RESEARCH PAPER

Design, Molecular Docking, and Biological Evaluation of Imidazo[2,1-b]thiazole Derivatives

Ram Sevak Verma[1*], Nitin Mittal[2], Bhumika Yogi[3], Sujeet Kumar Gupta[4]

[1,2]Lords University, Alwar-Bhiwadi Highway, Chikani, Alwar - 301028, Rajasthan, India
[3,4]J.S Singh Institute of Pharmacy, Sitapur-261207, Uttar Pradesh, India
***Corresponding author: Ram Sevak Verma, Lords University, Alwar-Bhiwadi Highway, Chikani, Alwar - 301028, Rajasthan, India**
Email: rajputrs933@gmail.com

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ABSTRACT

The research includes all stages of development, which include designing, synthesizing, molecular docking work, and testing anticancer properties of imidazo[2,1-b]thiazole compounds (5A–5E). The molecular docking studies showed that the complex formed between the two proteins displayed strong binding capacity to epidermal growth factor receptor and estrogen receptor alpha. The researchers used the MTT assay to test the cytotoxic activity of synthesized compounds against MCF-7 and MDA-MB-231 cell lines, which demonstrated that cell viability decreased with increasing concentration. The compound 5E showed the strongest cytotoxic properties among all tested compounds, while 5C displayed the second-highest activity level based on its lowest IC_{50} value. The flow cytometric analysis demonstrated that compound 5E caused cells to undergo apoptosis while blocking their normal cell cycle progression, which resulted in an increased SubG1 population. The studies revealed that compound 5E served as the main lead candidate for development as an anticancer drug.

Keywords: Imidazo[2,1-b]thiazole; Anticancer activity; Molecular docking; EGFR; ERα; Cytotoxicity; IC_{50}; Apoptosis; Cell cycle analysis.

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1. Introduction

The medical field still struggles against breast cancer because it remains one of the most frequently diagnosed cancers among women. The existing chemotherapy and targeted treatment methods fail because they encounter three major problems, which require scientists to create better treatment solutions.[16] Heterocyclic compounds have gained considerable attention in medicinal chemistry due to their diverse pharmacological activities. Imidazo[2,1-b]thiazole derivatives serve as effective scaffolds that exhibit multiple biological activities that include antimicrobial, anti-inflammatory, and anticancer effects.[5] Their structural flexibility enables scientists to create chemical changes that improve their biological effects and ability to target specific proteins.[1]

Current research demonstrates that directing therapy towards crucial signaling pathways that drive cancer development needs to be approached through targeted research on epidermal growth factor receptor (EGFR) and estrogen receptor alpha (ERα) pathways, which specifically affect breast cancer progression. Molecular docking serves as an effective computational tool to predict the interaction of small molecules with biological targets, which helps researchers develop new drugs through rational design methods. [28]

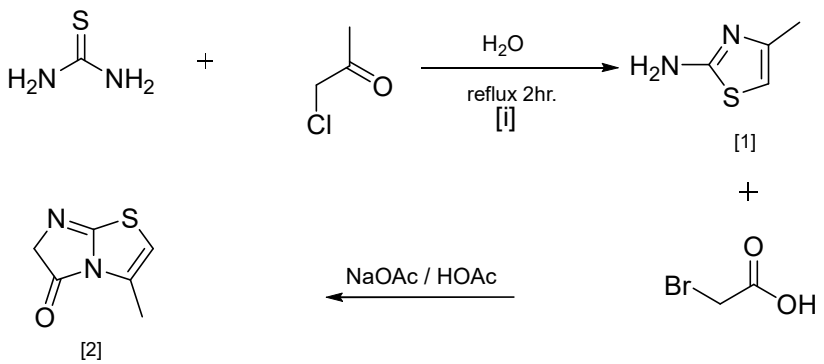
The present study investigates imidazo[2,1-b]thiazole derivatives through their design process and their chemical synthesis, together with their biological testing. The researchers tested the synthesized compounds for their ability to kill cancer cells through in vitro cytotoxic assays against MCF-7 and MDA-MB-231 cell lines. The researchers used molecular docking studies and apoptosis analysis to investigate their mechanism of action.[4]

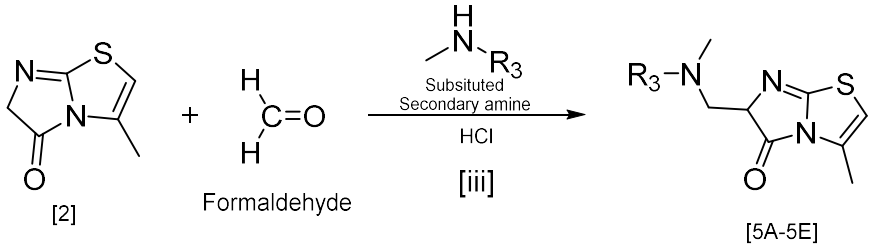
2. Materials and Methods

2.1 Chemistry

The study used analytical grade reagents and solvents, which were applied directly without any purification process except in cases where specific requirements existed. The laboratory conducted organic reactions under its established controlled conditions. The researchers tracked reaction development through thin-layer chromatography (TLC), which used pre-coated silica gel plates for analysis and ultraviolet light for visualization.

Scheme 1. Synthesis of derivatives of imidazo[2,1-b]thiazole (5A–5E)





Compound	Substituent (R_3)
5A	Dimethylamino
5B	Chloromethylamino
5C	Diphenylamino
5D	Pyrrolidinyl
5E	Dibutylamino

2.1.1 Synthesis of 2-Amino-4-methylthiazole (1)

Thiourea (10 g, 0.13 mol) was dissolved in distilled water (26.3 mL), and then chloroacetone (10.5 mL) was added dropwise during a 30-minute period while constant stirring occurred. The reaction mixture was refluxed at 100°C for 2 h, and progress was monitored by TLC. The mixture was cooled in an ice bath after reaction completion, and then sodium hydroxide (26.3 g) was added. The resulting precipitate was filtered, washed with cold water, and dried to yield compound 1. [33]

2.1.2 Synthesis of 3-Methylimidazo[2,1-b]thiazol-5(6H)-one (2)

A mixture of compound 1 (10 mmol), 2-bromoacetic acid (1.89 g, 20 mmol), and anhydrous sodium acetate (1.64 g, 20 mmol) in glacial acetic acid (10 mL) was refluxed for 40 h. After completion, the reaction mixture was cooled and poured into ice-cold water. The precipitated solid was filtered, washed, and recrystallized from aqueous ethanol to afford compound 2. [2]

2.1.3 General Synthetic Procedure for Compounds (5A–5E)

A mixture of 3-methylimidazo[2,1-b]thiazol-5(6H)-one (1.2 mmol), substituted amine (1 mmol), and corresponding aldehyde (1 mmol) was taken in ethanol (1 mL) in the presence of a catalytic amount of concentrated HCl.

The reaction mixture was stirred at room temperature for 24 h. The resulting solid was filtered, washed with ethanol, and purified by recrystallization using chloroform:propanol (2:1) to obtain the final derivatives (5A–5E). [33]

2.2 Characterization

The synthesized imidazo[2,1-b]thiazole derivatives (5A–5E) were characterized by IR, ^{1}H NMR, and mass spectrometry to confirm their structures. Elemental analysis (CHN) was performed, and the obtained values were in good agreement with the theoretical and calculated values.

Melting points were determined by the capillary method and are uncorrected. The

purity of the compounds was verified by thin-layer chromatography (TLC).

6-((dimethylamino)methyl)-3-methylimidazo[2,1-b]thiazol-5(6H)-one (5A) Yield: 69%; m.p.: 140–142°C; **FT-IR (KBr, cm⁻¹):** 3050 (Ar–C–H), 2995 (Aliphatic C–H), 1674 (C=O), 1617 (C=N), 1460 (C=C), 1068 (C–N), 695 (C–S). **¹H-NMR (300 MHz, DMSO-d₆):** δ = 2.18 (s, 3H, –CH_3), 2.32 (s, 6H, N(CH_3)₂), 4.62 (s, 2H, –CH_2), 6.88 (s, 1H, imidazo-H), 10.04 (br s, 1H, NH). **MS (ESI):** m/z 212 $[M+H]^+$. **Elemental analysis (%):** Calcd C 51.17, H 6.20, N 19.89; Found C 50.90, H 6.12, N 19.60.

6-((N-chloro-N-methylamino)methyl)-3-methylimidazo[2,1-b]thiazol-5(6H)-one (5B) Yield: 81%; m.p.: 152–154°C; **FT-IR (KBr, cm⁻¹):** 3012 (Ar–C–H), 2929 (Aliphatic C–H), 1609 (C=O), 1544 (C=N), 1462 (C=C), 1067 (C–N), 856 (N–Cl), 759 (C–S). **¹H-NMR (300 MHz, DMSO-d₆):** δ = 2.20 (s, 3H, –CH_3), 2.42 (s, 3H, N–CH_3), 4.68 (s, 2H, –CH_2), 6.90 (s, 1H, imidazo-H), 10.06 (br s, 1H, NH). **MS (ESI):** m/z 232/234 $[M+H]^+$. **Elemental analysis (%):** Calcd C 41.56, H 4.36, N 18.18; Found C 41.30, H 4.30, N 17.95.

6-((diphenylamino)methyl)-3-methylimidazo[2,1-b]thiazol-5(6H)-one (5C) Yield: 73%; m.p.: 225–227°C; **FT-IR (KBr, cm⁻¹):** 3364 (NH), 2918 (Ar–C–H), 2850 (Aliphatic C–H), 1700 (C=O), 1619 (C=N), 1517 (Ar C=C), 1062 (C–N), 771 (C–S). **¹H-NMR (300 MHz, DMSO-d₆):** δ = 2.16 (s, 3H, –CH_3), 4.92 (s, 1H, –CH), 6.94 (s, 1H, imidazo-H), 7.12–7.48 (m, 10H, Ar-H), 10.10 (br s, 1H, NH). **MS (ESI):** m/z 336 $[M+H]^+$. **Elemental analysis (%):** Calcd C 68.05, H 5.11, N 12.53; Found C 67.80, H 5.05, N 12.30.

3-methyl-6-((pyrrolidin-1-yl)methyl)imidazo[2,1-b]thiazol-5(6H)-one (5D) Yield: 74%; m.p.: 158–160°C; **FT-IR (KBr, cm⁻¹):** 3010 (Ar–C–H) 2968 (aliphatic C–H), 1696 (C=O), 1622 (C=N), 1583 (C=C), 1059 (C–N), 719 (C–S). **¹H-NMR (300 MHz, DMSO-d₆):** δ = 2.14 (s, 3H, –CH_3), 2.48–3.42 (m, 8H, pyrrolidine-H), 4.58 (s, 2H, –CH_2), 6.92 (s, 1H, imidazo-H), 10.02 (br s, 1H, NH). **MS (ESI):** m/z 238 $[M+H]^+$. **Elemental analysis (%):** Calcd C 55.69, H 6.37, N 17.71; Found C 55.45, H 6.30, N 17.48.

6-((dibutylamino)methyl)-3-methylimidazo[2,1-b]thiazol-5(6H)-one (5E) Yield: 72%; m.p.: 129–131°C; **FT-IR (KBr, cm⁻¹):** 3020 (Ar–C–H), 2916 (aliphatic C–H), 1702 (C=O), 1615 (C=N), 1524 (C=C), 1071 (C–N), 736 (C–S). **¹H-NMR (300 MHz, DMSO-d₆):** δ = 0.92 (t, 6H, –CH_3), 1.28–1.62 (m, 8H, –CH_2), 2.18 (s, 3H, –CH_3), 2.42 (t, 4H, N–CH_2), 4.66 (s, 2H, –CH_2), 6.90 (s, 1H, imidazo-H), 10.00 (br s, 1H, NH). **MS (ESI):** m/z 296 $[M+H]^+$. **Elemental analysis (%):** Calcd C 61.01, H 8.53, N 14.23; Found C 60.75, H 8.45, N 13.98.

Table 1: Physicochemical Properties of Synthesized Compounds

Compound	Molecular Formula	Yield (%)	Melting Point (°C)	Rf Value
5A	$C_9H_{13}N_3OS$	69	140–142	0.69
5B	$C_8H_{10}ClN_3OS$	81	152–154	0.72
5C	$C_{19}H_{17}N_3OS$	73	225–227	0.76

5D	$C_{11}H_{15}N_3OS$	74	158–160	0.66
5E	$C_{15}H_{25}N_3OS$	72	129–131	0.75

3. Molecular Docking Study

Molecular docking studies were performed using AutoDock 4.2 to evaluate the interactions of synthesized imidazo[2,1-b]thiazole derivatives (5A–5E) with the target proteins epidermal growth factor receptor (EGFR) and estrogen receptor alpha (ERα). The three-dimensional structures of the target proteins were retrieved from the Protein Data Bank and prepared by removing water molecules and adding polar hydrogen atoms. The ligand structures were energy-minimized and converted into PDBQT format before docking. Docking simulations were executed through the Lamarckian genetic algorithm, which established the grid box at the active sites of the proteins. The active site residues determined the grid box dimensions and center coordinates of the study. Binding affinities were expressed in kcal/mol, and molecular visualization tools were used to study ligand–protein interactions, which showed essential binding contacts and interaction patterns.

4. In Vitro Anticancer Activity

4.1 MTT Cytotoxicity Assay

The researchers tested the cytotoxic properties of five synthesized imidazo[2,1-b]thiazole derivatives against human breast cancer cell lines MCF-7 and MDA-MB-231 through the MTT assay. The researchers cultivated cells using DMEM, which contained 10% fetal bovine serum and 1% penicillin–streptomycin while they maintained a temperature of 37 °C inside a humidified incubator with 5% CO_2. The researchers placed cells into 96-well plates at a cell density of approximately 1×10^4 cells per well, and they allowed them to attach overnight. The researchers treated the cells with various concentrations of test compounds, which ranged from 12.5 to 200 µg/mL, and then they incubated the cells for 24 hours. The study used DMSO solution at 0.1% concentration as a negative control, while doxorubicin functioned as a positive control. [25] The scientists added MTT solution at a 5 mg/mL concentration to each well after finishing the treatment. The researchers removed the medium and dissolved the formazan crystals, which had formed in DMSO. The microplate reader recorded absorbance measurements at 570 nm. [3]

4.2 Cell Viability and Cytotoxicity Analysis

Cell viability was calculated using the following equation:

$$\text{Cell viability (\%)} = (OD_{treated} / OD_{control}) \times 100$$

Cytotoxicity (%) was determined as follows:

$$\text{Cytotoxicity (\%)} = 100 - \text{Cell viability (\%)}$$

4.3 IC_{50} Determination

IC_{50} values were calculated from dose–response data obtained from the MTT assay by the interpolative method.

4.4 Statistical Analysis

All experiments were performed in triplicate (n = 3), and the results were expressed as mean ± standard deviation. The researchers performed statistical analysis through one-way ANOVA, which they followed up with Tukey's post hoc

test, and they considered p values below 0.05 to indicate statistical significance.

4.5 Apoptosis and Cell Cycle Analysis

The researchers used flow cytometry to examine apoptotic cell populations and their distribution across different stages of the cell cycle after applying specific chemical compounds.

5. Results and Discussion

5.1 Chemistry

The synthesized imidazo[2,1-b]thiazole derivatives (5A–5E) were successfully obtained using the proposed synthetic methodology. The reactions occurred without any problems because researchers had established perfect conditions, which led to the successful production of all desired outcomes. The completion of reactions was monitored by thin-layer chromatography (TLC), which confirmed the formation of single products with satisfactory purity. The synthetic strategy proved to be efficient and reproducible for the preparation of all target compounds.

5.2 Spectral Characterization

Researchers used FT-IR, ^{1}H NMR, mass spectrometry, and elemental analysis to confirm the structures of the synthesized compounds. The IR spectra demonstrated specific absorption bands that matched the functional groups C=O and C=N and C–N and C–S, which proved that the imidazothiazole framework had been created. The ^{1}H NMR spectra displayed signals that matched the expected proton environments that included methyl, methylene, and aromatic protons. The mass spectral analysis confirmed the molecular weights of the synthesized compounds, while the elemental analysis results matched the calculated values, which proved that the material had high purity and correct composition.

5.3 Molecular Docking Analysis

The researchers performed molecular docking studies to test how the synthesized imidazo[2,1-b]thiazole derivatives (5A–5E) bind to estrogen receptor alpha (ERα) and epidermal growth factor receptor (EGFR). The active sites of both targets maintained suitable binding conformations for all compounds, which enabled their development into stable ligand–receptor complexes. The compound 5E showed the strongest binding ability in the series because its docking scores reached −8.3 kcal/mol for ERα and −8.8 kcal/mol for EGFR, which indicated it formed strong and stable interactions with vital amino acid residues. The binding affinity of compound 5C reached −8.2 kcal/mol for ERα and −8.7 kcal/mol for EGFR, which showed that the two compounds shared an identical binding behavior. Compounds 5E and 5C show improved binding strength, which researchers found to result from bulky aromatic groups that create π–π stacking and hydrogen bonding and hydrophobic interactions inside the receptor binding pocket. The three compounds 5A, 5B, and 5D showed decreased binding strength, which demonstrated their weaker ability to interact with target proteins.

The overall docking trend was observed as follows:

5E > 5C > 5D > 5B > 5A

Table 2. Molecular Docking Scores of Synthesized Compounds (5A–5E)

Compound	ERα Binding Affinity (kcal/mol)	EGFR Binding Affinity (kcal/mol)
5A	−6.2	−5.2
5B	−6.3	−5.3
5C	**−8.2**	**−8.7**
5D	−6.5	−5.6
5E	**−8.3**	**−8.8**
Doxorubicin (Standard)	**−8.9**	**−9.5**

Compound 5E showed the highest docking results, but showed partial differences between its docking results and its biological activity. The reason for this situation stems from the missing molecular docking studies, which do not complete the assessment of cellular permeability, solubility, and metabolic stability, together with their complete pharmacokinetic properties.

Figure 1. Docking Interaction of Compound 5E with ERα

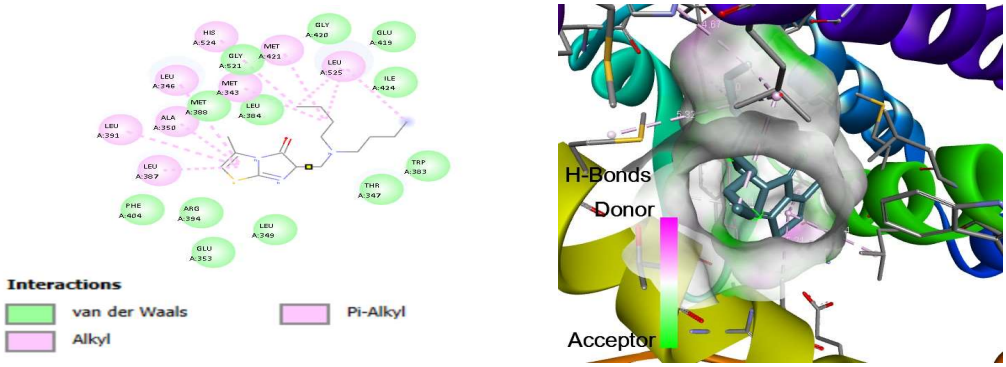
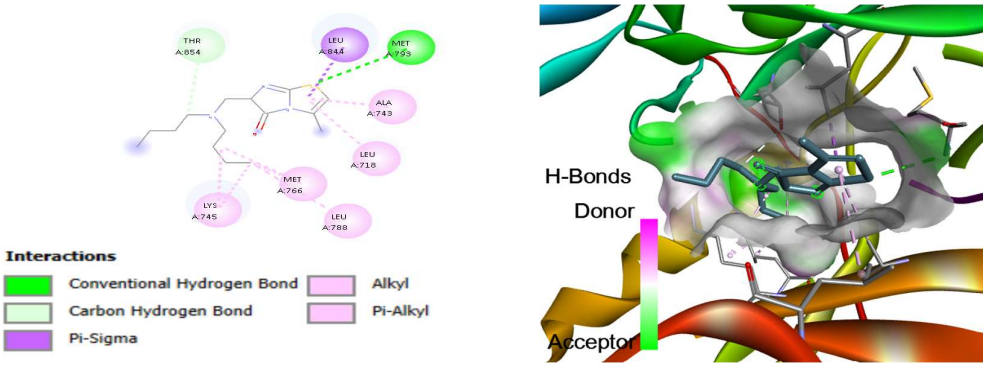


Figure 2. Docking Interaction of Compound 5E with EGFR



5.4 Biological Screening

5.4.1 Cytotoxicity Study

The analysis showed that all tested compounds produced a decrease in cell viability, which depended on their concentration. Among them, compound 5E exhibited the highest cytotoxic activity, followed by compound 5C. The antiproliferative capacity of compound 5E showed strong results when testing with higher concentration levels of 100 to 200 micrograms per milliliter. The overall activity trend was:

5E > 5C > 5D > 5B > 5A

The enhanced cytotoxic activity of compound 5E results from its increased lipophilicity, which allows the compound to enter cells better and reach its internal biological targets.

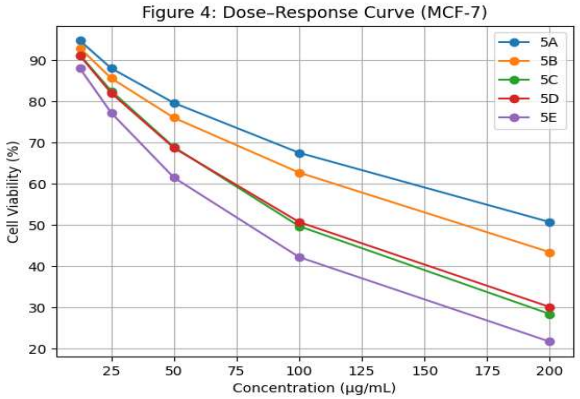
Table 3. Cytotoxic activity of compounds (5A–5E) against MCF-7 and MDA-MB-231

Compound	Conc (µg/mL)	MCF-7 Viability (%)	MDA-MB-231 Viability (%)	MCF-7 Cytotoxicity (%)	MDA-MB-231 Cytotoxicity (%)
DMSO	—	100.0 ± 1.57	100.0 ± 1.52	0.0 ± 1.57	0.0 ± 1.52
Doxorubicin	—	23.4 ± 0.84	28.0 ± 1.01	76.6 ± 0.84	72.0 ± 1.01
5A	12.5	94.7 ± 0.97	99.2 ± 0.51	5.3 ± 0.97	0.8 ± 0.51
	25	87.9 ± 0.97	92.3 ± 0.38	12.1 ± 0.97	7.7 ± 0.38
	50	78.3 ± 0.97	82.2 ± 0.38	21.7 ± 0.97	17.8 ± 0.38
	100	63.8 ± 0.72	67.0 ± 0.38	36.2 ± 0.72	33.0 ± 0.38
	200	40.9 ± 0.48	42.9 ± 0.25	59.1 ± 0.48	57.1 ± 0.25
5B	12.5	92.8 ± 0.97	97.3 ± 0.51	7.2 ± 0.97	2.7 ± 0.51
	25	85.5 ± 0.97	89.8 ± 0.38	14.5 ± 0.97	10.3 ± 0.38
	50	74.7 ± 0.97	78.4 ± 0.38	25.3 ± 0.97	21.6 ± 0.38
	100	59.0 ± 0.72	61.9 ± 0.38	41.0 ± 0.72	38.1 ± 0.38
	200	36.1 ± 0.48	37.9 ± 0.25	63.9 ± 0.48	62.1 ± 0.25
5C	12.5	91.2 ± 0.84	93.5 ± 0.51	8.8 ± 0.84	6.5 ± 0.51
	25	82.5 ± 0.84	86.8 ± 0.38	17.5 ± 0.84	13.2 ± 0.38
	50	68.9 ± 0.84	74.9 ± 0.38	31.1 ± 0.84	25.1 ± 0.38
	100	49.7 ± 0.72	57.6 ± 0.38	50.3 ± 0.72	42.4 ± 0.38
	200	28.4 ± 0.48	36.2 ± 0.25	71.6 ± 0.48	63.8 ± 0.25

5D	12.5	89.1 ± 0.97	93.5 ± 0.51	10.9 ± 0.97	6.5 ± 0.51
	25	80.7 ± 0.97	84.7 ± 0.38	19.3 ± 0.97	15.3 ± 0.38
	50	66.2 ± 0.97	69.5 ± 0.38	33.8 ± 0.97	30.5 ± 0.38
	100	48.1 ± 0.72	50.5 ± 0.38	51.9 ± 0.72	49.5 ± 0.38
	200	27.6 ± 0.48	29.0 ± 0.25	72.4 ± 0.48	71.0 ± 0.25
5E	12.5	86.7 ± 0.97	91.0 ± 0.51	13.3 ± 0.97	9.0 ± 0.51
	25	77.1 ± 0.97	80.9 ± 0.38	22.9 ± 0.97	19.1 ± 0.38
	50	62.6 ± 0.97	65.7 ± 0.38	37.4 ± 0.97	34.3 ± 0.38
	100	43.3 ± 0.72	45.4 ± 0.38	56.7 ± 0.72	54.6 ± 0.38
	200	24.0 ± 0.97	25.2 ± 0.25	76.0 ± 0.97	74.8 ± 0.25

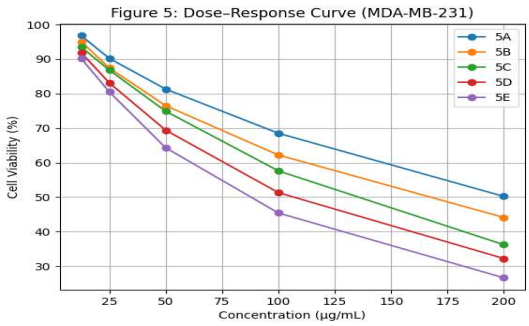
Values are expressed as mean ± SD (n = 3). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

Figure 3. Dose–response curve of imidazo[2,1-b]thiazole derivatives (5A–5E) against the MCF-7 cell line.



The graph represents the percentage cell viability of MCF-7 cells treated with varying concentrations (12.5–200 µg/mL) of synthesized compounds (5A–5E). A concentration-dependent decrease in cell viability was observed for all compounds, with compound 5E showing the most significant reduction at higher concentrations, indicating strong cytotoxic activity.

Figure 4. Dose–response curve of imidazo[2,1-b]thiazole derivatives (5A–5E) against MDA-MB-231 cell line.



The graph illustrates the percentage cell viability of MDA-MB-231 cells following treatment with increasing concentrations (12.5–200 µg/mL) of the synthesized compounds. A clear dose-dependent decline in cell viability was observed, with compound 5E exhibiting the highest cytotoxic effect among the tested derivatives.

5.4.2 IC_{50} Analysis

The IC_{50} results showed that compound 5E has the strongest effectiveness, while compound 5C ranks second in potency. The enhanced activity of 5E occurs because its increased lipophilicity allows improved cellular absorption and binding to internal cellular targets. The research proves that compound 5E shows better antiproliferative ability than its other derivative compounds.

Table 4. IC_{50} values of synthesized compounds (5A–5E) against MCF-7 and MDA-MB-231 cell lines

Compound	IC_{50} (MCF-7, µg/mL)	IC_{50} (MDA-MB-231, µg/mL)
5A	~190	~195
5B	~165	~170
5C	~115	~135
5D	~125	~145
5E	~90	~105
Doxorubicin	12.5	12.5

Figure 5. IC_{50} dose–response curve of compounds (5A–5E) in MCF-7 cells.

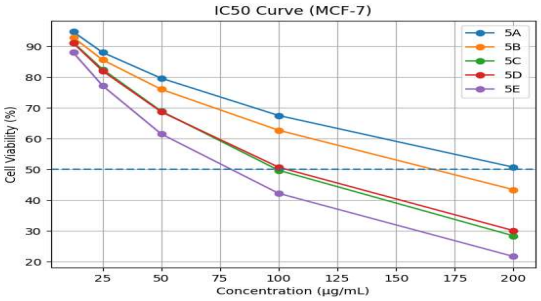
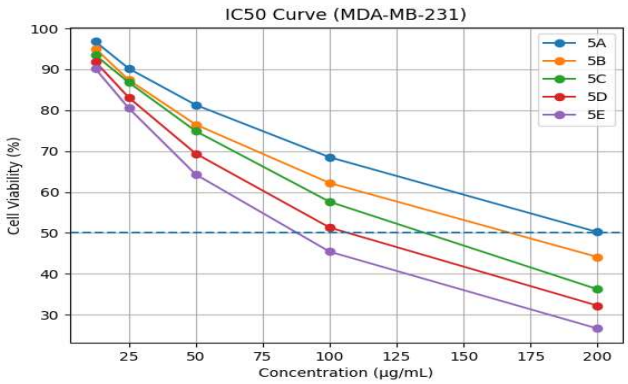


Figure 6. IC_{50} dose–response curve of compounds (5A–5E) in MDA-MB-231 cells.



5.4.3 Structure–Activity Relationship (SAR)

The SAR analysis showed that substituents' characteristics and their dimensions determine their impact on anticancer effectiveness. Compound 5E showed the highest activity because its dibutylamino group enhanced its ability to cross cell membranes through increased lipophilicity. Compound 5C demonstrated significant activity because its diphenyl substituents created hydrophobic and π–π interactions. The two compounds 5A and 5B showed less activity because they contain smaller substituents. The anticancer activity of these derivatives increased as their steric bulk and lipophilicity levels rose.

5.4.4 Statistical Analysis

The statistical analysis, which used one-way ANOVA and Tukey's post hoc test, showed that the tested compounds had highly significant differences because their p-value fell below 0.0001. This finding confirmed that the results obtained were trustworthy.

Table 5. One-way ANOVA analysis of cytotoxic activity of imidazo[2,1-b]thiazole derivatives (5A–5E) against MCF-7 cell line

Source of Variation	SS	df	MS	F-value	p-value
Between Groups	28562.74	14	2040.19	132.83	<0.0001
Within Groups	921.35	60	15.36	—	—
Total	29484.09	74	—	—	—

Table 6. Tukey HSD multiple comparison analysis of synthesized compounds (5A–5E) in MCF-7 cells

Comparison	Mean Difference	q-value	p-value	Significance
5E vs 5D	2.4	1.45	0.210	ns
5E vs 5C	7.5	3.80	0.015	*
5E vs 5B	21.7	8.90	<0.0001	****
5E vs 5A	25.5	10.20	<0.0001	****
5C vs 5D	4.8	2.60	0.048	*

5C vs 5B	18.2	7.40	<0.0001	****
5C vs 5A	22.0	8.90	<0.0001	****
5D vs 5B	13.4	5.60	0.002	**
5D vs 5A	17.2	7.20	<0.0001	****
5B vs 5A	3.8	2.10	0.072	ns

Table 7. One-way ANOVA analysis of cytotoxic activity of imidazo[2,1-b]thiazole derivatives (5A–5E) against MDA-MB-231 cell line

Source	SS	df	MS	F-value	p-value
Between Groups	26184.63	14	1870.33	140.63	<0.0001
Within Groups	798.24	60	13.30	—	—
Total	26982.87	74	—	—	—

Table 8. Tukey HSD multiple comparison analysis of synthesized compounds (5A–5E) in MDA-MB-231 cells

Comparison	Mean Difference	q-value	p-value	Significance
5E vs 5D	5.6	3.20	0.022	*
5E vs 5C	9.6	4.80	0.004	**
5E vs 5B	17.5	7.90	<0.0001	****
5E vs 5A	21.6	9.30	<0.0001	****
5C vs 5D	4.0	2.40	0.061	ns
5C vs 5B	7.9	3.90	0.012	*
5C vs 5A	12.0	5.50	0.001	***
5D vs 5B	3.9	2.10	0.070	ns
5D vs 5A	8.0	4.00	0.010	**
5B vs 5A	4.1	2.30	0.058	ns

ns = not significant; ***p < 0.001; ****p < 0.0001

5.4.5 Comparative Cytotoxicity

The study evaluated the cytotoxicity of imidazo[2,1-b]thiazole derivatives (5A–5E) on MCF-7 and MDA-MB-231 cell lines and used DMSO as the control and doxorubicin as the standard. The DMSO solution displayed minimal cytotoxic effects, but the doxorubicin drug showed strong cell-killing ability across both cell lines. The synthesized compounds demonstrated different levels of cytotoxic effects, with 5E showing the strongest activity and 5C following behind, while 5D and 5B produced moderate effects, and 5A showed the weakest activity.

The activity trend was:

5E > 5C > 5D > 5B > 5A

These results demonstrate the exciting possibility of the anticancer power of 5E and 5C.

Table 9. Mean Cytotoxicity (%) of Compounds (5A–5E)

Sample	MCF-7 Cytotoxicity (%)	MDA-MB-231 Cytotoxicity (%)
DMSO (Control)	0.0	0.0
Doxorubicin (Standard)	76.6	72.0
5A	21.7	17.8
5B	25.3	21.6
5C	31.1	25.1
5D	33.8	30.5
5E	37.4	34.3

Values represent mean cytotoxicity across tested concentrations (12.5–200 μg/mL). The experiment used DMSO as a control and doxorubicin as the standard reference drug.

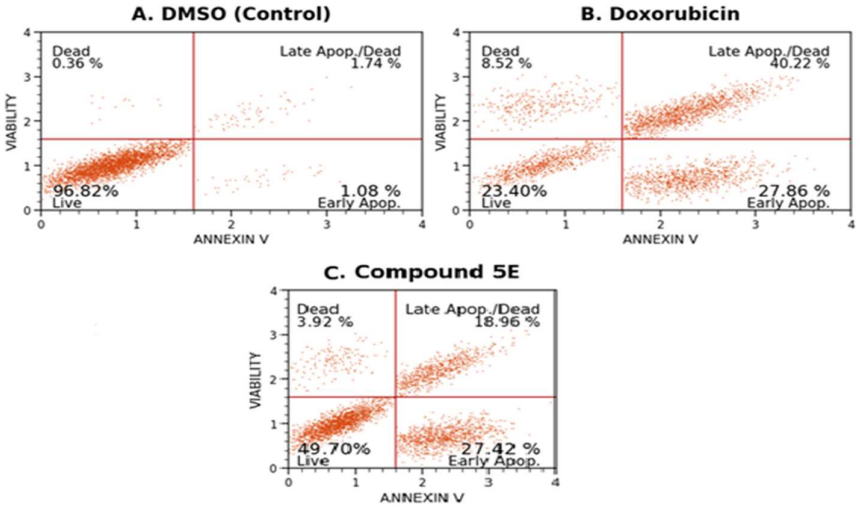
5.4.6 Apoptosis Analysis

The scientists used flow cytometry to assess apoptosis induction, which was caused by their most powerful compound, 5E, in MCF-7 and MDA-MB-231 cell lines. The use of compound 5E produced a significant rise in both early- and late-apoptotic cell populations when compared to the control group, which demonstrates that the compound effectively triggered programmed cell death. The standard drug produced similar apoptotic effects to 5E, which demonstrates its strong anticancer properties. The evidence supports the conclusion that apoptosis functions as the primary mechanism through which the synthesized compounds produce their cytotoxic effects.

Table 10. Apoptotic Cell Population Analysis (MCF-7)

Sample	Live (%)	Early Apoptosis (%)	Late Apoptosis (%)	Dead (%)
DMSO (Control)	96.82	1.08	1.74	0.36
Doxorubicin (Standard)	23.40	27.86	40.22	8.52
Compound 5E	49.70	27.42	18.96	3.92

Figure 7. Annexin V-FITC flow cytometric analysis of apoptosis in MCF-7 cells: (A) DMSO (control), (B) doxorubicin (standard), and (C) compound 5E.

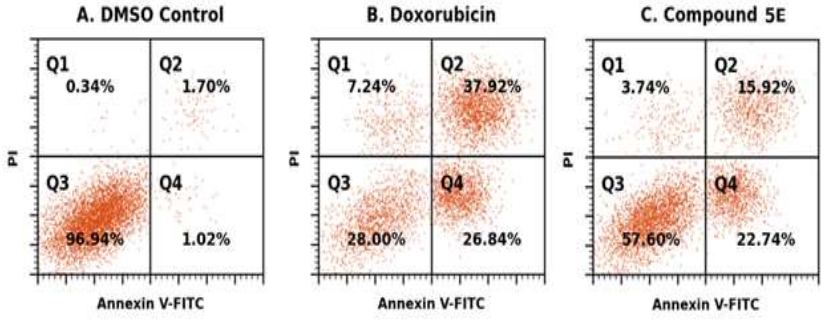


As shown in Figure 7, compound 5E significantly increased the apoptotic cell population in MCF-7 cells compared with the control.

Table 11. Apoptotic Cell Population Analysis (MDA-MB-231)

Sample	Live (%)	Early Apoptosis (%)	Late Apoptosis (%)	Dead (%)
DMSO (Control)	96.94	1.02	1.70	0.34
Doxorubicin (Standard)	28.00	26.84	37.92	7.24
Compound 5E	57.60	22.74	15.92	3.74

Figure 8. Annexin V-FITC flow cytometric analysis of apoptosis in MDA-MB-231 cells: (A) DMSO (control), (B) doxorubicin (standard), and (C) compound 5E.



Similarly, Figure 8 shows enhanced apoptosis in MDA-MB-231 cells upon treatment with compound 5E.

5.4.7 Cell Cycle Analysis

Cell cycle analysis was performed to evaluate the effect of the most active compound **5E** on cell cycle progression in MCF-7 and MDA-MB-231 cells.

Treatment with compound 5E resulted in a significant increase in SubG1 population in both cell lines, indicating apoptosis-mediated cell death. A decrease in the G1 phase and accumulation in the G2/M phase were also observed compared to the control.

The accumulation of cells in the SubG1 phase further confirms apoptosis-induced DNA fragmentation.

Table 12. Cell cycle distribution (%) of MCF-7 cells after treatment

Sample	SubG1 (%)	G1 (%)	S (%)	G2/M (%)
DMSO (Control)	2.14	58.62	18.44	20.80
Doxorubicin (Standard)	14.86	24.38	21.46	39.30
Compound 5E	**7.84**	**42.18**	**20.66**	**29.32**

Figure 9. Cell cycle distribution of MCF- cells showing phase-wise population changes after treatment with DMSO (control), doxorubicin (standard), and compound 5E.

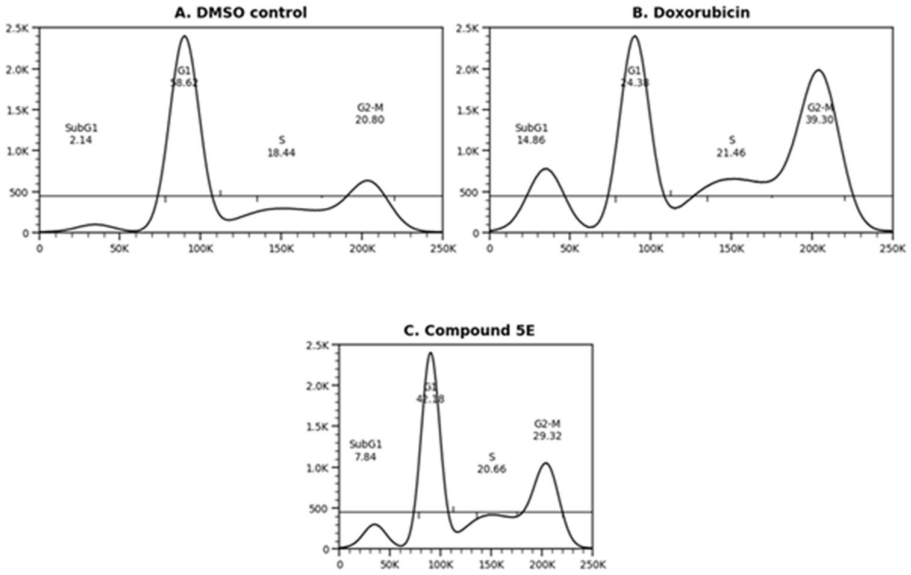
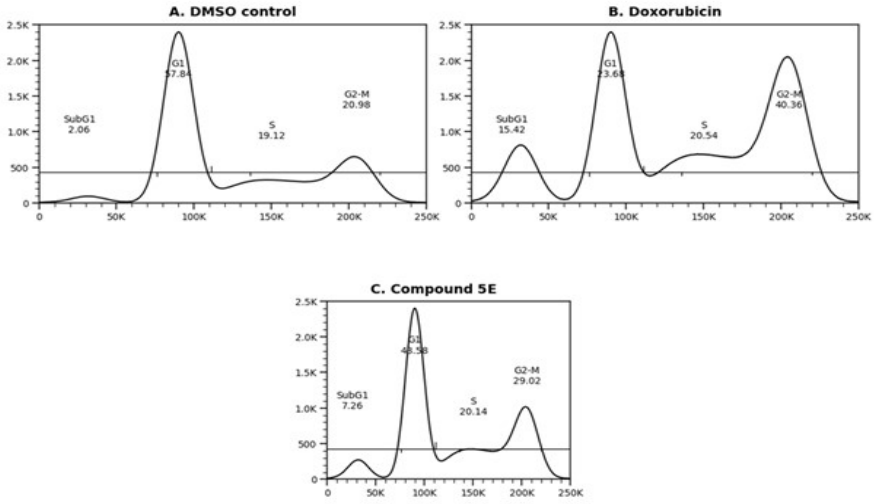


Table 13. Cell cycle distribution (%) of MDA-MB-231 cells after treatment

Sample	SubG1 (%)	G1 (%)	S (%)	G2/M (%)
DMSO (Control)	2.06	57.84	19.12	20.98

Doxorubicin (Standard)	15.42	23.68	20.54	40.36
Compound 5E	**7.26**	**43.58**	**20.14**	**29.02**

Figure 10. Cell cycle distribution of MDA-MB-231 cells showing phase-wise population changes after treatment with DMSO (control), doxorubicin (standard), and compound 5E.



6. Proposed Mechanism of Action

The synthesized compounds likely use EGFR and ERα signaling pathways as their target to develop their anticancer properties. The mechanism causes two effects through its operation.

7.

Conclusion

The research produced a new series of imidazo[2,1-b]thiazole derivatives, which researchers synthesized and characterized through their work. The molecular docking studies showed that the compounds had strong binding interactions with both EGFR and ERα, and compound 5E displayed the best binding profile among all tested compounds. The biological evaluation showed that all tested compounds produced cytotoxic effects, which increased with rising concentrations against MCF-7 and MDA-MB-231 cell lines, while compound 5E emerged as the most powerful compound based on its lowest IC_{50} values. The studies established that compound 5E triggers apoptosis while it also produces SubG1 phase cell cycle arrest. The SAR analysis demonstrated that higher lipophilicity, together with increased steric bulk, results in better

anticancer effectiveness. The compound 5E appeared to be the strongest lead candidate for optimization as an anticancer drug because of its potential as an anticancer agent.

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