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Original Research Article

Evaluation of Schiff Bases Against Two Cancer Cell Lines: A Combined Experimental and Theoretical Approach

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ABSTRACT

The rising global cancer rate necessitates the development of new therapeutic agents, with Schiff bases, compounds containing primary amines and aldehydes, gaining attention for their anticancer properties. The present study investigated the anticancer potential of three Schiff base derivatives against two cancer cell lines, MDA-MB231 (breast cancer) and HepG2 (liver cancer), employing both experimental and theoretical approaches. Schiff bases were synthesized using condensation reactions and characterized employing ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry. Cytotoxicity was evaluated using the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, and determined IC_{50} values. Computational studies using density functional theory (DFT) were employed to optimize molecular structures and assess electronic properties of Schiff bases, computing HOMO-LUMO energy gaps, dipole moments, and heat of formation. The three Schiff bases synthesized included (E)-4-(((4-chloro-2-nitrophenyl)imino)methyl)-3-methoxyphenol (1), 2-((2,4-dichlorophenylimino)methyl)-5-(diethylamino)phenol (2), and (E)-4-(((4-chloro-2-nitrophenyl)imino)methyl)-3-methoxy-N,N-dimethylaniline (3). Compound 2 demonstrated the highest activity against the HepG2 cell line with an IC_{50} value of 43.17 µg/mL, while compounds 1 and 3 exhibited lower activities (IC_{50} values of 70.29 and 73.69 µg/mL, respectively). The compounds also showed varying degrees of activity against the MDA-MB231 cell line, with compound 2 being the most effective. Molecular docking simulations further supported the experimental results, highlighting significant binding interactions between compound 2 and breast cancer-related proteins, with the strongest binding observed to Akt (5KCV). Similarly, compound 1 demonstrated effective binding to liver cancer proteins. This combined experimental and theoretical study provides valuable insights into the anticancer activity of Schiff bases and highlights their potential as promising candidates for cancer therapy.

Keywords: Schiff base, Antiproliferative, Breast cancer, Molecular docking.

Introduction

A significant alteration in the genetic makeup of normal cells causes a subset of cells to multiply uncontrollably, leading to the multistage progressive disease known as cancer.[1–3] Mutations in genes controlling cellular development, reproduction, and cell cycle can transform healthy cells into malignant ones. These cells proliferate, increase in mass, avoid apoptosis, get around cell checkpoints, and begin to invade adjacent and accessible organs.[4–6]

Primary amines and aldehydes undergo condensation reactions to form Schiff bases, also known as azomethines, which exhibit various biological functions.[7,8] These functions include pesticidal,[9,10] cytotoxic,[11,12] anticancer,[11,13] antimicrobial,[14,15] urease inhibitory,[16,17] anti-inflammatory,[18,19] anti-ulcerogenic,[20,21] antioxidant,[22,23] and DNA damage. Additionally, Schiff bases have been effectively employed in research as incredibly effective and selective sensing materials for electrochemical, membrane, and optical sensors.[24,25] Schiff bases exhibit diverse characteristics and biological applications, with their pharmacologic properties primarily ascribed to the azomethine (C=N) functional group.[26,27]

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Schiff bases have gained increasing attention in cancer drug discovery due to their strong antiproliferative activity against various cancer cell lines. They also exhibit the capacity to suppress tumour growth in animal models and contribute to the mitigation of multidrug resistance.[28,29]

The present study aimed to evaluate the anticancer potential of selected Schiff bases against two cancer cell lines through experimental assays and theoretical studies.

Materials and Methods

Sources of materials and instrumentation

Sigma-Aldrich provided all the chemicals and solvents used in the synthesis of Schiff bases. Trypsin and ethylenediaminetetraacetic acid (EDTA) were obtained from Capricorn, USA. Gibco, USA supplied the RPMI 1640 and fetal bovine serum. MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) dye was bought from Sigma, USA. Thermo Fisher Scientific, USA, supplied the cell culture plates, while Cypress Diagnostics, Belgium, provided the CO_2 incubator. All of the synthetic compounds' dot spots were visualized when exposed to UV light at 254 nm. A Gallenkamp melting point device was used to determine the melting points in capillary tubes. A Bruker Inova AV-400 spectrometer (Iraq) was used to record ^{1}H-NMR spectra at room temperature in dimethyl sulfoxide (DMSO)-d6 as the solvent. The ^{1}H-NMR spectra's signal peak was located at δ 2.50 ppm. The coupling constant values (J) were reported in Hz, and chemical shifts are provided in ppm (δ scale). The abbreviations for the splitting pattern are s: singlet; d: doublet; t: triplet; and m: multiplet. The absorbance was reordered between 600-4000 cm^{-1} after Fourier transform infrared (FTIR) spectra were performed using KBr (1%) discs on the Shimadzu IRAffinity-1 spectrophotometer (Iraq).

A Micro Mass LCT in Electrospray mode (ES-MS; Iran) was used to perform accurate mass measurements.

Cell lines and cultivation
The National Cell Bank of Iran (Pasteur Institute, Iran) supplied a human hepatocarcinoma cell line HepG2 and a human breast cancer cell line MCF-7. Cells were treated with antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin) after being grown in RPMI-1640 media (Gibco) with 10% FBS (Gibco). Humid air with 5% CO and 37°C was used to culture the cells, and then the cells were cultured using phosphate-buffered saline (PBS) solution and trypsin/ EDTA (Gibco) solution.

MTT cell viability assay in MCF7 Cells
[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium Bromide] (Sigma-Aldrich) was used to assess cell proliferation and viability by the MTT assay. Trypsin was used to digest the cells, then collected and adjusted to a density of 0.14×10^5 cells per well and then cultured on 96-well plates. containing 200 µl of new media per well for 24 hours. Upon the formation of a monolayer, the cells were subjected to treatment with 25-400 µg/ml of the synthesized compounds at 37 °C for 24 hrs. in a 5% CO_2 atmosphere. After 24 hours, the upper fluid was removed and 200 µl of the solution was added (0.5 mg/ml in phosphate-buffered saline [PBS]) and the plate was incubated for an additional 4 hrs. at 37 °C. Cells were incubated on a shaker at 37 °C until the crystals were fully dissolved. An ELISA reader (Model wave xs2, BioTek, USA) was used to assess cell viability by measuring absorbance at 570 nm. IC50 can be defined as the concentration of synthesized compounds that caused 50% cell mortality which was determined from the dose-response curves.

General procedure for the synthesis of Schiff base
The Schiff base compounds were synthesized by combining 0.01 mol of benzaldehyde derivatives with 1.45 g (0.01 mol) of 2-chloro-4-nitro aniline in a 25 ml ethanolic solution. After that, the mixture was stirred for 26 hours. Filtration using a Buchner funnel yielded 60–81% of the precipitate, which was subsequently recrystallized from ethanol and allowed to dry at ambient temperature.

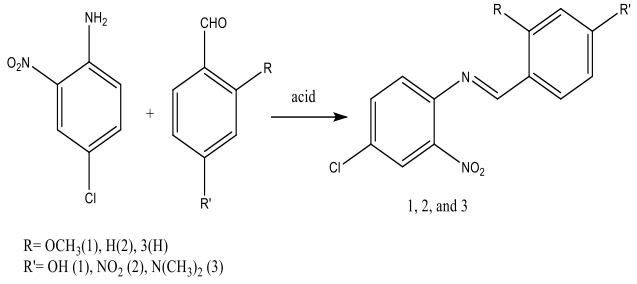

Figure 1: The schematic diagram for preparing the Schiff base derivatives.

(E)-4-(((4-chloro-2-nitrophenyl) imino) methyl)-3-methoxyphenol (1)
Yield 70%. m.p. 79 – 81 °C. 1H NMR (*DMSO-d6*, ppm): δ = 3.843 [s, 3H, CH_3], 6.953-7.954 [m, 6H; Haromatic], 9.774 [s; 1H; H_{OH}], 10.294 [s, 1H; $H_{azometane}$]. 13CNMR (400 MHz, DMSO-d6, ppm): δ =56.03, 111.10, 115.84, 118.75, 12167, 124.54, 126.56, 129.16, 130.42, 136.14, 145.57, 148.61, 153.48; MS m/z= 305.3 $[M-H]^+$.

(E)-N-(4-chloro-2-nitrophenyl)-1-(4-nitrophenyl) methanimine (2)
Yield 65%. m.p. 110 –112 °C. 1H NMR (*DMSO*-d6, ppm): δ = 7.757-8.430 [m, 7H, $H_{aromatic}$], 10.166 [s, 1H; $H_{azomethane}$]. 13CNMR (400 MHz, DMSO-d6, ppm): δ =124.44, 124.77, 127.69, 131.11, 131.38, 140.52. 151.08; MS m/z= 304.4 $[M-H]^+$.

(E)-4-(((4-chloro-2-nitrophenyl)imino)methyl)-3-methoxy-N,N-dimethylaniline (3)
Yield 73%. m.p. 63–65 °C. 1H NMR (*DMSO*-d6, ppm): δ = 3.324 [s, 6H, $2CH_3$], 6.787-7.956 [m, 7H, $H_{aromatic}$], 9.676 [s; 1H; $H_{azomethane}$], 13CNMR (400 MHz, DMSO-d6, ppm): δ =15.05, 111.54, 148, 147, 138, 131, 130, 121 and 108; MS m/z= 303.4 $[M]^+$.

Computational modeling and molecular docking analysis
Using the Material Studio-DMol3 (Version 2017 program) with a standard density functional theory (DFT), the density functional theory (DFT) approach was used to calculate the geometries and energies for the synthesized compounds (1-3) at the BLYP level of theory, and DNP as a basis set. Also, a molecular docking analysis of chemical 2 was carried out by employing MOE 2019. The protein crystal structures were obtained from the Protein Data Bank (PDB).

Results and Discussion

Characteristics of the synthesized compounds
The Schiff base derivatives were prepared by refluxing 4-chloro-2-nitro aniline and benzaldehyde derivatives 1, 2, and 3 in ethanol as solvent, as shown in Figure 1. The Schiff bases (1-3) were synthesized and then characterized using ^{1}H-NMR, ^{13}C-NMR, and mass spectroscopic techniques. The NMR spectra of synthesized compounds 1–3 were recorded in DMSO. The experimental section provided the chemical shifts of the various protons and carbons. The azomethine proton (-N=CH-) was represented by a singlet sharp signal that appeared at 10.294, 10.166, and 9.676 ppm, respectively, supporting the synthesis of 1–3. In addition to integrating protons in the aromatic and aliphatic regions, it is identical to the number of protons in the synthesized compounds 1-3. The azomethine carbon (CH=N) is represented by the peaks in the ^{13}C-NMR spectra at 191.48, 92.8, and 190.35 ppm.

Cytotoxicity of the synthesized compounds
The efficacy of the target derivatives in inhibiting the proliferation of breast cancer cells MDA-MB231 and antiproliferative HepG2 cancer cells was confirmed through cell viability screening.[25] After 24 hours of incubation with 200 µM for each derivative, compounds **1**, **2**, and **3** exhibited average performances against cancer and liver HepG2 cell lines (Table 1). The target compounds' cytotoxicity was evaluated using an MTT assay. For calculating the IC_{50} values and analyzing the dose-response, GraphPad Prism 8.1 software was ultimately employed. The results are displayed in Figure 1.[26] Compound 2 showed high activity against the liver carcinoma (HepG2) cell line with a value of 43.17 µg/mL, while compounds **1** and **3** demonstrated lower activity with IC_{50} values of 70.29 and 73.69, respectively. On the other hand, all synthesized compounds demonstrated activity against breast cancer, exhibiting IC_{50} values of 138.85, 71.55, and 95.30, respectively, as illustrated in Figure 2. All IC_{50} values were compared with 5-fluorouracil (5-FU), the reference drug, which exhibited IC_{50} values of 6.44 µg/mL and 28 µg/mL, respectively. The effect of functional groups, which include hydroxy and methoxy groups in compound **1**, a nitro group in compound **2**, and an N-dimethyl group in compound **3**, could explain the difference in activity values. These functional groups are highly expected to be responsible factors for enhancing cytotoxicity.

Table 1: The IC_{50} values of compounds 1-3 against different cancer cell lines.

Compound	Cancer cell line	
	HepG-2	MCF-7
1	70.29	138.85
2	43.17	71.55
3	73.69	95.30
5-fluorouracil	6.44	28

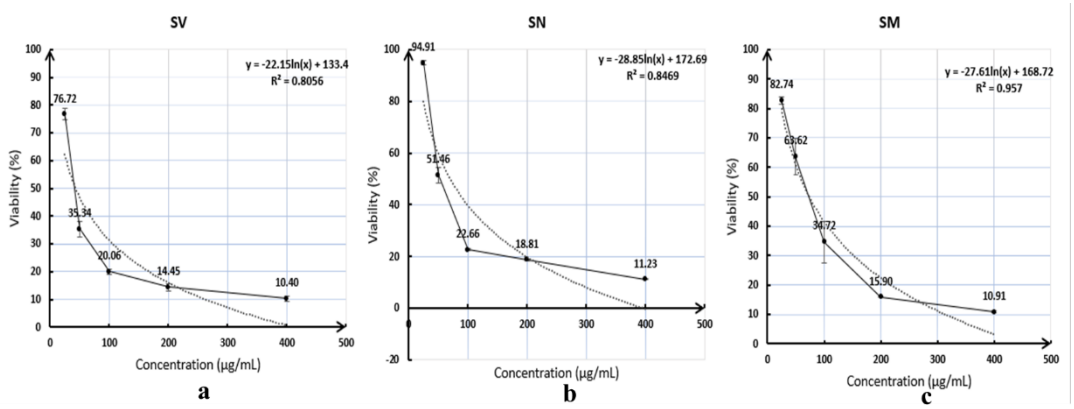

Figure 1: Effect of concentrations (0–400 µg/ml) of compounds 1-3 on the viability of the human liver cancer (HepG-2) cell line after 24 hours of incubation.

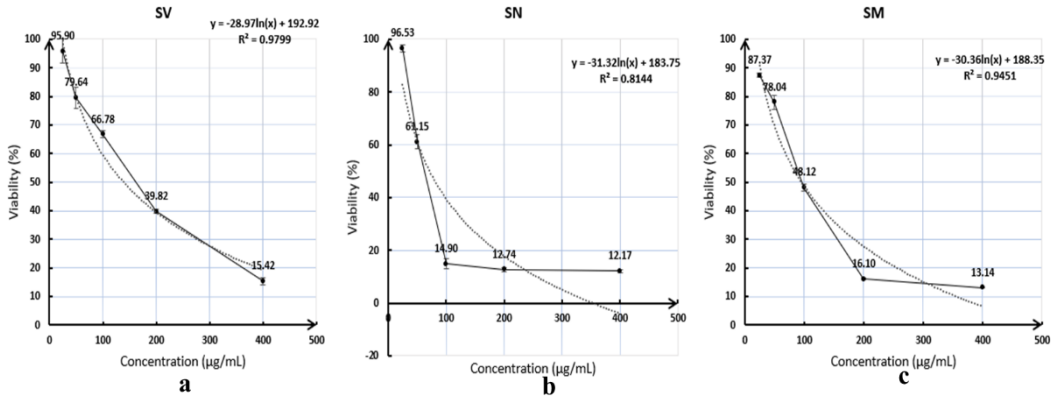

Figure 2: Effect of concentrations (0–400 µg/ml) of compounds 1-3 on the viability of the human breast cancer (MCF-7) cell line after 24 hours of incubation.

Computational analysis of molecular properties and reactivity

The geometry optimization of molecules **1-3** was conducted by the DFT. Figure 3 shows the optimized structure of the studied molecules. Table 2 summarizes the binding energy, gap of energy HOMO-LUMO, heat of formation, and dipole moment of the synthesized compounds. By the values of dipole moment and energy gap (ΔE LUMO-HOMO), the chemical activity of the synthesized compounds can be shown.[31]

According to the values listed in Table 2, higher reactivity was associated with molecule 2 (ΔE LUMO-HOMO =1.878 eV. and µ =8.530 Debye), which aligns with the results of the biological anti-cancer activity. This could be attributed to the presence of nitro groups, as well as the biologically active chlorine group. Concerning the values of the heat of formation and binding energy, it was observed also that molecule **2** is the most stable.

Table 2: Calculated energy values of the studied molecules.

Molecules	HOMO energy (eV.)	HUMO energy (eV.)	$\Delta E_{LUMO-HOMO}$ (eV.)	µ (Debye)	ΔH_f (Kcal/mol)	Binding energy (eV.)
1	-5.174	-2.924	2.250	7.413	-26.0433	-158.280
2	-4.704	-2.826	1.878	8.530	52.001	-168.928
3	-6.006	-3.822	2.184	5.213	49.041	-145.628

Figure 4 shows the forms of HOMO orbitals and the electrostatic potential map (EPM) of the studied molecules, where it was found that the electron density of these compounds is concentrated on the nitro groups, in addition to chlorine atoms.[32]

Molecular docking analysis

Molecular docking studies are one of the most important methods used to determine the susceptibility of compounds to interact with different receptors, such as the protein that causes a particular disease, theoretically using molecular docking programs. The occurrence of a binding between the compound and the protein gives an indication of the biological activity of the compound. A compound that successfully binds to the disease-related protein can be used as an inhibitor or antidote for the disease. Molecular docking studies allow researchers to visually analyze compounds and predict binding interactions between the active groups of a ligand and a protein receptor. These studies also calculate binding energy and determine the number of hydrogen bonds formed, providing key insights into the ligand's potential as a therapeutic agent for diseases associated with the target protein.[33]

The molecular docking of compound **2** was evaluated against breast cancer-related proteins in the MCF-7 cell line, including PR (PDP: 4OAR), ERα (PDP: 3ERT), mTOR (PDP: 4DRH), EGFR (PDP: 2J6M),

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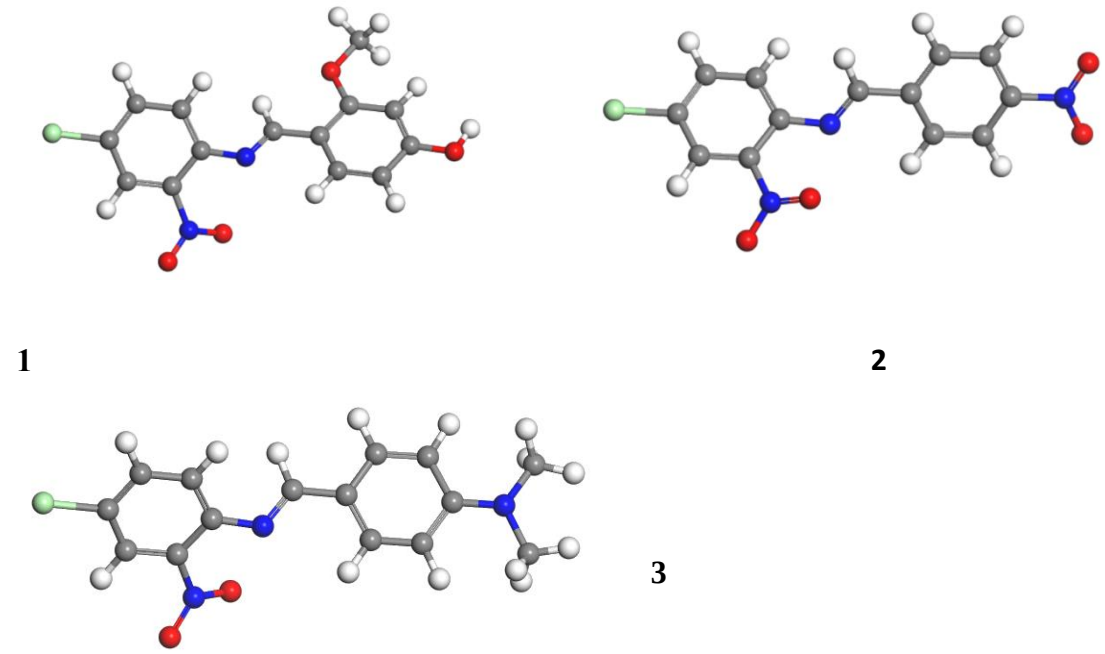

Figure 3: Geometric optimization of the structures of the studied molecules.
1:(E)-4-(((4-chloro-2-nitrophenyl)imino)methyl)-3-methoxyphenol; 2: 2-((2,4-dichlorophenylimino)methyl)-5 (diethylamino)phenol; 3: (E)-4-(((4-chloro-2-nitrophenyl)imino)methyl)-3-methoxy-N,N-dimethylaniline

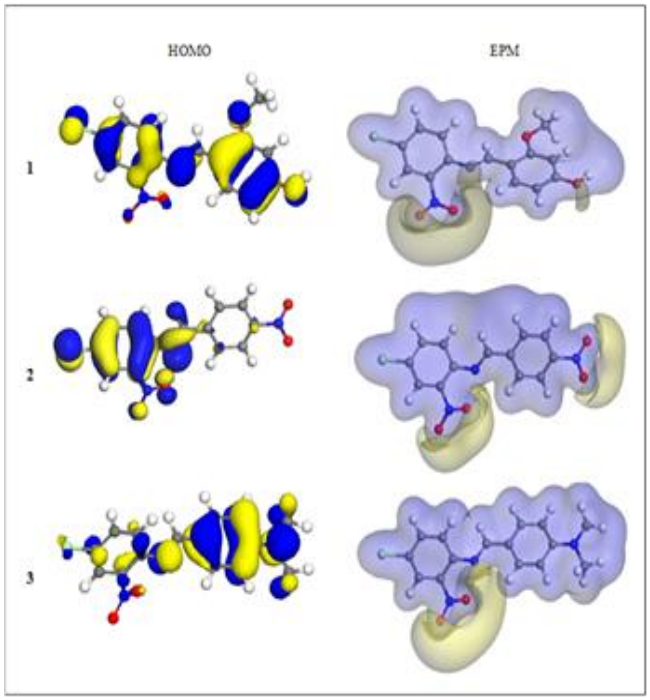

Figure 4: HOMO orbitals and the electrostatic potential maps of the studied molecules.

CDK6 (PDP: 3NUP), CDK2 (PDP: 4FX3), and Akt (PDP: 5KCV).[1,34,35] The strongest interaction was observed with the 5KCV protein, exhibiting the highest affinity energy (S) of -6.5867 kcal/mol and an RMSD value of 1.1264.[35] Table 3 shows the results of molecular docking analysis of compound **2** with the target proteins, and Figure 5 displays the interactions of 2D and 3D forms. The results indicate two key interactions between compound 2 and the 5KCV protein. The first is an acceptor hydrogen bond between the oxygen atom of the compound and SER-205, while the second is a π-π interaction with Trp-80.

The study also evaluated compound **2** against liver cancer proteins in the HepG2 cell line, including 3EJB, 3E7J, 2X39, 3W32, and 4NOS. The strongest binding was observed with protein 3EJB, exhibiting the lowest binding energy of -6.0729 kcal/mol and an RMSD value of 1.1264. Only one type of *pi*-H association was observed with the amino acid ILE-82, alongside ten amino acids forming Van der Waals interactions, as shown in Table 3 and Figure 5.

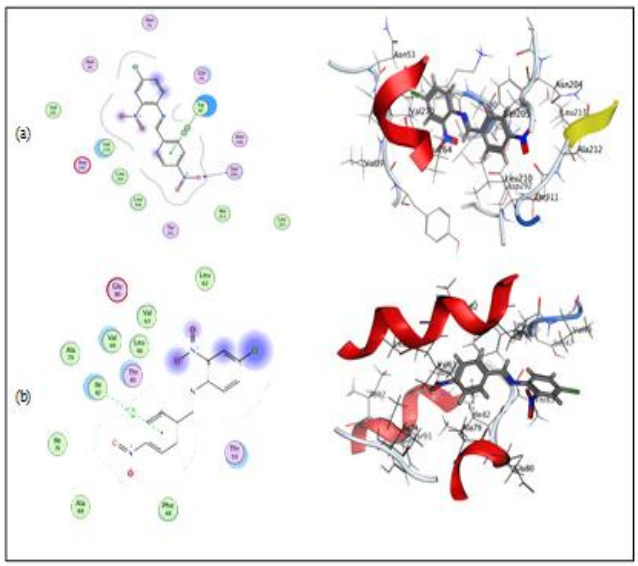

Figure 5: 2D and 3D forms of compound 2 with target proteins.
a: 5KCV; b: 3EJB

Table 3: Molecular docking of compound 2 with target 5KCV and 3EJB proteins.

Protein (Receptor)	RMSD	Affinity energy (S) Kcal/mol	Interaction		
			Type	Amino acid	Distance(Å)
5KCV	1.1264	-6.5867	H-acceptor *pi-pi*	SER205 Trp80	3.03 3.92
3EJB	1.4219	-6.0729	*pi* -H	ILE82	4.19

Conclusion

The findings of the present study demonstrated that the synthesized Schiff bases showed significant cytotoxicity against MDA-MB231 (breast) and HepG2 (liver) cancer cell lines, with synthesized compound 2 being the most potent. IC_{50} values suggested their potential as anticancer agents. Computational studies, including density functional theory and molecular docking, further supported the observed biological activity by revealing key structural characteristics and interactions with target proteins involved in cancer progression. These findings highlight the promising role of Schiff bases in cancer treatment, providing a foundation for further exploration in drug design and development.

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