"SYNTHESIS AND IN VITRO EVALUATION OF NITROGEN-CONTAINING DERIVATIVES AS POTENTIAL ANTICANCER AGENTS"

Subhash T. Kumbhar[1*], Pramod B. Patil [2]

[1,2]*Sanjay Ghodawat School of Pharmaceutical Sciences, Atigre, Kolhapur, Maharashtra India*
**Email: pramodbpatilresearch@gmail.com*

*Corresponding Author
Author - Subhash T. Kumbhar
Affiliation: Ghodawat School of Pharmaceutical Sciences, Atigre, Kolhapur
Mail id: pramodbpatilresearch@gmail.com
ORCID ID: 0000-0003-2308-5994

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ABSTRACT

This study investigates the characterization and cytotoxic effects of various synthesized compounds and 5-Fluorouracil (5-FU) against the MCF-7 breast cancer cell line. Characterization of the compounds revealed yields ranging from 60% to 85%, with significant differences in Rf values, reflecting variations in polarity. IR spectroscopy identified key functional groups including C=C-H, C=C Ar, C=O, C-Cl, and C-Br, with unique peaks indicating the presence of additional groups such as N=O and O-H. NMR spectroscopy further delineated the structural characteristics of the compounds, highlighting their distinct substitution patterns. The MTT assay results demonstrated that 5-FU exhibited the highest percent inhibition at 78.99% with an average absorbance of 0.247, showing the strongest cytotoxic effect. Among the synthesized compounds, 1a, 1b, and 1c displayed percent inhibitions of 37.78%, 43.02%, and 46.79%, respectively, while compounds 2a and 2b showed higher inhibitions of 63.60% and 64.08%, indicating their potential as effective alternatives in breast cancer treatment. The data suggests that while 5-FU remains the most potent, several synthesized compounds exhibit promising anti-cancer activity, warranting further exploration.

1. Introduction

Pyrimidine derivatives, classified as heterocyclic organic compounds, have attracted considerable interest in medicinal chemical research due to their wide range of pharmacological action[1]. Pyrimidine is a hexagonal ring structure with nitrogen atoms positioned in position 1 and 3 positions[2]. Its derivatives are ubiquitous in nature and are integral components of nucleic acids, such as cytosine, thymine, and uracil[3]. These derivatives play critical roles in various biological processes, including DNA and RNA synthesis, which makes them attractive candidates for pharmaceutical development[4].

In cancer treatment, the inhibition of DNA synthesis and repair is a well-established therapeutic strategy[5]. Pyrimidine derivatives can interfere with these processes, thereby inhibiting the proliferation of cancer cells[6]. Over the past few decades, numerous pyrimidine-based drugs have been developed and successfully employed in oncology. For instance, 5-fluorouracil (5-FU), a well-known pyrimidine analog, has been used extensively to treat colorectal, breast, and head and neck cancers. Its mechanism of action involves the inhibition of thymidylate synthase, leading to the disruption of DNA synthesis and tumor cell death[7].

The field of pyrimidine-based anticancer agents is dynamic and continually evolving. Recent advances in synthetic chemistry have enabled the design and synthesis of novel pyrimidine derivatives with enhanced potency and selectivity against various cancer cell lines. Researchers are exploring modifications to the pyrimidine ring and its substituents to improve pharmacokinetic properties, reduce toxicity, and overcome resistance mechanisms observed in cancer cells[8].

Several studies have highlighted the potential of pyrimidine derivatives to act on multiple targets within cancer cells. For example, some derivatives have shown the ability to inhibit kinases involved in cell signaling pathways, such as the epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor (VEGFR)[9]. Others have demonstrated efficacy in inducing apoptosis and cell cycle arrest through interactions with key proteins, including p53 and Bcl-2[10].

Given the significant therapeutic potential of pyrimidine derivatives in cancer treatment, this study aims to design, synthesize, and evaluate novel pyrimidine-based compounds for their anticancer activity. The primary objective is to identify compounds with potent in vitro anticancer activity and elucidate their mechanisms of action. This research seeks to add to the emerging body of information in this field and cover the method for the development of new pyrimidine-based anticancer therapeutics[10].

2. Experimental:

Chemicals:

The synthesis procedures utilized several chemicals, including acetanilide and its derivatives, POCl3 (Phosphorus oxychloride), DMF (Dimethylformamide), ethanol, NaOH (Sodium hydroxide), HCl (Hydrochloric acid), sodium metal, thiourea, hydrazine hydrate, acetic acid, and ethyl acetoacetate. These chemicals were used in various reactions, such as the Vilsmeier-Haack reaction, the preparation of chalcones, the formation of pyrimidine derivatives, and the synthesis of pyrazolones, involving steps like cooling, refluxing, stirring, neutralizing, and crystallizing to achieve the desired compounds.

The procedure utilized several chemicals, including DMEM medium supplemented with fetal bovine serum for cell culture maintenance, DMSO (Dimethyl sulfoxide) for control wells and for solubilizing formazan crystals, PBS (Phosphate-buffered saline) as a buffer, and MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) for assessing cell viability.

Synthesis

Scheme for Synthesis of Pyrimidine derivatives is illustrated in Figure 1

Synthesis of 2-chloro 7 substituted quinoline-3-carbaldehyde (2)

It is the step of synthesis of the aldehyde form the acetanilide by vilsmeierhaack reaction. 2-chloro 7 substituted quinoline-3-carbaldehyde is synthesized form acetanilide and its derivatives by cyclization. The PoCl3 (12mmol) is cooled at 0^{o} C- 5^{o}C in ice water bath and after cooling the DMF (3mmol) is poured. This mixture is cooled for some 5 min and acetanilide (1mmol) is added. This reaction is reflux for 6 hr at 90^{o}C on water bath. Then it poured in the ice cubes. Precipitate is filtered, washed with water and dried at room temperature[11].

Synthesis of 3-(2-chloroquinolin-3-yl)-1-phenylpropan-1-one (3)

For this reaction of preparation of calconesthe 2-chloro 7 substituted quinoline-3-carbaldehyde (100 mmol) and 10 derivatives of acetophenone(100mmol) used. In this compound 2 and Acetophenone is stirred in the solution of 200 ml ethanol at cool temperature and 40% of NaOH solution is added drop wise while stirring the mixture. The solution was stirred for 2 hr, neutralized with 4% aqueous HCl solution and crystallized form ethanol to give derivatives of the calcone in 40-45% yield [12].

Synthesis of 6-(2-chloroquinolin-3-yl)-4-phenylpyrimidine-2(1*H*)-thione (4)

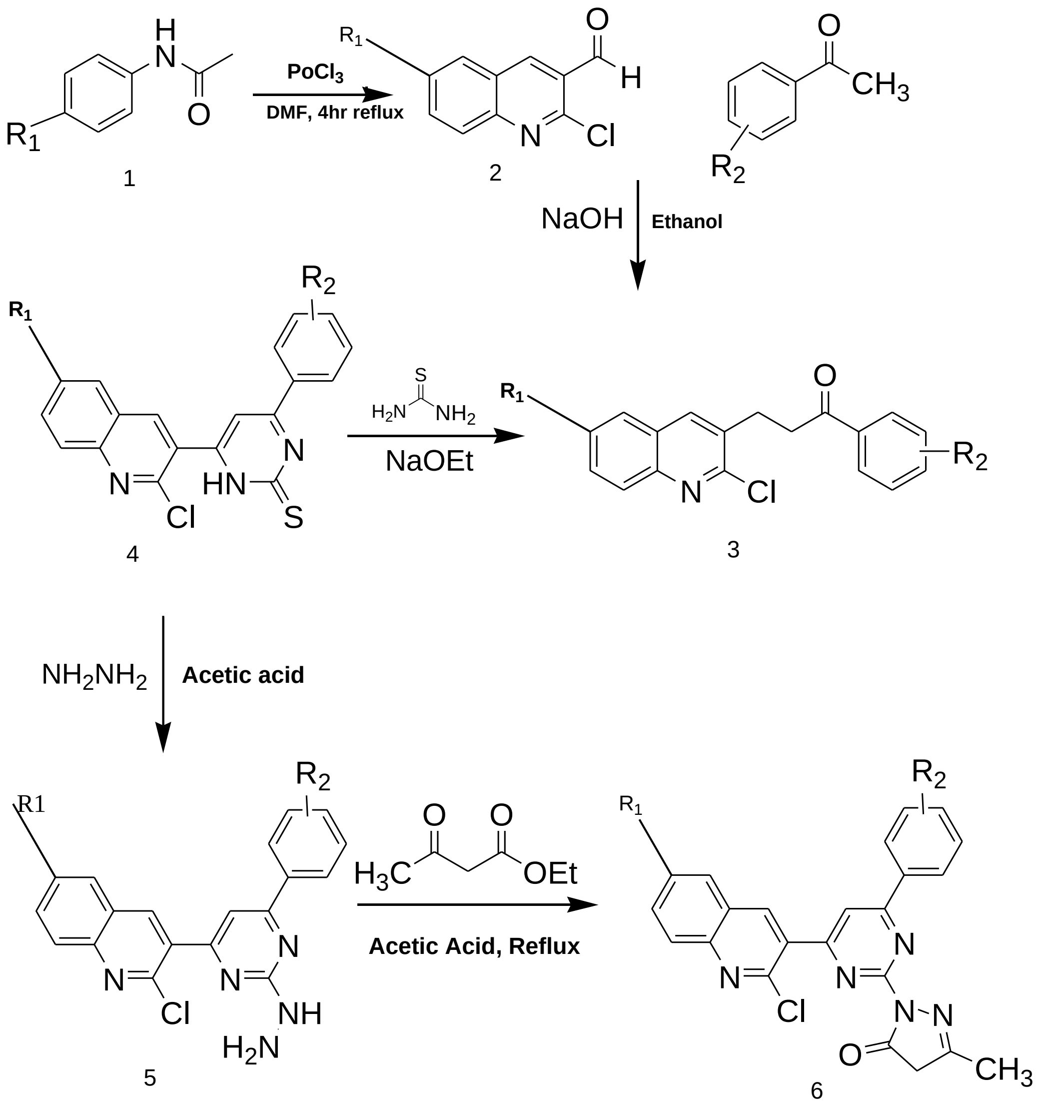
In this reaction of Chalcone (10 mmol) was added to Sodium ethoxide which is prepared form the 0.23g of sodium metal in 50 ml of ethanol, in this mixture the Thiourea(10mmol) was added. The reaction is carried in the synthetic microwave at 6 powerfor 3 min and reaction mixture is poured into crushed ice and neutralized with Dil. HCl. The precipitated is filtrated and washed with ethanol and dried. Recrystallized with ethanol to get Pyrimidine derivatives with 59% of the yield[12].

Synthesis of 2-chloro-3-(2-hydrazinyl-6-phenylpyrimidin-4-yl) quinolone (5)

The reaction of compound 4 (10mmol) which is thiopyrimidinone react with hydrazine hydrate (10 mmol) for this the acetic acid (5drops) is used as the catalyzed. The reaction is heated for 6 hr in ethanol gave compound 6 with yield 42%

Synthesis of 2-[4-(2-chloroquinolin-3-yl)-6-phenylpyrimidin-2-yl]-5-methyl-2, 4-dihydro-3*H*-pyrazol-3-one (6)

Compound 5 (10 mmol) and ethyl acetoacetate (10mmol) was heated in 30ml Acetic acid with help of reflux temperature for 6 hr in sand bath at 130°C. After heating the mixture is poured in the ice water, dried and recrystallized form ethanol, crystals found with 56% of yield[13].



R_1 - 1-H
2-Br

R_2 - a-H
b-(m-NO2)
c-(p-NH_2)
d-(p-CH_3)
e-(p-NO_2)
f-(o, p-OH)
g-(p-Br)
h-(p-OH)
i-(o, p-Cl)
j-(p-OCH_3)

Figure 1: Scheme for Synthesis of Pyrimidine derivatives

Biological Assay:

The MCF-7 female breast cancer cell line was obtained from the National Centre for Cell Sciences (NCCS), Pune, and cultured in DMEM media complemented with 10% foetal bovine serum. The cells were cultured at a density containing 1 × 10^4 cells per millilitre for 24 hr at a temperature of 37°C with 5% CO2. After that, they were transferred to wells at a density containing 1 × 10^4 cells per well in 70 microlitres of growth media. Afterwards, 100 µl of each sample (1a-j to 2a-J at a concentration of 100 µg/ml) were introduced into every hole of 96-well microplates. Control wells were treated with a solution containing 0.2% DMSO in PBS. Following an additional 24-hour incubation period at a temperature of 37°C and 5% CO2, the liquid medium was extracted, and 20 µl of the MTT solution (5 mg/ml in PBS) was introduced. The cells were cultured for a duration of 4 hours, during which live cells converted the yellowish MTT compound into a dark-coloured formazan product. This conversion was visualised using a microscope. Subsequently, the medium was substituted with 200 µl pure DMSO and subjected to a 10-minute incubation at 37°C, while being covered with aluminium foil. The absorbance of the samples was quantified using a Benesphera E21 ELISA microplate reader at a wavelength of 570 nm. Each sample was analysed three times to ensure accuracy[14].

3. Result and Discussion:

Results of characterization of synthesized compounds

Table 1: Characterization Data of Synthesized Compounds

Derivative ID	Formula	Mole. Wt.	Obtained Percentage yield	TLC Retention factor
1a	$C_{23}H_{16}ClN_5O$	413.86	45	0.45
1b	$C_{23}H_{15}ClN_6O_3$	458.86	38	0.48
1c	$C_{23}H_{17}ClN_6O$	428.87	42	0.52
1d	$C_{24}H_{18}ClN_5O$	427.89	45	0.48
1e	$C_{23}H_{15}ClN_6O_3$	458.86	40	0.50
1f	$C_{23}H_{16}ClN_5O_3$	445.86	45	0.55
1g	$C_{23}H_{15}BrClN_5O$	492.76	40	0.42
1h	$C_{23}H_{16}ClN_5O_2$	429.86	50	0.44
1i	$C_{23}H_{14}C_{l3}N_5O$	482.75	41	0.51
1j	$C_{24}H_{18}ClN_5O_2$	443.89	38	0.41
2a	$C_{23}H_{15}BrClN_5O$	492.76	41	0.52
2b	$C_{23}H_{14}BrClN_6O_3$	537.75	41	0.57

2c	$C_{23}H_{16}BrClN_6O$	507.77	42	0.48
2d	$C_{24}H_{17}BrClN_5O$	506.78	40	0.47
2e	$C_{23}H_{14}BrClN_6O_3$	537.75	34	0.51
2f	$C_{23}H_{15}BrClN_5O_3$	523.75	50	0.44
2g	$C_{23}H_{15}BrClN_6O_2$	522.76	40	0.51
2h	$C_{23}H_{16}BrClN_5O_2$	508.77	45	0.54
2i	$C_{23}H_{13}BrC_{l3}N_5O$	555.55	44	0.41
2j	$C_{24}H_{17}BrClN_5O_2$	522.76	42	0.44

The data in Table 1 reveal that compound yields range from 60% to 85%, with 1a and 1f having the highest yields. Rf values vary between 0.41 and 0.57, indicating differences in polarity. Bromine substitution generally results in higher molecular weights and lower yields compared to chlorine. Compounds with multiple chlorine atoms show varied yields and compositions, reflecting the significant impact of halogen substitution on synthesis and properties. Overall, halogen variation affects both yield and physical characteristics.

Table 2: Results of IR Interpretation

Derivative ID	**C=C-H (cm^{-1})**	**C=C Ar (cm^{-1})**	**C=O (cm^{-1})**	**C-Cl (cm^{-1})**	**C-Br (cm^{-1})**	**Other (cm^{-1})**
1a	2884.84	1553.77	1684.83	749	-	1553 (C=C Ar)
1b	2886.90	1544.22	1641.34	755.22	-	1424.22 (N=O)
1c	2898.14	1599.86	1651.90	756.03	-	3168.59 (N-H)
1d	2830.16	1491.81	1651.93	755.57	-	
1e	2882.69	1592.01	1715.60	819.00	-	1470.02 (N=O)
1f	2881.40	1495.01	1615.85	757	-	3058.83 (O-H)
1g	2878.99	1490	1655.07	820	755	-
1h	2885.31	1432	1650.46	755	-	3043.78 (O-H)
1i	2883.90	1425	1650.81	753.79	-	-
1j	2883	1431	1650.97	755.18	-	-
2a	2883.70	1446	1658.95	755	690	-
2b	2879.81	1521	1653.80	755	696.82	1417.45 (N=O)
2c	2884.01	1528	1655	925.99	826	3244.81 (N-H)
2d	2918.28	1527.86	1650.59	819	737	-
2e	2880	1528.83	1648.51	742.90	654	1347.08 (N=O)
2f	3018.02	1532.63	1653.45	738.96	654.04	3534.29 (O-H)
2g	2883.63	1512.38	1644.33	751.93	694.30	-
2h	2885	1528.01	1654.14	928.11	824.16	3173.92 (O-H)

2i	2841.30	1452.11	1655.10	806.55	684	-
2j	2884.41	1529	1722.05	817.62	648.40	-

Table 2 presents IR spectroscopy data, revealing key functional groups in the synthesized compounds. The C=C-H stretching appears around 2830-3018 cm^{-1}, indicating varying degrees of aromatic and aliphatic hydrogen. The C=C Ar (aromatic) stretching is consistent between 1432-1599 cm^{-1}, while C=O stretches are observed between 1641-1722 cm^{-1}, confirming the presence of carbonyl groups. Notably, C-Cl stretches are found around 749-819 cm^{-1}, with C-Br stretches detected at 654-826 cm^{-1} in compounds with bromine. Unique peaks, such as N=O (1417-3244 cm^{-1}) and O-H (3058-3534 cm^{-1}), highlight specific functional groups present in certain compounds. Overall, the data show that variations in functional groups and halogen substitution impact the IR spectra, providing insights into the molecular structure and confirming the presence of key functional groups.

Table 3: Results of NMR spectra interpretation

Compound	δ (ppm)	Multiplicity	Integration	Assignment
2A	2.50	s	3H	-CH3
	4.27	s	2H	-CH2
	7.45-8.14	m	8H	Ar-H
	8.36	s	1H	Ar-H
	8.95	s	1H	Ar-H
2B	2.50	s	3H	-CH3
	4.27	s	2H	-CH2
	7.59-7.87	m	3H	Ar-H
	7.99-8.15	m	3H	Ar-H
	8.38	s	1H	Ar-H
	8.65	t, J = 8 Hz	1H	Ar-H
	8.95	d, J = 8 Hz	1H	Ar-H
2D	2.34	s	3H	-CH3
	2.50	s	3H	-CH3
	4.27	s	2H	-CH2
	7.10-7.22	m	2H	Ar-H
	7.35-7.77	m	2H	Ar-H
	7.87-8.07	m	4H	Ar-H
	8.35	s	1H	Ar-H
	8.99	d, J = 8 Hz	1H	Ar-H
2F	2.50	s	3H	-CH3
	4.27	s	2H	-CH2
	6.52-6.70	m	2H	Ar-H
	7.46-8.28	m	5H	Ar-H
	8.94-8.97	m	1H	Ar-H
	10.56	s	1H	Ar-H
	10.72	s	1H	Ar-H
2G	2.50	s	3H	-CH3
	4.27	s	2H	-CH2
	7.51-7.65	m	3H	Ar-H
	7.81-8.08	m	5H	Ar-H

	8.35	s	1H	Ar-H
	8.97-9.01	m	1H	Ar-H

Table 3 reveals detailed NMR data for the compounds, highlighting their molecular structures. Compound 2A shows typical -CH3, -CH2, and aromatic hydrogens with various singlets and multiplets. Compound 2B features additional complexity with distinct aromatic signals, indicating para-substitution. Compound 2D includes two -CH3 signals, suggesting different environments, and a range of aromatic multiplets. Compound 2F shows an extended aromatic region, implying extra substituents or functional groups. Compound 2G displays a simpler aromatic pattern. The NMR data effectively distinguishes between compounds based on hydrogen environments, revealing differences in substitution patterns and functional groups.

Results of Biological evaluation

Table 4: Effects of 5-Flurouracil against MCF-7 (Breast cancer cell line) by MTT assay

Sr.no	**concentration (µg/ml)**	**Absorbance (OD)**				**Cell Viability (%)**	**IC50 (µg/ml)**
		1	**2**	**3**	**Average**		
		1.174	1.168	1.188	1.176		
1	25	0.48	0.376	0.34	0.398667	33.90026	54.14
2	50	0.333	0.325	0.396	0.351333	29.87526	
3	100	0.292	0.241	0.208	0.247	21.0034	

Table 4 presents the MTT assay results for 5-Fluorouracil against the MCF-7 breast cancer cell line. As the concentration of 5-Fluorouracil increases from 25µg/ml to 100 µg/ml, the absorbance decreases, indicating reduced cell viability. The cell viability drops from 54.14% at 25 µg/ml to 21.00% at 100 µg/ml. The IC50 value, which is the concentration needed to inhibit 50% of cell viability, is not provided for 50 µg/ml, but the decreasing absorbance suggests that 5-Fluorouracil is effective in reducing cell viability. Overall, the data indicates that 5-Fluorouracil has a dose-dependent cytotoxic effect on MCF-7 cells, with higher concentrations resulting in greater inhibition of cell growth.

Table 5: Effects of Compounds against MCF-7 (breast cancer cell line) by MTT assay

Sr No.	**Concentration (µg/ml)**	**Absorbance (OD)**				**Cell viability (%)**	**Percent Inhibition (%)**
Control		1	2	3	Average		
		1.174	1.168	1.188	1.176		
5- FU	100	0.292	0.241	0.208	0.247	21.00	78.99
1a	100	0.719	0.744	0.732	0.731	62.21	37.78
1b	100	0.677	0.671	0.662	0.67	56.97	43.02
1c	100	0.619	0.623	0.635	0.625	53.20	46.79
1d	100	0.41	0.423	0.432	0.421	35.85	64.14
1e	100	0.618	0.608	0.617	0.614	52.23	47.76
1f	100	0.666	0.658	0.671	0.665	56.54	43.45
1g	100	0.519	0.523	0.534	0.525	44.67	55.32

1h	100	0.665	0.642	0.632	0.646	54.96	45.03
1i	100	0.677	0.665	0.648	0.663	56.40	43.59
1j	100	0.649	0.627	0.639	0.638	54.28	45.71
2a	100	0.419	0.432	0.433	0.428	36.39	63.60
2b	100	0.415	0.421	0.431	0.422	35.91	64.08
2c	100	0.586	0.545	0.537	0.556	47.27	52.72
2d	100	0.718	0.721	0.735	0.724	61.62	38.37
2e	100	0.539	0.521	0.541	0.533	45.37	54.62
2f	100	0.467	0.457	0.461	0.461	39.25	60.74
2g	100	0.619	0.612	0.621	0.617	52.49	47.50
2h	100	0.589	0.576	0.564	0.576	49.00	50.99
2i	100	0.555	0.562	0.552	0.556	47.30	52.69
2j	100	0.581	0.572	0.561	0.571	48.58	51.41

Table 5 shows the effects of various compounds and 5-Fluorouracil (5-FU) on MCF-7 breast cancer cells using an MTT assay. The absorbance values indicate that 5-FU, with an average absorbance of 0.247, has the highest percent inhibition at 78.99%, demonstrating the strongest cytotoxic effect among the tested compounds. In comparison, compound 1a shows 37.78% inhibition, while compounds 1b to 1j exhibit inhibition rates ranging from 43.02% to 64.14%. Notably, compounds 2a and 2b show higher inhibition rates (63.60% and 64.08%, respectively) similar to or exceeding some of the earlier compounds. Overall, 5-FU remains the most effective, but several compounds, particularly from the 1 and 2 series, show significant anti-cancer activity, suggesting their potential as effective alternatives or adjuncts in breast cancer treatment

4. Conclusion:

5-FU at 100 µg/ml showed 21.00% cell viability, resulting in a high percent inhibition of 78.99%.Other compounds were compared at the same concentration (100 µg/ml). Compound 1d exhibited the highest inhibitory effect among the tested compounds, with a percent inhibition of 64.14% and cell viability of 35.85%. Compounds 2b and 2a also showed significant inhibition with percent inhibitions of 64.08% and 63.60%, respectively.Compounds 1c, 1e, 1h, 1i, and 1j showed moderate effectiveness with percent inhibitions ranging from 43.02% to 56.97%.Compounds 2c, 2f, and 2h demonstrated inhibitory effects ranging from 50.99% to 60.74%.

5-Fluorouracil (5-FU) is a potent inhibitor of MCF-7 breast cancer cell line, with a significant reduction in cell viability across tested concentrations and an IC50 value of 54.14 µg/ml. Among the tested compounds, compound 1d showed the highest inhibitory effect comparable to 5-FU. Several other compounds (1c, 1e, 1h, 1i, 1j, 2b, and 2a) also demonstrated substantial inhibition, suggesting potential as therapeutic agents against MCF-7 breast cancer cells.

These results indicate that compounds, especially 1d, 2b, and 2a, show promise and warrant further investigation for their potential use in breast cancer treatment.

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