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IN SILICO ANTICANCER ACTIVITY PREDICTION OF PYRIMIDINE DERIVATIVES

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ABSTRACT

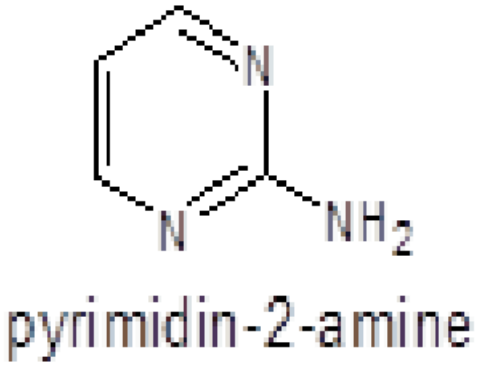
Cancer is described as the uncontrolled growth of abnormal cells. The outcome of anticancer therapy is chiefly depending upon electivity of targets. Many targets for the treatment of cancer cells are available namely stem cells, protein coupled receptors, ErbB receptors, growth factor, fibroblast growth factor. The protein ligand interactions play a significant role in structural steroid hormones, proteases, protein kinase, vascular endothelial growth factors, transforming based drug designing. In our present research work, we have chosen Angiogenin, vascular endothelial growth factors, transforming growth factors, fibroblast growth factor as targets to screen our proposed chemical structures of evaluation of anticancer activity. Using the docking software, depending on the energy values we have to identify the best drug analogues that have great binding efficiency towards targets. Several modifications have to be made to the probable functional groups which are interacting with receptor molecules. Analogues of the drug molecules are prepared using ACD chemsketch and docking. Starting material, (2-Amino Pyrimidine) is a potent anticancer drug. The Pyrimidine and its various derivatives have various pharmacological activities including anticancer, antitubercular, antibacterial and antimicrobial. These activities are confirmed in various research projects by using many evaluation factors viz., in-vitro and in-vivo evaluations. We have studied our drug on following targets by using computer-based drug designing approach i.e., MOLECULAR DOCKING by using Mcule dock software and further the activity is

confirmed by the in-vitro anti-cancer study on human colon cancer cell line for the compound 4C by MTT assay technique.

KEYWORDS: Insilco drug designing, anticancer activity, Chalcones, Michael adduct, Molecular docking, In-vitro anticancer activity.

INTRODUCTION

Pyrimidine-2-amine



Molecular Formula: $C_4H_5N_3$

Molecular Weight: 111.145

Pyrimidine-2-amine and its derivatives

In medicinal chemistry, many Pyrimidine-2-amine derivatives have been developed as chemotherapeutic agents and are widely used. Pyrimidine-2-amine moiety carrying compounds exhibit various activities like Anti tubercular, Anti-microbial, Anti-inflammatory etc. Targets used to study anticancer activity of Pyrimidine-2-amine are as follows:

Vascular endothelial growth factor (VEGF), originally known as vascular permeability factor (VPF), is a signal protein produced by cells that stimulates the formation of blood vessels.

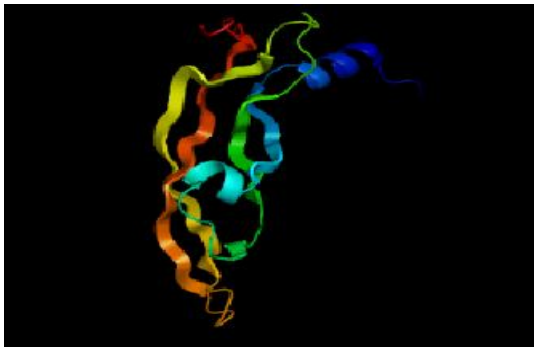


Fig 1: VEGF.

Protein tyrosine kinase(PTK) are the high-affinitycell surface receptors for many polypeptide growth factors, cytokines, and hormones. Receptor tyrosine kinases are part of the larger family of protein tyrosine kinases, encompassing the receptor tyrosine kinase proteins which contain a transmembrane domain, as well as the non-receptor tyrosine kinases which do not possess transmembrane domains.

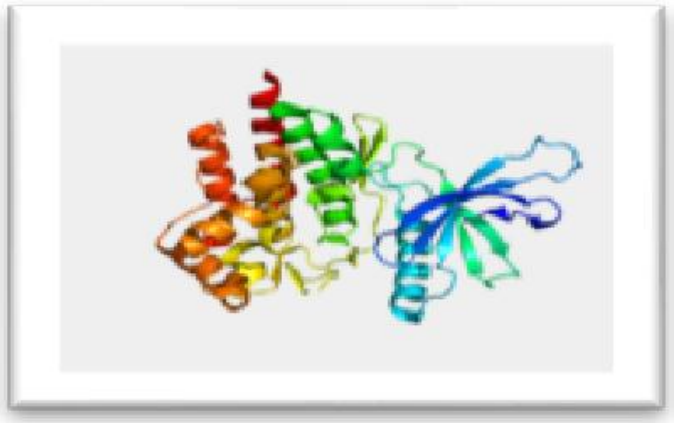


Fig 2: PTK.

Transforming growth factor beta (TGF-β) is a multifunctional cytokine belonging to transforming growth factor superfamily that includes four different isoforms (TGF- β 1 to 4, HGNC symbols TGF ß 1, TGF ß 2, TGF ß 3, and TGF-ß4) and many other signalling proteins produced by all white blood cells lineages.

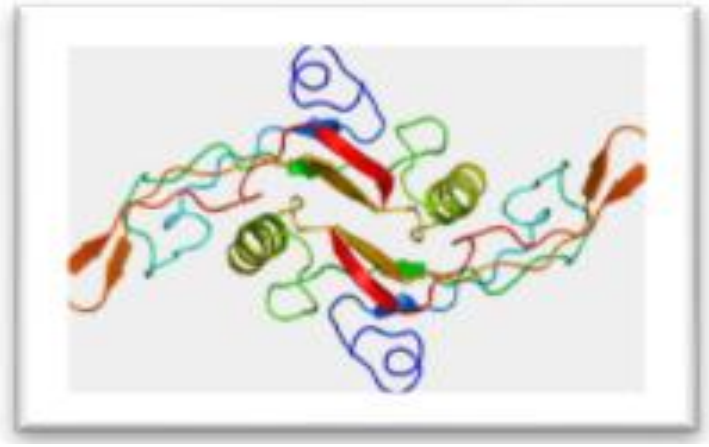


Fig 3: TGF-β.

Fibroblast growth factors, or FGFs, belong to family of growth factors, with members involvedinangiogenesis, wound healing, embryonic development, and various endocrine signalling pathways.

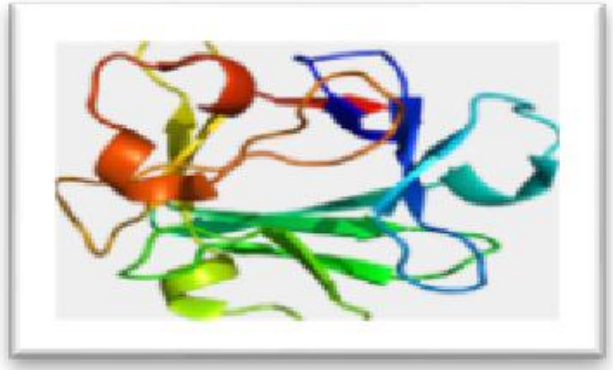
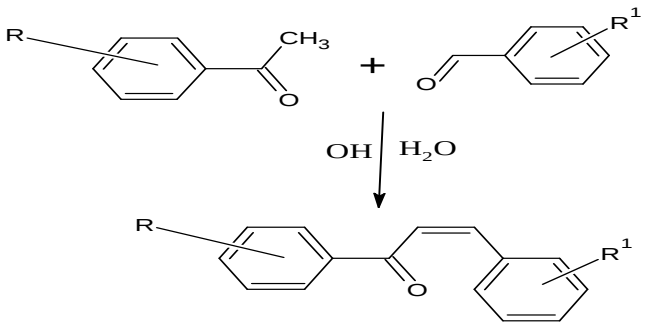


Fig. 4. FGF.

Chalcones

Chalcones, considered to be the precursor of flavonoids and isoflavonoids, are abundant in edible plants. They consist of open-chain flavonoids in which the two aromatic rings are joined by a three-carbon α, β-unsaturated carbonyl system. Studies revealed that compounds with a chalcone-based structure have anti-inflammatory, anti-bacterial, anti-fungal, and anti-tumour activities. The reaction is carried out at about 50 ^{o}C for 12-15 hours.

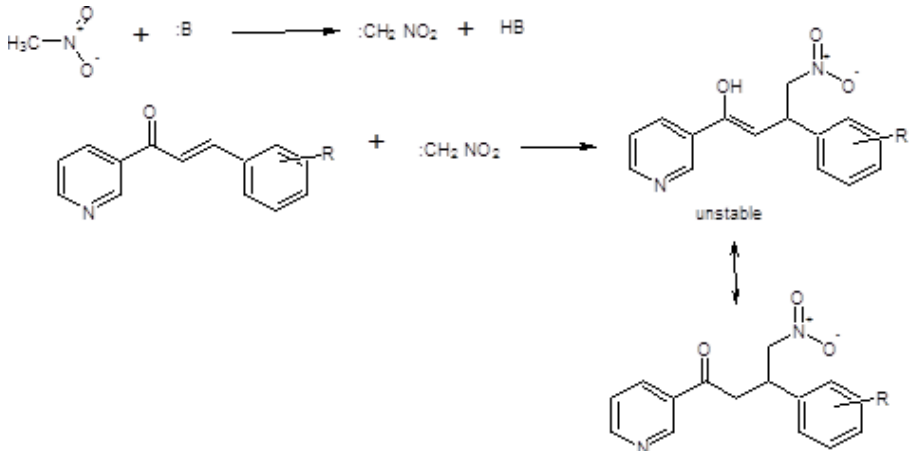


Michael Adducts pyridyl chalcones

Among all heterocyclic compounds, Michael Adducts pyrimidine chalcones are one of the most important heterocyclic structures exhibiting remarkable pharmacological activities because it is an essential constituent of all cells.

Mechanism

Michael Adducts Reaction



Experimental work

Step 1: preparation of chalcone

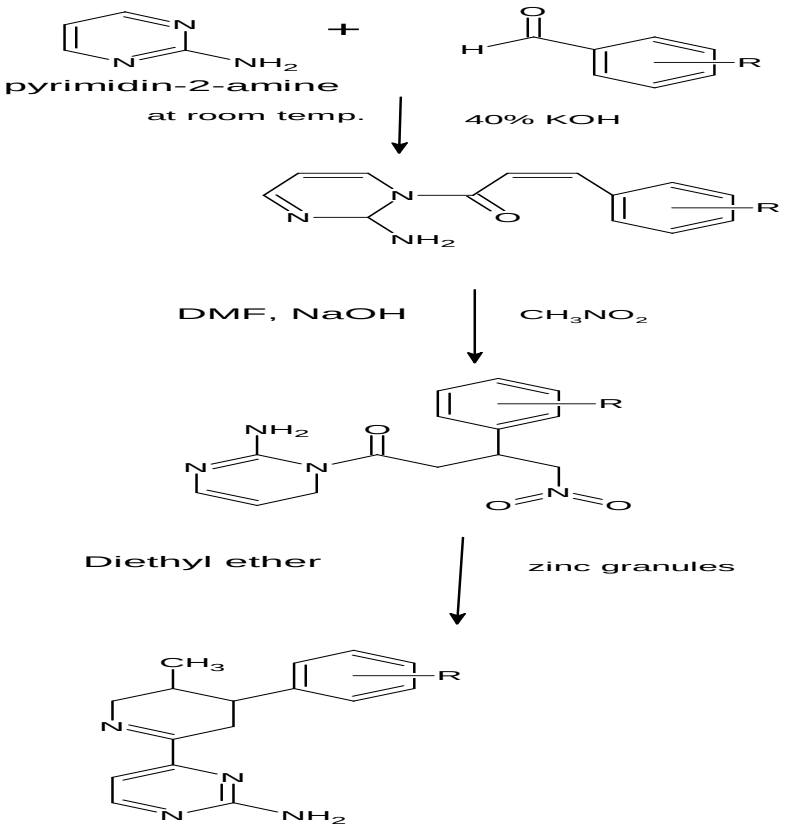
- Equimolar mixture of XYZ & different substituted aldehydes dissolved in 15ml of ethanol and added to 40% KOH & stirred the entire reaction mixture for 6 hours.
- Then the mixture is kept for overnight at room temp.
- Then pour the above mixture in crushed ice, then acidified with HCl.
- The obtained chalcone was recrystallized with alcohol.

Step 2: preparation of michael adduct

- NAOH 1.0 M was added to the stirred solution of chalcones (2.08g, 10 mmol) at room temperature in DMF (10 mL) & Nitromethane (0.61g, 10mmol), the resulting mixture was stirred until the reaction was complete. (TLC)

Step 3: reduction and ring cyclization

- Then granular zinc (3.27g, 50mmol) was added to the mixture and was stirred at 80^0c & concentrated HCL (20 mL) was added very slowly.
- The mixture was stirred at 80^0c under the reducing conditions for about 90 min., & then allowed to come at room temperature.
- Neutralized with saturated aqueous NaHCO, (30ml) & extracted with diethyl ether (3×20ml), filtered & concentrated.
- The crude product was purified by silica gel chromatography.



MATERIALS AND METHODS

Tools and Materials Used: - For our present study we used bioinformatics tools, biological database like PDB (protein data bank) and software like ACD chem. sketch, organic chemistry portal and molecule docking. ACD chemsketch can convert "SMILES" notations to structure and vice versa. Docking allows the scientist to virtually screen a database of compounds and predict the strongest binders based on various scoring functions. It explores ways in which two molecules, such as drugs and an enzyme or receptor fit together and docks to each other well, like pieces of a three-dimensional zigzag puzzle. All the parameters used for molecule docking are selected by default.

Table 1: Substitutions of Michael adducts pyrimidine derivatives and Smiles File.

Sr. No.	Substitutions	Smiles file
1	4-Cl	CC1CN=C(CC1c1ccc(Cl)cc1)c1ccnc(N)n1
2	3-Cl	CC1CN=C(CC1c1cc(Cl)ccc1)c1ccnc(N)n1
3	2-Cl	CC1CN=C(CC1c1ccccc1Cl)c1ccnc(N)n1
4	Biphenyl Carbaldehyde	CC1CN=C(CC1c1ccc(cc1)c1ccc(C=O)cc1)c1ccnc(N)n1
5	Dimethyl Benzaldehyde	CC1CN=C(CC1c1cc(ccc1C=O)N(C)C)c1ccnc(N)n1
6	Benzaldehyde	c1cnccc1C1=NC(C(C1)c1ccc(cc1)C=O)C

Table 2: Drug like properties predicted from Molsoft, Molinspiration Property calculator the following are the properties of Michael Adducts Pyrimidine derivatives.

Properties	1C	2C	3C	4C	5C	6C
Molecular formula	$C_{16}H_{17}ClN_4$	$C_{16}H_{17}ClN_4$	$C_{16}H_{17}ClN_4$	$C_{23}H_{22}N_4O$	$C_{19}H_{23}N_5O$	C_7H_6O
Molecular weight	300.11	300.11	300.11	370.18	337.19	106.04
Number of HBA	3	3	3	4	4	1
Number of HBD	2	2	2	2	2	0
MolLogP	3.49	3.49	3.37	4.37	2.57	1.65
MolLogS A.(moles/L) B.(mg/L)	A.-4.1 B.222.58	A.-4.10 B.23.94	A.-3.88 B.39.17	A.-5.96 B.0.41	A.-3.81 B.51.75	A-1.7 B.1753.59
Mol. PSA ($A^{2)}$	48.42	48.42	48.42	62.31	65.39	14.40
Mol. Vol. ($A^{3)}$	272.32	272.39	272.11	359.17	333.10	104.08
Number of stereo centers	2	2	2	2	2	0
Drug likeliness	1.03	0.73	0.75	0.46	-0.08	-1.82

Table 3: Docking score predicted from Mcule the following are the properties of Michael Adducts Pyrimidine derivatives.

Compound code	Log P	Drug likliness	Docking score			
			FGF	PTK	VEGF	TGF
1C	3.49	1.03	-8.1	-7.2	-8.1	-8.5
2C	3.49	0.73	-8.1	-7.3	-8.3	-8.9
3C	3.37	0.75	-8.2	-7.2	-7.6	-9.0
4C	4.37	0.46	-8.3	-8.3	-8.5	-8.0
5C	2.57	-0.08	-7.8	-7.1	-7.7	-8.2
6C	2.19	0.86	-7.8	-7.4	-7.6	-8.7

Targets for the anti-cancer study using Molecular Docking

1. VEGF-1Y6A: vascular endothelial growth factor.
2. TGF-1py5: Transforming growth factor.
3. FGF-1agw: Fibroblast growth factor.
4. PTK-1a08: Protein tyrosine kinase.

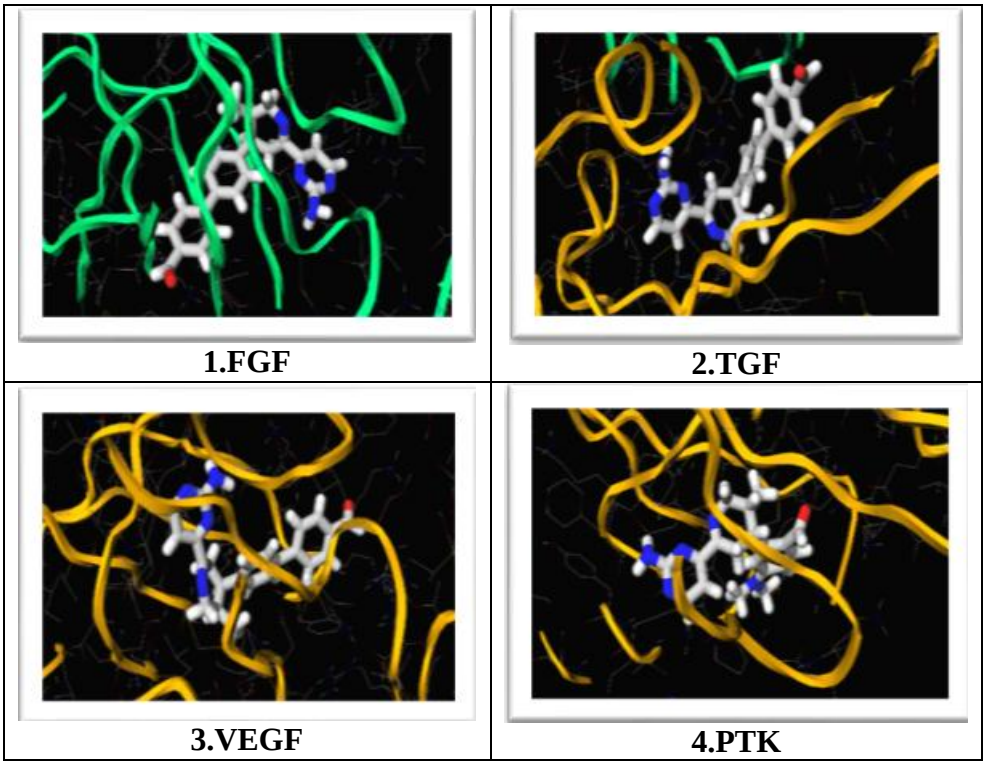


Fig 5: Docking pose of 4C with the Target FGF, TGF, VEGF & PTK.

From the above results, we can conclude that the target shows good ligand-protein interactions than the other targets and give better docking score than the standard drug pyrimidine.

In-vitro anti-cancer activity evaluation of the synthesized compounds

Introduction

Cancer is a family of diseases that are hazardous which involve abnormal cell growth with the potential to unfold to alternative components of the body and destroy the normal functioning of cells. To prove the anticancer activity of the synthesized drug products which was predicted by Molecular docking, the In-vitro anticancer study was carried out for the compound 4C.

Experimental protocol:

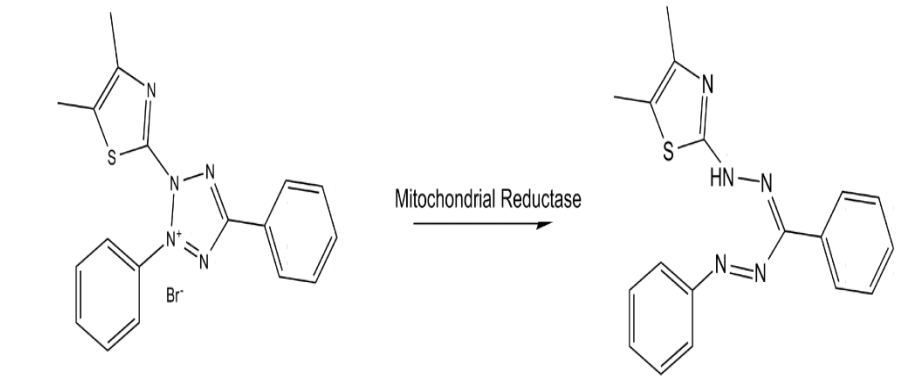
Cytotoxicity

Formula: Using graph Pad Prism Version5.1, we calculate the IC 50 of compounds

$$\text{Surviving cells (\%)} = \frac{\text{Mean OD of check compound}}{\text{Mean OD of Negative management}} \times 100$$

Inhibiting cells (%) =100- Surviving cells

MTT



3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
(MTT)

(*E*,*Z*)-5-(4,5-dimethylthiazol-2-yl)-1,3-diphenylformazan
(Formazan)

RESULT AND DISCUSSION

All the synthesized products were analysed for their chemical and physical characterization. Among all the compounds 4C was having better drug score according to Mcule Docking. Hence, 4C was subjected to In-vitro anticancer study. All the data generated from experiment for compounds is tabulated below.

Table 4: Physico-chemical characterization data of synthesised compounds.

Sr. No.	Code	Formula	Mol.weight	% yield	Melting point	Rf value	Colour
1	1C	$C_{16}H_{17}ClN_4$	300.78598	58.72	318-320	0.65	Creamish White
2	2C	$C_{16}H_{17}ClN_4$	300.78598	81.22	298-300	0.65	Creamish Brown
3	3C	$C_{16}H_{17}ClN_4$	300.78598	68.37	312-314	0.54	Creamish White
4	4C	$C_{19}H_{23}N_5O$	337.41882	83.33	252-254	0.58	Creamish White
5	5C	$C_{23}H_{22}N_4O$	370.44698	63.56	325-327	0.67	Brown
6	6C	$C_{17}H_{18}N_4O$	310.35042	70.86	308-310	0.65	Creamish Brown

Table 5: Spectral characterization of chalcones.

Sr. No.	Comp. Code	IR		^{1}H NMR	
		Bond	**Frequency (cm^{-1})**	**Bond**	**δ (ppm)**
1.	1C	AromaticC-Cl	754.18	Secondary alkane $(CH_3)2$-CH_2	1.2
		Aromatic C-Cl	759.00	Tertiary alkane $(CH_3)3$-CH	1.5
		Aromatic C-N Stretch	843.87	Aromatic H	2.6
		Aromatic stretch C=C	1419.63	Aromatic C-H	7.2
2.	2C	Aromatic C-Cl	659.67-	Tertiary alkane $(CH_3)3$-CH	1.6
		Aromatic C-Cl	696.37	Aromatic H	2.6
		Aromatic C-N Stretch	751.29	Aromatic C-H	7.2
		Aromatic stretch C=C	844.84	--	--
3.	3C	AromaticC-Cl	668.5-707.89	Tertiary alkane $(CH_3)3$-CH	1.6
		Aromatic C-Cl	754.15-	Aromatic H	2.6
		Aromatic C-N Stretch	844.84	Aromatic C-H	7.2
		Aromatic stretchC=N	1373.34-	--	--
		AromaticstretchC=C	1500.64Cm^-	--	--
4.	4C	Aromatic C-H Stretch	652.92-678.95	H-C-Cl	3.1
		Aromatic C-H Bend	680.60-761.90	H-C-Cl	3.9
		Aromatic C-N Stretch	1328.9-1381.06	Ar-H	7.5
		Aromatic stretch C=C	1500.6-1666.33	--	--
5.	5C	Aromatic stretch C=N	1416.74	Secondary alkane $(CH_3)2$-CH_2	1.2
		Aromatic stretch C=C	1418.67	Tertiary alkane $(CH_3)3$-CH	1.5
		Aromatic stretch C=C	1483.28	--	--
		Aromatic stretch C=C	1423.49	--	--
6.	6C	Aromatic C-NStretch	844.84	Secondary alkane $(CH_3)2$-CH_2	1.2
		Aromatic stretch C=C	1436.52	Tertiary alkane (CH3)3-CH	1.5
		Aromatic stretch C=C	1497.75	Aromatic H	2.6
		Aromatic stretch C=C	1500.64	Aromatic C-H	7.2

Results of anti-cancer activity of the synthesized compounds.

Table 6: IC_{50} value of compounds IN µg/ml.

Cmp	HT-29
V	116.6
Paclitaxel	239.09

Table 7: Cell viability of compound 4C in different concentrations.

Cell Viability	
Conc µl/ml	**V**
400.0	19.72
200.0	37.80
100.0	49.78
50.0	69.93
25.0	93.36
12.5	95.64
NC	100

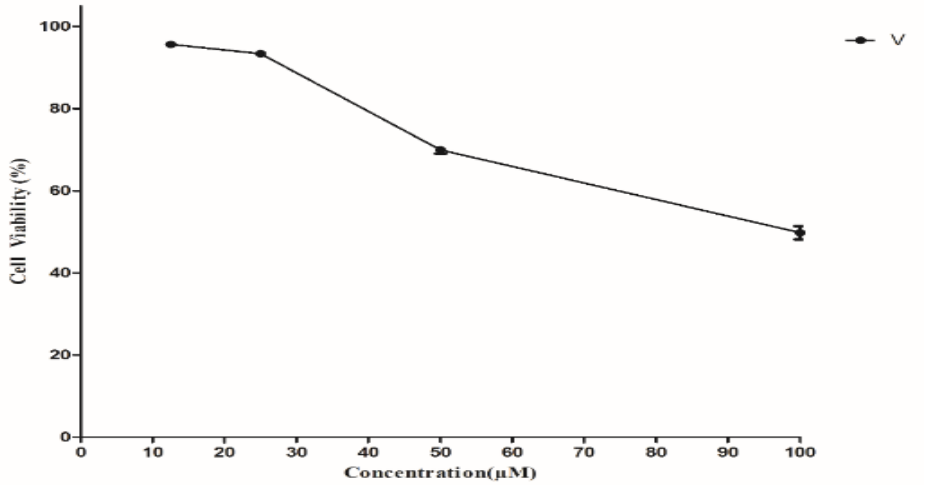


Fig. 6: **Activity graph of compound 4C.**

Graph: Activity graph of the compound 4C in accordance to concentration of the compound and its activity in terms of cancer cell viability at particular conc. The above graph shows that the results predicted by Mcule docking are found positive for the compound 4C and it proves it can be a great Drug discovery approach in comparison with lengthy and expensive Traditional drug discovery system.

CONCLUSION

Thus, the present study reports that the methods employed for synthesis and the analysis of compounds are often suitable and give great results for the proposed establishment of data. The computer aided drug designing approach i.e., Molecular docking which was used to predict the anticancer activity of the synthesized compounds held better in terms of anti-cancer activity results for compound 4C.

This study proves that Molecular docking can be a great approach of drug development in upcoming years of clinical research.

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