JOURNAL OF CLINICAL AND BIOMEDICAL SCIENCES

ORIGINAL ARTICLE

OPEN ACCESS

Received: 22-01-2025
Accepted: 06-03-2025
Published: 26-09-2025

Citation: Chakraborty AK, Singh A, Maity P. Design, Synthesis and Biological Evaluation of Some Novel Benzthiazole Containing 4H-Chromene-4-one Derivatives. J Clin Biomed Sci 2025; 15(3): 184-190. https://doi.org/10.58739/jcbs/v15i3.25.40

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Funding: None

Competing Interests: None

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Published By Sri Devaraj Urs Academy of Higher Education, Kolar, Karnataka

ISSN
Print: 2231-4180
Electronic: 2319-2453

Design, Synthesis and Biological Evaluation of Some Novel Benzthiazole Containing 4H-Chromene-4-one Derivatives

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Abstract

Background: Chromene derivatives are gaining attention in the pharmaceutical industry, while substituted benzothiazole derivatives have shown potential in various therapeutic areas, including anti-ulcer, anti-hypertensive, anti-viral, anti-fungal, anti-cancer, and anti-histaminic treatments. **Material and Methods:** Benzimidazole derivatives, when combined with other heterocyclic compounds such as pyrazole, thiadiazole, triazole, thiazole, coumarin, and 2-azetidinone, exhibit a wide range of pharmacological properties. In our research, we developed novel 2-(2-(5-nitro-1H-benzo[d]thiazol-2-yl)vinyl)-4H-chromen-4-one derivatives through a reaction involving 2-methyl-1H-benzo[d]thiazole and substituted chromone-3-carbaldehyde in the presence of glacial acetic acid (GAA). **Results:** The synthesized compounds were characterized using techniques such as IR spectroscopy, ^{1}H NMR, ^{13}C NMR, mass spectrometry, and elemental analysis. Their in-vitro anti-cancer activity was assessed against the A-549 human cancer cell line using the SRB assay method. Among the tested compounds, R1 and R6 demonstrated significant inhibitory activity, reducing cell growth by 21.9% and 39.4%, respectively, at a concentration of 80 μg/mL, while other derivatives allowed 57–94% cell growth. **Conclusion:** The biological activity was influenced by the substitutions on the coumarin nucleus, with unsubstituted (R4), fluoro-substituted (R6), and chloro-substituted (R1) coumarin derivatives showing notable potency.

Keywords: Synthesis; Heterocyclic; Benzthiazole; Chromene; Derivatives; Cancer cell line

1 Introduction

Heterocycles represent a crucial class of compounds, accounting for more than half of all known organic substances[1]. They are integral to a wide array of applications, appearing in numerous drugs, vitamins, natural products, biomolecules, and biologically active agents, including antitumor, antibiotic, anti-inflammatory,

antidepressant, antimalarial, anti-HIV, antimicrobial, antibacterial, antifungal, antiviral, antidiabetic, herbicidal, fungicidal, and insecticidal compounds. Furthermore, heterocycles play a significant role as structural components in synthetic pharmaceuticals and agrochemicals[2].These compounds also exhibit unique properties such as solvatochromism, photochromism, and bioluminescence, making them valuable in materials science. They are widely utilized in the production of dyes, fluorescent sensors, brightening agents, data storage materials, plastics, and analytical reagents. Heterocycles are especially important in supramolecular and polymer chemistry, including the development of conjugated polymers.

For medicinal chemists, the true advantage of heterocycles lies in their versatility, allowing for the creation of extensive compound libraries from a single core scaffold. These libraries can then be screened across various receptors, often yielding multiple active molecules. The potential for designing nearly infinite combinations of fused heterocyclic structures enables the development of novel polycyclic frameworks with diverse physical, chemical, and biological characteristics. Consequently, devising efficient strategies to synthesize polycyclic structures from biologically relevant heterocyclic templates is a priority for both organic and medicinal chemists[3]. The ultimate aim of medicinal chemistry is to innovate and discover new therapeutic compounds.

1.1 Drug discovery and development process

The process of drug discovery and development is intricate and highly demanding, often carrying life-and-death implications for patients. Their well-being depends on the dedication of scientists and clinicians who work to discover, develop, and administer medications aimed at preventing, managing, and treating diseases, injuries, and various health conditions. This journey, from initial discovery to an approved drug, typically spans 12–15 years and involves a financial commitment of roughly one billion dollars. Of the millions of molecules screened, only a small fraction advance to late-stage clinical trials and eventually reach patients as approved treatments[4].

1.2 Benzthiazole

Thiazole was first identified by Hantzsch and Weber in 1887, with its structure later confirmed by Popp in 1889. In the thiazole ring, the numbering begins at the sulfur atom. The basic structure of benzothiazole consists of a benzene ring fused to the 4th and 5th positions of a thiazole ring. Thiazole is a five-membered heterocyclic compound containing one sulfur and one nitrogen atom. Both thiazole and its derivatives, including benzothiazole, serve as fundamental frameworks in the synthesis of numerous thiazole-based compounds. These derivatives exhibit a wide range of pharmacological activities and have proven effective in the treatment of various diseases[5].

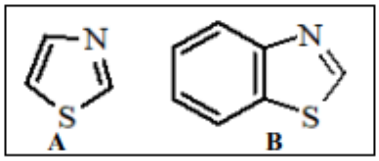


Fig 1. Structure of A) Thiazole and B) Benzothiazole

1.3 Chromene

Oxygen-containing heterocycles occupy a prominent position among heterocyclic compounds due to their natural abundance and significant roles in biology and medicine[6]. Within this group, chromene-based scaffolds stand out for their widespread occurrence in natural products and their diverse and potent biological activities. The term ”chromones” originates from the Greek word chroma, meaning ”color,” highlighting the vivid range of colors displayed by many chromone derivatives. A 4H-chromene (4H-1-benzopyran) is defined as a compound in which a benzene ring is fused with a 4H-pyran ring.

2 Materials and methods

2.1 Chemicals and equipment

All chemicals used in this study were sourced from Sigma Aldrich (USA). Analytical thin-layer chromatography (TLC) was performed on silica gel G/UV-254 precoated sheets (0.2 mm thick) from Macherey-Nagel (Germany), employing analytical grade solvents. Spots were visualized using either iodine spray (10% w/w I_2 in silica gel) or UV light. Additionally, bioinformatics tools and databases, such as the Protein Data Bank (PDB), alongside software like Autodock and ACD ChemSketch, were utilized. The PDB, established at Brookhaven National Laboratories (BNL), serves as the global repository for structural data of biological macromolecules, including structures determined through X-ray crystallography and NMR methods.

Melting points were measured using capillary tubes and are uncorrected. Infrared (IR) spectra were recorded as KBr pellets for solids on a Perkin Elmer Spectrum FT-IR instrument. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained in DMSO-d with TMS as an internal standard, using a Bruker NMR spectrometer. Spin multiplicities are reported as singlet (s), doublet (d), triplet (t), or multiplet (m), with coupling constants (J) provided in hertz. Mass spectra were acquired using a Thermo Finnigan LCQ Advantage MAX 6000 ESI spectrometer.

2.2 General procedure for the synthesis of title compounds[7–11]

Step 1: Synthesis of 2-methyl-1H-benzo[d]thiazole

In a round-bottom flask, combine 5.43 g of 2-mercaptoaniline, 20 mL of water, and 5.4 g of acetic acid. Heat the mixture under reflux using a water bath for 45 minutes. Allow the reaction mixture to cool to room temperature. Gradually add a 10% ammonia solution to the cooled mixture while stirring continuously until a precipitate forms. Filter the precipitate and purify it by recrystallization from 10% aqueous ethanol, using activated charcoal to remove impurities.

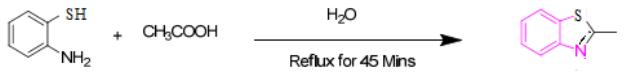


Fig 2. Synthesis of 2-methyl-1H-benzo[d]thiazole

Step 2: Synthesis of 2-methyl-5-nitro-1H-benzo[d]thiazole

Add 5 mL of concentrated nitric acid (HNO_3) and 5 mL of concentrated sulfuric acid (H_2SO_4) to 2-methyl-1H-benzo[d]thiazole in a reaction flask. Heat the mixture under reflux for 30 minutes. Afterward, pour the reaction mixture into cold water while shaking vigorously. A yellow precipitate of 2-methyl-5-nitro-1H-benzo[d]thiazole will form.

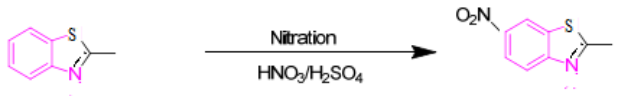


Fig 3. Synthesis of 2-methyl-5-nitro-1H-benzo[d]thiazole

Step 3: Synthesis of 2-(2-(5-nitro-1H-benzo[d]thiazol-2-yl) vinyl)-4H-chromen-4-one Derivatives

Mix 1 mmol of 2-methyl-1H-benzo[d]imidazole with 1 mmol of different substituted chromen-3-carbaldehydes dissolved in ethanol. Add 10 mL of glacial acetic acid and heat the mixture under reflux for 1 hour. Monitor the reaction progress by TLC using a 50:50 mixture of ethyl acetate and petroleum ether as the mobile phase. Once the reaction is complete, filter the mixture, wash it with acetic acid or ethanol, and dry it under vacuum.

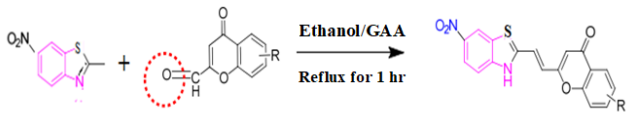


Fig 4. Synthesis of 2-(2-(5-nitro-1H-benzo[d]thiazol-2-yl) vinyl)-4H-chromen-4-one Derivatives

2.3 Synthesized derivatives of 2-(2-(5-nitro-1H-benzo[d]thiazol-2-yl vinyl -4H-chromen-4-one

Table 1. List of substituents of newly synthesized derivatives

Sr. No.	Compound Code	R
1	R1	6-Cl
2	R2	7-F
3	R3	6-CH3
4	R4	-H
5	R5	6,7-Cl
6	R6	6-F
7	R7	6-OCH3
8	R8	6-Br

2.4 In-Vitro Anticancer Activity by SRB Assay

To evaluate the anti-tumor activity of a new compound, it is essential to determine its cytotoxic concentration. Cytotoxicity tests identify the highest concentration of the compound that does not harm the cell line. After treatment with the drug, cell death and viability are measured. The results are further confirmed through additional metabolic assays, such as the SRB assay.

3 Results

3.1 Characterization of synthesized compound

The purity of the synthesized compounds was confirmed by measuring the melting point and conducting TLC using a 50:50 ethyl acetate: petroleum ether solvent system. The structures of the compounds were validated through FT-IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry, with the results matching the anticipated structures.

3.2 Spectral data for synthesized compound

IR spectral data and NMR spectral data:

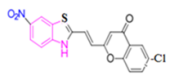
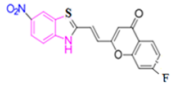
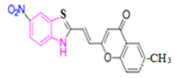
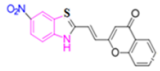
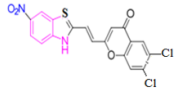
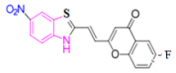
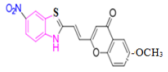
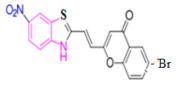
Com code	Structure	Molecular Formula	Mol weight	Melting point	Appearance
R1		C18H10ClSN2O4	367	218-220°C	Orange solid
R2		C18H10FSN2O4	351	210-212°C	Yellow solid
R3		$C_{19}H_{13}N_2SO_4$	347	200-202°C	Orange solid
R4		$C_{18}H_{11}N_2SO_4$	333	220-222°C	Orange solid
R5		$C_{18}H_9Cl_2N_2SO_4$	402	220-222°C	Orange solid
R6		$C_{18}H_{10}FN_2SO_4$	351	219-221°C	Orange solid
R7		$C_{19}H_{13}N_2SO_5$	363	216-218°C	Orange solid
R8		$C_{18}H_{10}BrN_2SO_4$	412	218-220°C	Orange solid

Fig 5. Physico-chemical properties of synthesized compound

Table 2. Solubility data of synthesized compounds

Compound code	Water	Acetone	Chloroform	DMSO	Ethanol	Methanol	Ethyl acetate
R1	-	++	+++	+++	+	++	+
R2	-	++	+++	+++	+	++	+
R3	-	++	+++	+++	+	++	+
R4	-	++	+++	+++	+	++	+
R5	-	++	+++	+++	++	++	+
R6	-	++	+++	+++	+	++	+
R7	-	++	++	+++	+	++	+
R8	-	++	++	+++	+	++	+

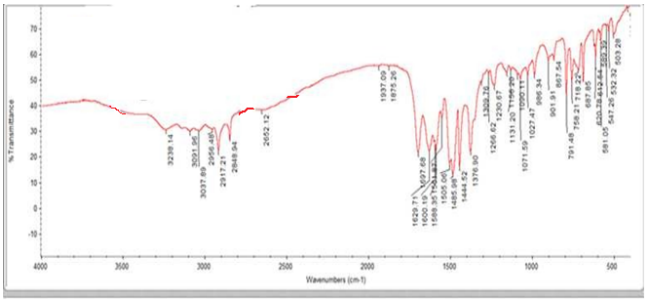


Fig 6. IR Spectra for compound R1

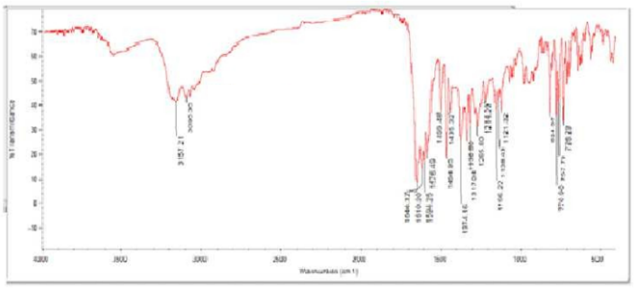


Fig 7. IR Spectra for compound R2

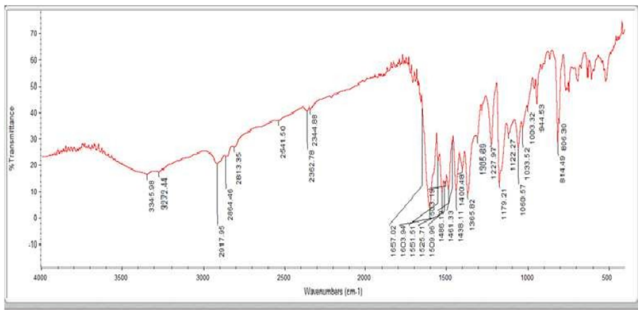


Fig 8. IR Spectra for compound R3

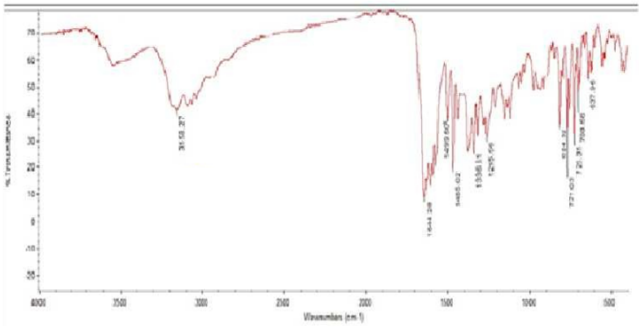


Fig 9. IR Spectra for compound R4

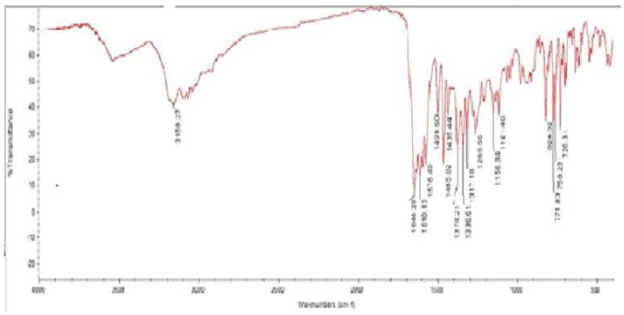


Fig 10. IR Spectra for compound R5

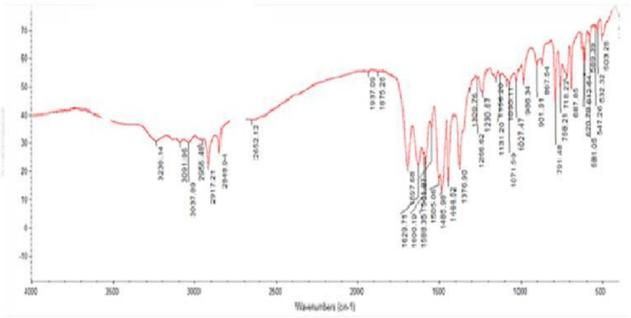


Fig 11. IR Spectra for compound R6

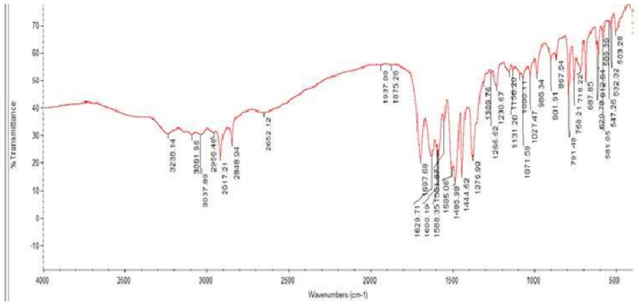


Fig 12. IR Spectra for compound R7

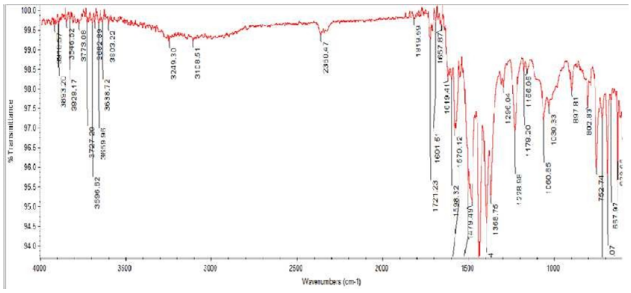


Fig 13. IR Spectra for compound R8

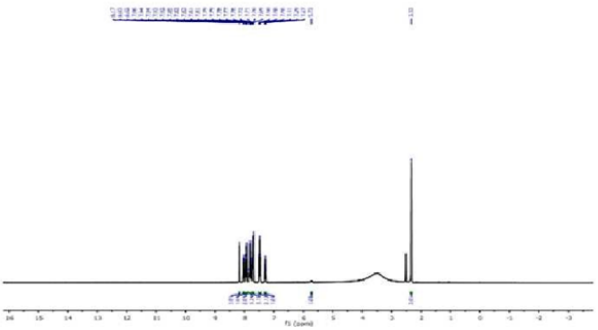


Fig 14. Fig 12. ^{1} H NMR spectrum of compound R1

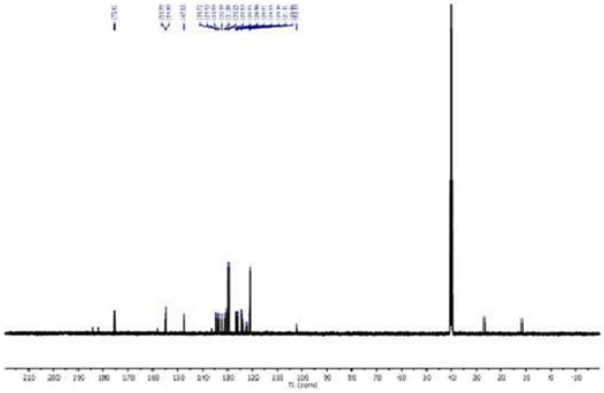


Fig 15. ^{13} C NMR spectrum of compound R1

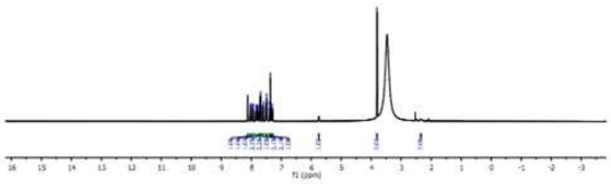


Fig 16. ^{1} H NMR spectrum of compound R6

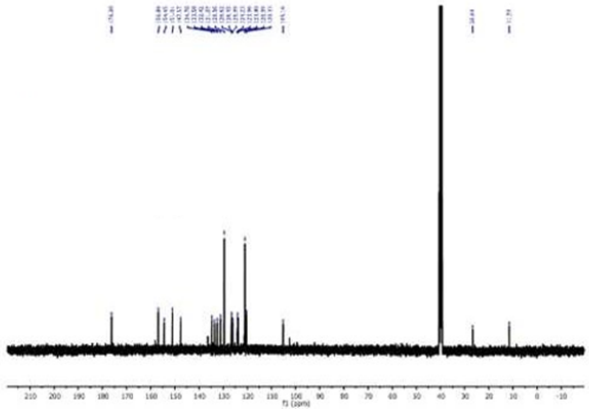


Fig 17. Fig 15. ^{13} C NMR spectrum of compound R6

3.3 In vitro Anti-Cancer Activity

The synthesized compounds R1, R2, R3, R4, R5, R6, R7, and R8 were assessed for anti-cancer activity using the SRB assay against the A-549 human lung cancer cell line. All the compounds demonstrated moderate to strong growth inhibition of A-549 cells when compared to the control drug, Adriamycin.

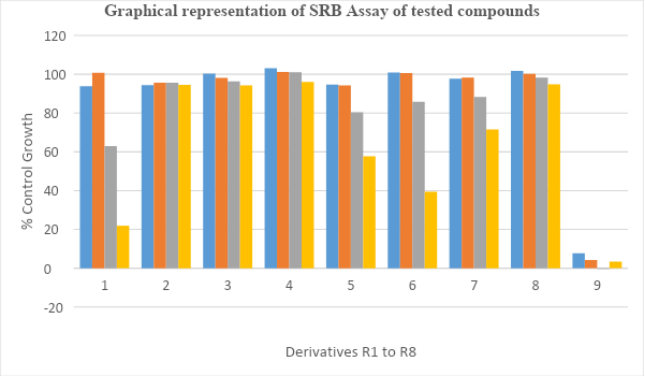


Fig 18. Graphical representation of SRB Assay data of tested compounds

4 Discussion

We synthesized 2-(2-(5-nitro-1H-benzo[d]thiazol-2-yl)vinyl)-4H-chromen-4-one derivatives by reacting 2-methyl-1H-benzo[d]thiazole with substituted chromone-3-carbaldehyde in the presence of glacial acetic acid (GAA). The compounds were characterized using IR, 1H NMR, 13C NMR, mass spectrometry, and elemental analysis. Their in-vitro anti-cancer activity was evaluated using the SRB assay against the A-549 human cancer cell line.

Table 3. The SRB Assay of tested compounds against Human Lung Cancer Cell Line A-549

Code	Human Lung Cancer Cell Line A-549															
	% Control Growth															
	Drug Concentrations (μg/ml)															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
R1	86.7	96.7	62.5	18.3	101.9	113.2	84.1	34.7	93.2	92.4	42.5	12.8	93.9	100.8	63	21.9
R2	91.2	89.5	85.6	95.3	101.3	104.9	112.6	93	90.8	92.4	88.9	95.2	94.4	95.6	95.7	94.5
R3	95.2	92.5	90.6	90.3	108.3	106.9	105.6	102.6	97.8	95.4	92.9	90.2	100.4	98.2	96.3	94.3
R4	99.2	96.5	95.6	94.3	110.3	108.9	112.6	102	99.8	98.4	94.9	92.2	103.1	101.2	101	96.1
R5	91.2	87.6	75.6	66.9	102.7	104.9	104.3	56.3	90.1	90.6	61.2	49.9	94.7	94.3	80.4	57.7
R6	96.2	88.5	77	33.5	110.4	111.7	98.6	51	96.2	102	81.7	33.5	100.9	100.7	85.8	39.4
R7	94.9	85.6	80	43.3	104.8	97.6	102.6	75.1	93.6	111.8	82.3	96.2	97.8	98.3	88.3	71.5
R8	96.2	94.5	93.6	90.3	111.3	109.9	106.6	102	97.8	96.4	94.9	92.2	101.7	100.2	98.3	94.8
ADR	10	5.1	-8.4	-1.2	8.4	5	10.4	6.1	4.7	2.6	-3.1	5.4	7.7	4.2	-0.4	3.4

The results showed that compounds R1 and R6 reduced cell growth by 21.9% and 39.4%, respectively, at a concentration of 80 μg/mL, while other compounds allowed 57-94% growth. These findings suggest that substitutions on the coumarin nucleus significantly influence biological activity, with unsubstituted (R4), fluoro (R6), and chloro (R1) substituted coumarin derivatives showing significant potency.

5 Conclusion

The synthesized compounds were characterized by IR, 1H NMR, 13C NMR, and mass spectrometry. Their solubility was tested in different solvents: they were freely soluble in DMSO, soluble in chloroform and methanol, slightly soluble in acetone, ethyl acetate, and ethanol, and insoluble in water. The compounds were assessed for in-vitro anti-cancer activity using the SRB assay against the A-549 human cancer cell line. The results revealed that compounds R1 and R6 inhibited cell growth by 21.9% and 39.4%, respectively, at a concentration of 80 μg/mL, while the other compounds showed 57-94% growth. These findings suggest that substitutions on the coumarin nucleus have a significant impact on biological activity, with unsubstituted (R4), fluoro (R6), and chloro (R1) coumarin derivatives exhibiting considerable potency.

6 Acknowledgments

We acknowledged the contributions of the Laboratory staff of B.R Nahata College Pharmacy, Faculty of Pharmacy, Mandsaur University for creating a friendly environment for the synthesis of derivatives and evaluation of Anti-inflammatory activity studies.

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