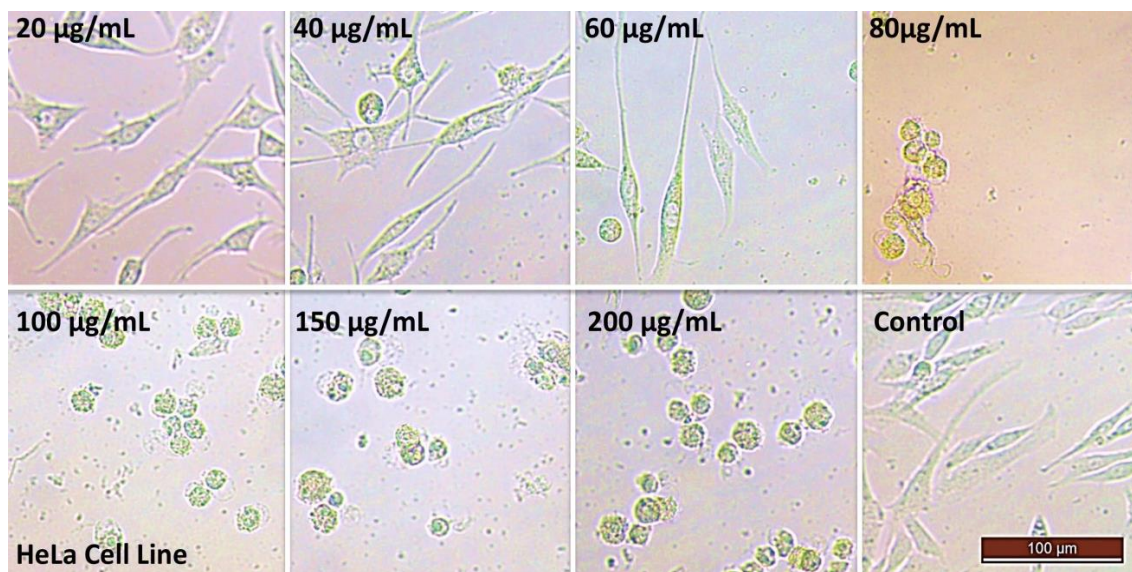


Figure 7: The effect of **3d** on the morphology of HeLa cell line. Exponentially growing cells were incubated by adding various concentrations of **3d** at 37 °C overnight. Control cells were treated with only DMSO.

CONCLUSIONS

In brief, a new series of amino acid methyl ester Schiff bases (**3a-i**) was prepared and characterized by spectroscopic methods (FT-IR, UV-Vis, ^1H - and ^{13}C -NMR). Schiff bases were synthesized by condensation reactions of salicylaldehyde derivatives and amino acid methyl esters in alkaline chloroform media. All analysis results were in accordance with suggested Schiff base structures. All Schiff bases were validated against A549, HeLa, HT29, Hep3B, MCF7 and C6 cell lines by the MTT assay. According to the results, all of the imine compounds exhibited selectivity for aforementioned carcinoma cell lines. Especially, compound **3d** was the most active compound against all tested cell lines with good TGI and IC50 values of 5.81-52.36 µg/mL and 5.72-46.35 µg/mL, respectively. GI50, TGI and LC50 values of the Schiff bases **3a-i** emphasized that they satisfied the NCI-60 criteria to pass into further pharmacological investigations. Although full mechanism underlying the cytotoxic activity could not be explained, it is known that some organic substituents like phenyl, halogens, hydroxy and methoxy enhance cytotoxic activity. It can be concluded that the existence of two phenyl rings and bromo substituent and the position of bromo group highlight compound **3d** as a strong anticancer agent.

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