RESEARCH ARTICLE

Synthesis and Evaluation of *In-vitro* Anticancer Activity of Novel Pyrimidine Derivatives

Machchhindra R. Holam[1*], M. Komala[2]

[1]*School of Pharmaceutical Science, VELs Institute of Science, Technology and Advanced Studies, Palavaram, Chennai, Tamil Nadu, India.*

[2]*Department of Pharmaceutics, School of Pharmaceutical Science, VELs Institute of Science, Technology and Advanced Studies, Chennai, Tamil Nadu, India.*

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ABSTRACT

A series of substituted 1-methyl-6-oxo-2-[(4-oxo-1,3-thiazolidin-3-yl)amino]-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile were synthesized by multicomponent type of reaction using ethyl cyanoacetate, thiourea and aromatic aldehyde. The anticancer potential of every produced substance were evaluated through HT29 and A549 cell lines. In the given series, compounds 7m and 7r showed the highest anticancer potential on HT29 and A549 cell lines with IC_{50} of 10.0 and 17.2 µg/mL. The in-silico ADME of 7m and 7r showed good pharmacokinetic properties.

Keywords: Anticancer, Pyrimidine derivatives, Thiazolidine, CDK4.

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INTRODUCTION

Cancer is a complex group of diseases that can develop in almost any organ or body tissue. These morphological and pathophysiological changes are brought on when aberrant cells proliferate threateningly, cross normal bodily boundaries, and spread to other organs or neighboring body parts. The second greatest cause of death worldwide is cancer. In 2020, The number of people diagnosed with cancer increased to 19.3 million and mortality rates reached 10.0 million.[1] In some cases, malignancies can now be averted by avoiding risk factors and employing currently available evidence-based preventative strategies. Surgery, radiation, chemotherapy, and hormone therapy are all possible cancer treatments. The prognosis, disease stage, available treatments, side effects, and patient preferences all influence the course of treatment. Lung cancer will continue to rise through 2035 in the majority of nations, making it a significant global public health concern.[2] Chemotherapy is essential in the fight against cancer. The prognosis, disease stage, available treatments, side effects, and patient preferences all influence the course of treatment.[3] Chemotherapy is essential in the fight against cancer. Another mechanism in which cancer cells metabolize carbon is being targeted by cancer treatment research to reduce side effects. The cell population is kept stable via cellular death. The permeabilization of the mitochondrial outer membrane, cytochrome C, and cellular stress are key regulators of this apoptotic process. The primary protein involved in the apoptotic process belongs to the B-cell lymphoma-2 protein family.[4]

Chemistry

The compound 7c-r were synthesized by treating intermediate 2-hydrazinyl-3-methyl-4-oxo-6-phenyl-1,2,3,4-tetrahydropyrimidine-5-carbonitrile with various substituted aromatic aldehyde in glacial acetic acid and in a subsequent step the intermediate compound were treated with thioglycolic acid (Table 1). All compounds were recrystallized using glacial acetic acid and absolute ethanol and obtained 55–66% yield. Different spectral studies that confirmed TLC and their structure monitored the reaction's progress. *In-vitro* anticancer activity was checked by SRB assay given in Tables 2 and 3. ADME data showed that the compound 7m and 7r shows promising kinetic properties.[5,6]

MATERIALS AND METHODS

Merk Company's chemicals and reagents were utilized. The compounds' melting point was checked by Prolab India digital melting point apparatus. The compound structure were confirmed by FTIR spectrum recorded using KBr discs on Bruker, Alpha-T IR spectrophotometer. ^{1}H NMR spectra were chronicled on jeol JNM-ECZ400S.

General procedure to synthesize of substituted 1-methyl-6-oxo-2-[(4-oxo-1,3-thiazolidin-3-yl)amino]-4-phenyl-1,6-

**Author for Correspondence: macholam10@gmail.com*

dihydropyrimidine-5-carbonitrile compound (7c-r) (Figure 1). The compounds (5c-r) (0.01 mol) and thioglycolic acid (0.01 mol) were added in CH3COOH(50 mL), stirred for 15 to 20 minutes. The resultant solution was refluxed for 5–6 hours. Two-thirds in volume decreased the reaction mixture by heating it on a hot plate before being poured into cold water. A solid residue was collected, filtered, and then recrystallized from ethanol.[7,8]

Anticancer Activity (*In-vitro*)

Anticancer Assay

The anticancer activity of the synthesized 7c-r compound was evaluated in HT-29 and A549 cells. The cells were cultured in a mixture containing 2 mM L-glutamine and 10% foetal bovine serum. To do this, ninty six-well microtiter plates were seeded and then incubated for 24 hours at 37°C, 5% carbon dioxide, 95% air, and 100% relative humidity. The 1-mg/mL test solution was made up of distilled water kept in the freezer until use. The test solutions were diluted with complete medium to final 100, 200, 400, and 800 µg/mL concentrations. The 10, 20, 40, and 80 µg/mL drug concentrations were obtained by adding 10 µL of each dilution to 90 µL of medium in labeled wells. After an additional 48 hours of incubation at room temperature, cold TCA was added and the plates were put back in the fridge for 60 minutes at 4°C. SRB solution (0.4% (w/v) in 50 µL of 1% CH_3COOH) was added to each well, and the plates were incubated for 20 minutes at RT. After the staining process, the remaining dye was removed with 5 washes in 1% acetic acid. The absorbance was checked at both the standard 540 nm and the longer 690 nm. The rate of expansion was determined,[9,10]

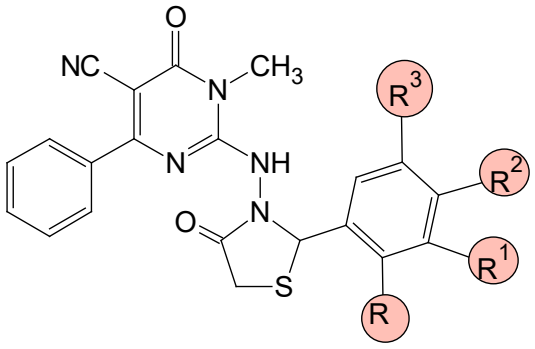


Figure 1: Compound 7c to 7r

RESULTS AND DISCUSSION

The intermediate compounds (5a-r) were obtained by treatment of compound 4 with various substituted aldehydes. The absence of NH_2 peak at 3300 in the FTIR spectrum confirmed formation of the expected product. Further intermediates was treated with thioglycolic acid in glacial acetic acid it furnished a product identified as substituted 1-methyl-6-oxo-2-[(4-oxo-1,3-thiazolidin-3-yl)amino]-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile(7a-r). All the derivatives were confirmed by FTIR and ^{1}H-NMR spectral studies. The compounds were recrystallized using absolute methanol and acetic acid. The peak around 2922 cm^{-1} in compounds, 5a–r confirmed the N–H str vibrations, and strong absorption bands around 2200 cm^{-1} due to CN str vibration in the synthesized compounds. The ^{1}H-NMR spectra present resonances assigned to the SCH2 signal represented as singlet at 4.12 ppm due to the methylene protons.[11,12]

Anticancer activity

Cytotoxicity

Sulforhodamine B protein (SRB) assay procedures were used to evaluate the cytotoxic effects of the recently synthesised chemical 7c-r on the proliferation of colon (HT-29) and lung (A549) cancer cell lines[13] in order to assess their anticancer effects. Ninty six-well plates used to seed the cell and incubated at 37°C for 24 hours. Different concentration of compound 7c-r (10, 20, 40 and 80 µg/mL) was made and incubated extra for 48 hours then finally used to treat the cells. DMSO was added to solubilize of the tested compounds in aqueous media. Adriamycin (ADR) was used as control for the test. The results, in terms of GI_{50} (Figure 2), TGI, and LC_{50}, are summarized in Tables 2 and 3. Using ADR as a control, the data showed that the 50% cell growth inhibition values (GI_{50}) have moderate to good activity on colon and lung cancer,

Table 1: Data of derivatives 7c to 7r

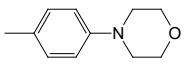
Comp id	*MF*	*MW*	*R*	*R1*	*R2*	*R3*	*MP (°C)*
7c	C22H19N5O4S	449.48	H	OCH3	OH	H	196–198
7e	C21H15BrClN5O3S	532.79	OH	Br	H	Cl	212–214
7f	C23H22N6O2S	446.52	H	H	-N(CH3)2	H	198–200
7g	C21H16lN6O4S	448.45	H	NO2	H	H	208–210
7l	C22H18BrN5O4S	528.37	H	OCH3	OH	Br	214–216
7m	C21H17N5O3S	419.45	OH	H	H	H	210–212
7n	C21H15Br2N5O2S	561.24	H	Br	H	Br	210–212
7o	C25H24N6O3S	488.56	H	H		H	196–198
7p	C23H19N5O3S	445.49	H	H	-COCH3	H	202–204
7r	C29H24N6O3S	536.60	H	H	NHCOCH2C6H5	H	228–230

Table 2: Anticancer activity of pyrimidine derivatives (7c-r) on colon cancer

Human colon cancer cell Line HT-29					Drug concentration µg/mL calculated from graph			
	Drug concentration µL/mL				Anticancer parameters			
	% Control growth				HT-29	LC50	TGI	GI50
Compound	10	20	40	80	Compound			
7c	75.2	21.7	-54.7	-49.2	7c	59.2	32.8	9.1
7e	82.3	69.9	33.7	-59.1	7e	78.6	44.9	28.7
7f	86.2	26.8	-12.4	-31.3	7f	>75	51.4	11.0
7g	86.2	79.2	71.3	39.5	7g	NE	>80	66.4
7l	29.8	-33.6	-31.2	-50.6	7l	56.1	11.0	<10
7m	13.9	-23.4	-29.5	-59.1	7m	66.1	15.0	<10
7n	58.4	55.0	32.1	10.1	7n	>70	90.2	21.1
7o	71.3	12.2	-29.0	-49.4	7o	68.4	31.4	8.2
7p	88.6	43.3	-35.6	-56.8	7p	63.7	39.8	14.5
7r	89.3	88.4	78.6	61.4	7r	NE	NE	>80
ADR	18.5	42.6	48.5	42.4	ADR	NE	<10	NE

Table 3: Anticancer activity of pyrimidine derivatives (7c-r) on lung cancer

Human colon cancer cell line A549					Drug concentration µL/mL calculated from graph			
	Drug concentration µL/mL				Anticancer parameters			
	%Control growth				A549	LC_{50}	TGI	GI_{50}
Compound	10	20	40	80	Compound			
7c	78.6	76.2	48.3	-50.2	7c	>80	47.4	33.6
7e	56.2	22.6	-39.6	-71.4	7e	57.3	29.6	8.4
7f	85.4	78.4	58.3	-31.4	7f	>80	55.2	36.4
7g	56.4	42.6	88.4	76.3	7g	NE	>80	>80
7l	78.4	82.6	25.2	-49.1	7l	68.5	46.2	29.3
7m	37.4	13.2	-16.4	-42.2	7m	>80	18.4	<10
7n	68.4	54.9	52.4	12.5	7n	>80	>80	38.3
7o	68.2	59.1	26.2	-34.4	7o	>80	45.3	28.2
7p	56.2	36.2	41.1	-44.2	7p	43.1	52.4	25.3
7r	86.1	67.2	36.2	54.4	7r	NE	NE	>80
ADR	-3.1	-7.9	-10.4	-23.0	ADR	NE	<10	<10

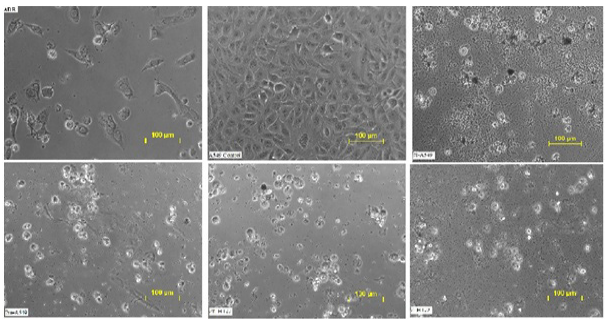


Figure 2: *In-vitro* anticancer activity (Growth Inhibition).

with values ranging from L > M > O > C>F >P>N>E>G>W and M>E>O>C>P>L>F>N>G>W, respectively. Meanwhile, the concentration of 7m and 7r drug that produces growth inhibition of cells (TGI) for colon cancer is 10 and 17.2 µg/mL. Moderate activity was found in the remaining compounds, whereas low activity was shown by 7g and 7e. Among four dose levels of compounds, maximum inhibitory activity was found at 80 µg/mL. Figure 2 shows the percentage control growth results of 7c-r on HT-29 and A549 cell lines, respectively, with reference ADR.[14,15]

CONCLUSIONS

The results concluded that among all newly synthesized pyrimidines derivatives (7m-7r) derivatives, the most potent anticancer activity was displayed by 7l and 7p on HT-29 and 7m on A549 cell lines, respectively. Thus it is confirmed that the compound 7m and 7r is a promising approach for the design of new anticancer moiety with strong anticancer potential. To prove its effectiveness in the treatment of cancer, however, more *in-vivo* animal trials are needed.

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