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Original Article

ANTIOXIDANT, ANTICANCER AND MOLECULAR DOCKING STUDIESOF NOVEL 5-BENZYLIDENE SUBSTITUTED RHODANINE DERIVATIVES

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ABSTRACT

Objective: The primary objective was to study the *in vitro* antioxidant and anticancer evaluation of novel 5-benzylidene substituted rhodanine derivatives and molecular docking studies of the most active compounds with 3 different anticancer targets.

Methods: Antioxidant potential of 5-benzylidene substituted rhodanine derivatives were studied by DPPH assay, anticancer evaluation was done by MTT assay and Computational evaluation were done using various softwares such as ACD Lab Chemsketch 12.0, molinspiration and Discovery Studio 2021.

Results: Compound 3j exhibited the highest antioxidant activity with an IC_{50} value of 31.21. Other compounds 3b, 3d and 3f also showed moderate antioxidant potential. The Antioxidant study showed a good correlation with molecular docking studies. *In vitro* anticancer assay results showed that compound 3a has an IC_{50} value<62.5 against HeLa cell lines. All the other compounds showed only moderate activity. Out of the ten synthesized derivatives, compounds 3d and 3j showed good docking scores with 3 different anticancer targets.

Conclusion: Ten novel rhodanine derivatives which has been studied can be developed into potent antioxidant and anticancer agents in future.

Keywords: Antioxidant, Anticancer, Rhodanine derivatives, Computational evaluation

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INTRODUCTION

Cancer is reported to be the second most significant health problem in which cells are growing out of control resulting in tissue growth regulation failure [1, 2]. Worldwide, an estimated 19.3 million new cancer cases and almost 10.0 million cancer deaths occurred in 2020 and the global cancer burden is expected to be 28.4 million cases in 2040 [3]. Female breast cancer became the most commonly diagnosed cancer with an estimated 2.3 million new cases in 2020, representing 11.7% of all cancer cases [4]. The large increase in the number of cancer cases and high mortality rate reveals the fact that the present anticancer treatment is inadequate.

Thiazolidinedione (TZD) and Rhodanine derivatives have become very important groups of heterocyclic compounds in drug design and discovery [5]. Due to the ability to demonstrate a broad range of biological activities rhodanine derivatives have been well recognized as a privileged scaffold in medicinal chemistry. Many studies have revealed the anticancer effects of rhodanines over the last few decades [6-11]. The five-membered rhodanine ring bearing a Sulphur and Nitrogen at the first and third positions are reported to have significant anticancer activity against different types of cancer, including breast cancer, prostate cancer, colorectal cancer, liver cancer, pancreatic cancer, gastric cancer, lung cancer, renal cancer, and leukaemia [12-15].

Reactive oxygen species (ROS) are thought to play an important role in the progression of many chronic diseases, including cancer [16-18]. ROS are produced in the human body from metabolic activity or exogenously from smoking, air pollutants, radiation, ozone, and industrial chemicals. Antioxidants help in scavenging or preventing the generation of ROS and control the formation of free radicals [18]. The intake of antioxidants has been shown to reduce the risk of cancer as well as neurological and cardiovascular pathologies among others [19].

Based on these findings, we were interested in investigating the antioxidant and anticancer effects of our previously reported new chemical entity (NCE), 3-[(dialkylamino) alkyl]-2-thioxothiazolidinone. In the present study, a series of ten 5-benzylidene substituted rhodanine derivatives were tested for antioxidant potential by DPPH assay and *in vitro* anticancer activity against HeLa and MDAMB-231 using the MTT assay. To correlate the mechanism of action of the most active compounds, molecular docking studies were also carried out.

Fewer efficacies in the present cancer therapy, patient non-compliance, drug resistance and uncertainty of current candidates in a clinical trial have led to the need for the development of potential anticancer agents.

MATERIALS AND METHODS

Chemistry

A new chemical entity (NCE), 3-[(dialkylamino) alkyl]-2-thioxothiazolidinone (fig. 1) by substituting various benzylidene derivatives at the 5th position was prepared previously 20] by the reaction of N–substituted Rhodanine, substituted benzaldehyde and ammonium acetate in a minimum amount of acetic acid (Scheme II). Compounds were characterized by melting points, IR, NMR and MASS Spectra and were purified by column chromatography to get excellent yield.

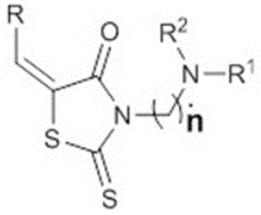


Fig. 1: General structure of the designed molecular framework

***In vitro* antioxidant studies by DPPH assay**

The DPPH radical scavenging activity (H/e-transferring activity of the compound was evaluated according to the method reported by Sunitha Dontha in 2016 with slight modification [21]. Briefly aliquot 100 µl of various concentrations (500, 250, 125, 62.5, 31.25, 15.625 µM) were dissolved in methanol. Added 130 µl (100 µl) of DPPH. 100 µl of methanol mixed with DPPH served as a negative control. The mixture was vortexed and then incubated in the dark for 30 min at room temperature. After the prescribed incubation, the absorbance was measured at 550 nm using a multiwall Elisa reader and plotted the graph by taking the concentration along the X-axis and the percentage scavenging activity along Y-axis.

$$\% \text{ Scavanging activity} = \frac{\text{Ac} - \text{As}}{\text{Ac}} * 100$$

Where Ac is Absorbance of Control and As is Absorbance of Sample

***In vitro* anticancer studies**

Reagents and cell line

MDAMB-231 (breast carcinoma) and HeLa (human cervical cancer) cells were procured from National Centre for Cell Sciences, Pune, India. The cells were cultured and maintained in a DMEM medium containing 10% fetal bovine serum at 37 °C, 5% CO_2 and saturated humidity. Other reagents used for the study were Dulbecco's modified Eagle's medium (DMEM) (Lonza), Fetal bovine serum, Trypsin, Antibiotic antimycotic (Gibco, USA), MTT (Merck), Sodium dodecyl sulphate (SDS) and Dimethyl formamide (DMF) (Sigma) and cell culture plastic wares (Eppendorff, Germany).

Cell proliferation by MTT assay

MTT assay was performed to study cell proliferation (http://www.organic-chemistry.org/prog/peo/). $5x10^3$ cells were seeded and incubated overnight in 96 well plates, the next day, the wells were treated with different concentrations of compounds (1000, 500, 250, 125 and 62.5 µg/ml) and incubated further for 48 and 72 h. MTT reagent was added after the specified incubations into each well at a concentration of 100 µg per well and incubated in the dark at 37 °C for two hours. Followed by the addition of lysis solution (20% SDS in 50% DMF) into each well, it was again incubated in the dark for further four hours. The optical densities were measured at 570 nm after the incubation, using an enzyme-linked immunosorbent assay reader (Tecan infinite M200 PRO) and the percentage of cytotoxicity was calculated using the equation,

$$\% \text{ Cytotoxicity} = 100 - \frac{\text{OD treated}}{\text{OD control}} * 100$$

IC_{50} was calculated using Easy plot software.

Microscopy

Phase-contrast microscopy (Magnus, Magcam, DC5) is used to assess the morphological changes of MDAMB-231 and HeLa cells. These cells were treated with the compounds at different concentrations for 48 and 72 h before morphological testing. The morphology of the untreated and treated cells was captured and compared for cytotoxic effects.

Statistical analysis

Data were expressed as the mean of three independent experiments for the cytotoxicity studies.

Docking simulations

Docking is a virtual screening of a database of compounds and predicting the strongest binding agents based on various scoring functions. The docking module LibDock using Discovery Studio 2021 was used for docking. Drug likeness scores of the compounds were evaluated with the help of Lipinski's rule of five. The ligands were positioned in the binding site by using Libdock. Libdock is a suitable algorithm to find various conformations of the ligands within the receptor. Receptor ligand interactions were optimized by molecular dynamics using CHARMM. Protein targets selected for the study are progesterone receptor 1A28, Estrogen receptor 3ERT which are involved in the progression and development of breast cancer and Aurora Kinase-4ZTR which comes under the tyrosine kinase receptor. Therefore these proteins were selected for the docking study. The docking study was done using Discovery studio 2021 software and target proteins (1A28, 3ERT and 4ZTR) were downloaded from the protein data bank.

Preparation of ligands

Structures of the biochemical compounds were obtained from Pub Chem (https://pubchem.ncbi.nlm.nih.gov/) compound database, the world's largest freely accessible chemical database that provides information regarding chemical and physical properties, biological activities, safety and toxicity, patents and literature citations of chemical molecules. The ligand structure procured from PubChem possess different protonation states and 3D geometries and is prepared for molecular interaction studies by correcting, editing and generating variations of the structures and optimizing them using Biovia Discovery Studio v.21.

Preparation of the target proteins

The three-dimensional X ray crystallographic structure of proteins was retrieved from Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). The Protein Data Bank (PDB) is a freely accessible structural database that provides three-dimensional X-ray crystallographic and NMR data of large biological molecules such as proteins and nucleic acids. From the crystallographic structure of the proteins, unwanted water molecules, heteroatoms and complex ligands were manually removed. The Protein Prepare protocol in Discovery Studio v.21 was used to insert the missing atoms, missing loop regions, delete alternate conformations, remove waters, standardise atom names and protonate titratable residues using predicted pKs. Energy minimization was also performed, and the minimized structure was used as the target structure for the docking studies.

Molecular docking analysis

The molecular interaction study between the targets and ligands was conducted using Biovia Discovery Studio v.21. Initially, the binding sites of the proteins were predicted using the 'define and edit binding site' option in the software based on the PDB site records. For the molecular interaction study LibDock protocol [22], a high throughput docking algorithm to find various ligand conformations in the protein active site based on polar interaction sites (hotspots) was used. CHARMM was the force field applied which uses positional relationships between atoms to determine the energy and forces acting on each particle of the system. The LibDock score and binding energy of the protein-ligand complexes were estimated and recorded.

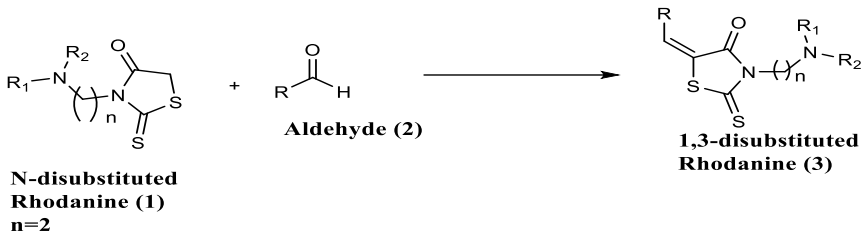
Pharmacokinetic screening

The compounds were evaluated for their acceptability as an oral drug based on Lipinski's rule of five [23] which is essential for drug-like pharmacokinetic profile in rational drug design [24]. The druggability of the ligand molecules was also predicted by ADMET analysis which computes the absorption, distribution, metabolism, excretion and toxicity potential of a pharmaceutical compound within an organism [25]. The 2D structures of the molecules were subject to analysis of solubility, intestinal absorption, and hepatotoxicity, plasma protein binding ability, blood-brain barrier (BBB) penetration, cytochrome P450 inhibition and AMES mutagenicity using ADMET descriptors in Discovery studio v.21.

RESULTS AND DISCUSSION

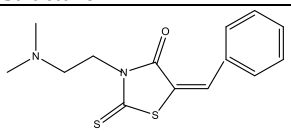
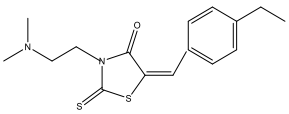
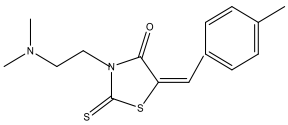
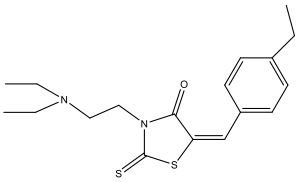
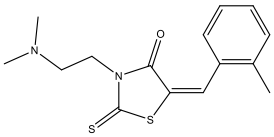
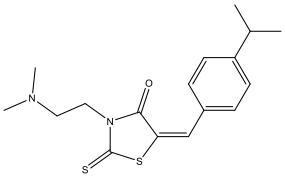
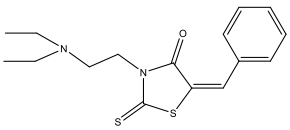
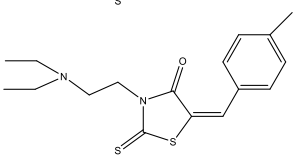
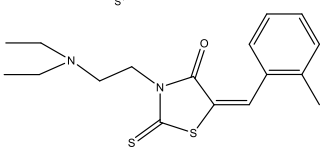
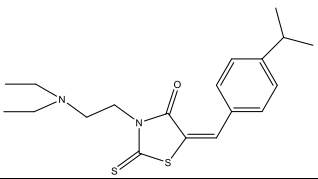
Chemistry

Synthesis of a new chemical entity (NCE), 3-[(dialkylamino) alkyl]-2-thioxothiazolidinone by substituting various benzylidene derivatives at the 5^{th} position (Scheme 1) was reported previously [20]. Different Rhodanine derivatives (3a-3j) synthesized are given in table 1.



Scheme II: **Reagents and condition: a) Ammonium acetate, Acetic acid, 80-85 °C**

Table 1: List of derivatives selected for synthesis using ACD Lab Chemsketch 12.0 and their molecular formula

Compound code	Structure	Chemical name	Molecular formula
3a		5-Benzylidene-3-(2-(dimethyl amino)ethyl)-2-thioxothiazolidin-4-one	$C_{14}H_{16}N_2OS_2$
3b		3-(2-(dimethyl amino) ethyl)-5-(4-ethylbenzylidene)-2-thioxothiazolidin-4-one	$C_{16}H_{20}N_2OS_2$
3c		3-(2-(dimethyl amino) ethyl)-5-(4-methylbenzylidene)-2-thioxothiazolidin-4-one	$C_{15}H_{18}N_2OS_2$
3d		3-(2-(diethylamino)ethyl)-5-(4-ethylbenzylidene)-2-thioxothiazolidin-4-one	$C_{18}H_{24}N_2OS_2$
3e		3-(2-(dimethylamino)ethyl)-5-(2-methylbenzylidene)-2-thioxothiazolidin-4-one	$C_{15}H_{18}N_2OS_2$
3f		3-(2-(dimethylamino)ethyl)-5-(4-isopropylbenzylidene)-2-thioxothiazolidin-4-one	$C_{17}H_{22}N_2OS_2$
3g		5-benzylidene-3-(2-(diethylamino)ethyl)-2-thioxothiazolidin-4-one	$C_{16}H_{20}N_2OS_2$
3h		3-(2-(diethylamino)ethyl)-5-(4-methylbenzylidene)-2-thioxothiazolidin-4-one	$C_{17}H_{22}N_2OS_2$
3i		3-(2-(diethylamino)ethyl)-5-(2-methylbenzylidene)-2-thioxothiazolidin-4-one	$C_{17}H_{22}N_2OS_2$
3j		3-(2-(diethylamino)ethyl)-5-(4-isopropylbenzylidene)-2-thioxothiazolidin-4-one	$C_{19}H_{26}N_2OS_2$

In vitro antioxidant studies by DPPH assay

DPPH assay is based on the measurement of the scavenging capacity of antioxidants towards it. The odd electron of the nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine [26]. Concentrations ranging from 15.625µg/ml-500 µg/ml of the synthesized rhodanine derivatives were tested for their antioxidant activity by using the DPPH scavenging method. Ascorbic acid was used as the standard. The antioxidant activity was estimated by IC_{50} value and the values are shown in table2. The scavenging activity of compounds 3a-3j is shown in fig. 2.

Among the compounds tested for antioxidant activity, compounds 3b, 3f and 3j exhibited the highest antioxidant activity with IC_{50} values of 170.49, 184.39 and 31.21 µg/ml respectively while IC_{50} of reference compound ascorbic acid was found to be less than 15.63µg/ml. Another moderately active compound, 3d showed an IC_{50} value of 453.17 µg/ml. All other compounds (3a, 3c, 3e, 3g, 3h, 3i) were not considered to have anti-oxidant activity since their IC_{50} value for quenching DPPH was more than 500 µg/ml. A series of benzylidene rhodanines were reported to have antioxidant potential by DPPH radical scavenging assay [27]. In our study, 4-ethylbenzylidene and 4–isopropyl benzylidene group at the 5th position of rhodanine showed highest antioxidant activity.

Table 2: Antioxidant activity of synthesized compounds

Compound	IC_{50}(µg/ml)
3a	>500
3b	170.49
3c	>500
3d	453.17
3e	>500
3f	184.39
3g	>500
3h	>500
3i	>500
3j	31.21
Ascorbic acid	<15.63

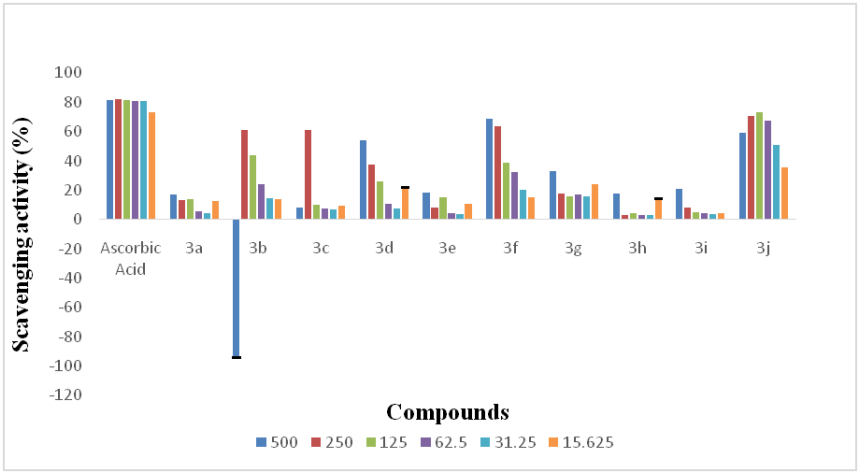


Fig. 2: Radical scavenging activity of compounds 3a-3j. Results are expressed as mean±SD (n = 3)

In vitro anticancer studies

The synthesized rhodanine derivatives were screened for their anticancer activity using an MTT assay against MDAMB-231 and HeLa cell lines. Untreated cells served as control. The positive control used for the study was Doxorubicin for MDAMB and Cisplatin for HeLa cells. The percentage of cell inhibition at different concentrations of compounds and IC_{50} value was determined. IC_{50} concentrations of the different compounds at 48 and 72 h on MDAMB-231 and HeLa cells are given in table 3. The percentage of cytotoxicity induced by the compounds (3a-3j) after 48 and 72 h on MDAMB-231 and HeLa cells are shown in fig. 3, 4, 5 and 6.

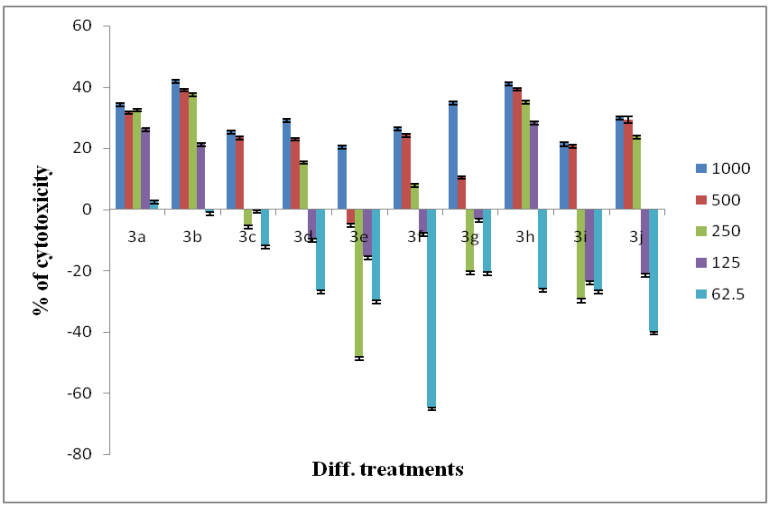


Fig. 3: Percentage cytotoxicity induced by the compounds (3a-3j) after 48 h on MDAMB-231 cells at different concentrations. Data given in mean±SD (n = 3)

Table 3: IC50 concentrations of the different compounds at 48 and 72 h on MDAMB-231 and HeLa cells

Compound	IC_{50} (µg/ml)			
	MDAMB-231		HeLa cells	
	48 h	72 h	48 h	72 h
3a	>1000	250-500	<62.5	<62.5
3b	>1000	296	208	622
3c	>1000	>1000	>1000	>1000
3d	>1000	>1000	700	820
3e	>1000	252	>1000	184
3f	>1000	984	488	>1000
3g	>1000	247	360	>62.5
3h	>1000	679	244	494
3i	>1000	>1000	185	>1000
3j	>1000	>1000	>1000	966

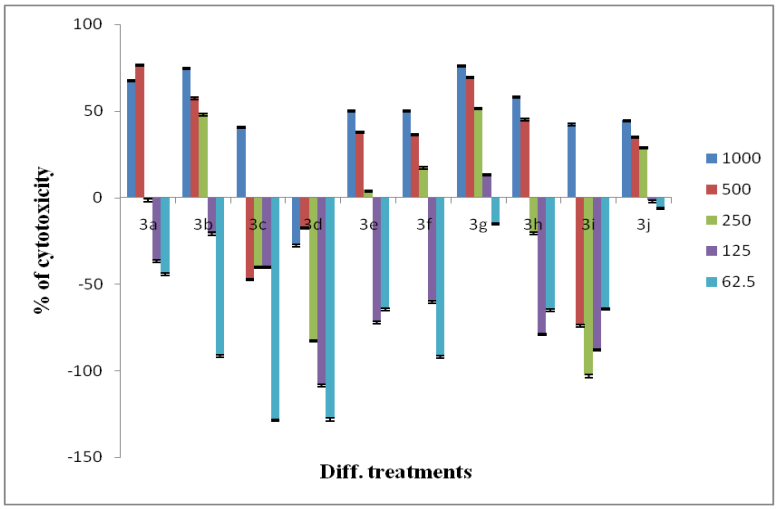


Fig. 4: Percentage cytotoxicity induced by the compounds (3a-3j) after 72 h on MDAMB-231 cells at different concentrations. Data given in mean±SD (n = 3)

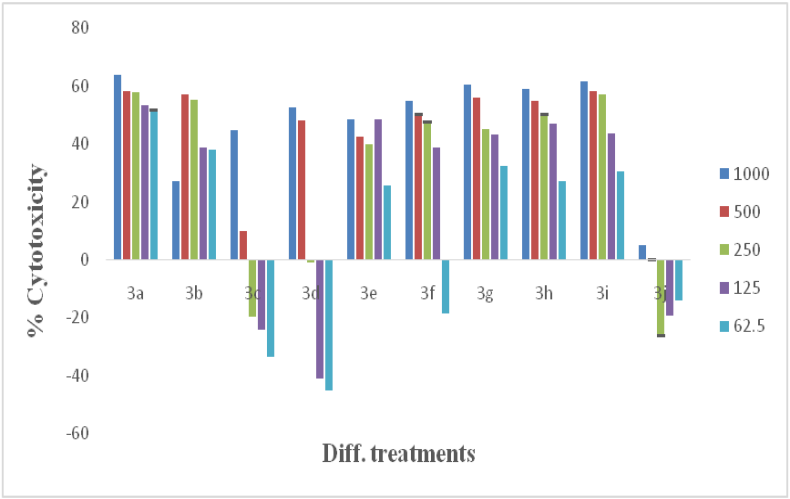


Fig. 5: Percentage cytotoxicity induced by the compounds (3a-3j) after 48 h on HeLa cells at different concentrations. Data given in mean±SD (n = 3)

Molecular modelling

Molecular descriptor analysis and drug likeness of Rhodanine derivatives (3a-3j) are shown in Tables 4, 5 and 6. Molar volume was calculated based on group contributors and analysed the transport characteristics of a molecule mainly gastro intestinal absorption (HIA) or Blood brain barrier (BBB). It was found that the calculated values of molar volumes were within the range (table 4). The measure of the total polarizability of molecules describing the steric effects and predicted polarizability of the compounds are within the range compared to the standard. All the compounds were expected to have good BBB and skin permeability. All the compounds were found to have a molecular weight of less than 500 Daltons and Log P value ranges from 1.92-4.19 (table 5).

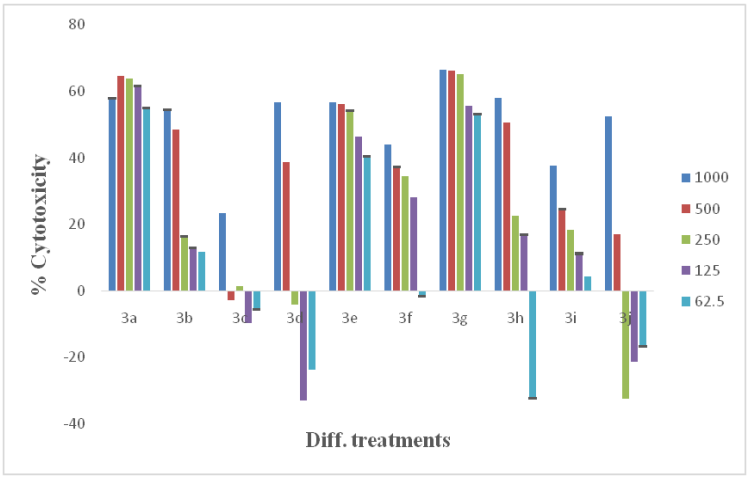


Fig. 6: Percentage cytotoxicity induced by the compounds (3a-3j) after 72 h on HeLa cells at different concentrations. Data given in mean±SD (n = 3)

Table 4: Molecular descriptor analysis by ACD lab chemsketch 12.0. Data given in mean±SD (n = 3)

Compound code	Molecular refractivity (cm^3)	Molar volume (cm^3)	Parachor (cm^3)	Surface tension (dyne/cm)
3a	84.44±0.4	225.0±5.0	635.4±6.0	63.5±5.0
3b	93.69±0.4	257.1±5.0	713.7±6.0	59.3±5.0
3c	89.06±0.4	240.8±5.0	673.7±6.0	61.2±5.0
3d	102.95±0.4	289.5±5.0	793.9±6.0	56.5±5.0
3e	89.06±0.4	240.8±5.0	673.7±6.0	61.2±5.0
3f	98.30±0.4	273.9±5.0	751.8±6.0	56.7±5.0
3g	98.30±0.4	273.9±5.0	751.8±6.0	56.7±5.0
3h	98.32±0.4	273.3±5.0	753.8±6.0	57.8±5.0
3i	98.32±0.4	273.3±5.0	753.8±6.0	57.8±5.0
3j	98.32±0.4	273.3±5.0	753.8±6.0	57.8±5.0

Table 5: Analysis of lipinski rule of five using molinspiration

Compound code	mi LogP	Molecular Weight	nON	nOHNH	N rotb	N Violation
3a	1.92	292.43	3	0	4	0
3b	2.84	320.48	3	0	5	0
3c	2.37	306.46	3	0	4	0
3d	3.59	348.54	3	0	7	0
3e	2.32	306.46	3	0	4	0
3f	3.44	334.51	3	0	5	0
3g	2.67	320.48	3	0	6	0
3h	3.12	334.51	3	0	6	0
3i	3.08	334.51	3	0	6	0
3j	4.19	362.56	3	0	7	0

The compound that exceeds molecular weight (Mw)>500Da, calculated log P>5, Hydrogen bond donors>5 and Hydrogen bond acceptors>10 is unlikely to be considered as a potential drug candidate because it would likely lack properties essential for its absorption, distribution, metabolism and excretion.

Table 6: Analysis of drug-likeness using molinspiration

Compound code	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
3a	-0.90	-1.84	-0.79	-1.22	-0.74	-0.40
3b	-0.73	-1.65	-0.70	-0.98	-0.57	-0.34
3c	-0.87	-1.84	-0.75	-1.14	-0.73	-0.43
3d	-0.63	-1.53	-0.65	-0.84	-0.48	-0.32
3e	-0.90	-1.84	-0.81	-1.08	-0.78	-0.50
3f	-0.68	-1.60	-0.63	-0.88	-0.54	-0.33
3g	-0.74	-1.70	-0.70	-1.00	-0.60	-0.37
3h	-0.73	-1.70	-0.69	-0.96	-0.60	-0.41
3i	-0.76	-1.70	-0.74	-0.90	-0.65	-0.47
3j	-0.60	-1.49	-0.60	-0.77	-0.47	-0.32

Molecular docking study with different anticancer targets

Ten compounds were selected for the study and 3D structures of standard drug and 5-Fluorouracil was downloaded from the PubChem database in. sdf format. The ligands were prepared to generate possible conformers and tautomers. 3D structures of proteins were procured from PDB. The protein structures was cleaned (water molecules and other hetero atoms removed),

prepared and minimized before docking. The docking module LibDock using Discovery Studio 2021 was used to study the interaction between the protein and ligand molecules.

Docking with progesterone receptor (PDB ID: 1A28)

The three-dimensional structure of the human progesterone receptor ligand-binding domain was downloaded from the PDB database with PDB ID: 1A28 with crystallographic resolution 1.80 A° (fig. 7). The protein consists of two polypeptide chain A and B. The protein chains consists of a total of 500 amino acids and has a molecular weight of 57452.3 Daltons. In the present study, the active site of protein interacting with the standardized ligand molecules was selected as the binding site.

90 poses of each selected ligand in the docked complexes were generated. The interacting molecular complexes among these having high LibDock scores and a maximum number of hydrogen bonds and active residues were selected. All 10 compounds have shown good interaction with the progesterone receptor in comparison with the standard drug, Adriamycin (PubChem ID: 31703) and 5-Fluorouracil (PubChem ID: 3385). Table 7 shows the Libdock scores of the best conformers of the ligands.

Fig. 7: Crystallographic structure of human progesterone receptor from PDB database (PDB ID: 1A28)

Table 7: Libdock score of ligands against human progesterone receptor (PDB ID: 1A28)

S. No.	PubChem ID	Lib dock score
1.	3a	80.7518
2.	3b	87.8036
3.	3c	83.2175
4.	3d	94.8511
5.	3e	83.3321
6.	3f	91.6506
7.	3g	89.3985
8.	3h	93.8283
9.	3i	91.7942
10.	3j	98.6231
Standard	5-Fluorouracil (PubChem ID: 3385)	59.6716

The ligands 3j and 3d showed top binding affinity. The docked complex of progesterone receptor (PDB ID: 1A28) with top score ligands and Standard ligands are shown in fig. 8 were analyzed to study the interactions between the target and the ligand molecule. The interacting residues, the nature of the interacting bond and the bond distance are given in table 8.

Table 8: Interactions between human progesterone receptor (PDB ID: 1A28) and ligands

S. No.		PubChem ID	Lib dock score	Interacting residues	Bond distance	Nature of bonding
1.	8468	3j	98.6231	A: MET756:SD–3j	5.45854	Other
				A: TYR890:C,O; CYS891:N–3j	5.0035	Hydrophobic
				3j: C20-A: LEU721	4.28126	Hydrophobic
				3j: C20-A: MET759	4.80199	Hydrophobic
				3j: C21-A: LEU763	3.45764	Hydrophobic
				3j: C23-A: LEU715	3.86146	Hydrophobic
				3j: C23-A: VAL903	5.0746	Hydrophobic
				3j: C24-A: CYS891	3.80059	Hydrophobic
				A: PHE778–3j: C21	4.78601	Hydrophobic
				A: PHE905–3j: C23	4.91859	Hydrophobic
				A: PHE905–3j: C24	4.1478	Hydrophobic
				3j-A: MET756	5.3591	Hydrophobic
				3j-A: VAL760	5.39639	Hydrophobic
				3j-A: MET801	4.72525	Hydrophobic
				3j-A: LEU887	4.9706	Hydrophobic
				3j-A: CYS891	4.55172	Hydrophobic
2.	78016	3d	94.8511	A: MET756:SD–3d	5.55576	Other
				A: TYR890:C,O; CYS891:N–3d	4.8023	Hydrophobic
				3d: C11-A: LEU721	4.82046	Hydrophobic
				3d: C11-A: LEU763	4.6011	Hydrophobic
				3d: C12-A: LEU721	3.85694	Hydrophobic
				A: PHE778–3d: C11	4.37467	Hydrophobic
				A: PHE905–3d: C20	4.57443	Hydrophobic
				3d-A: MET756	5.29031	Hydrophobic
				3d-A: MET801	4.76102	Hydrophobic
				3d-A: LEU887	4.85436	Hydrophobic
				3d-A: CYS891	4.21074	Hydrophobic
3.		5-Fluorouracil (PubChem ID: 3385)	59.6716	A: GLN725:HE21-3385:O3	2.48473	Hydrogen Bond
				A: ARG766:HH21-3385:O3	2.37618	Hydrogen Bond
				A: ARG766:HH22-3385:O3	2.29975	Hydrogen Bond
				3385:H11-A: GLN725:OE1	2.69285	Hydrogen Bond
				A: VAL760:HA-3385:O2	2.2983	Hydrogen Bond
				A: PHE778:HA-3385:O3	2.98497	Hydrogen Bond
				3385-A: MET759	4.91528	Hydrophobic
				3385-A: LEU763	4.9889	Hydrophobic

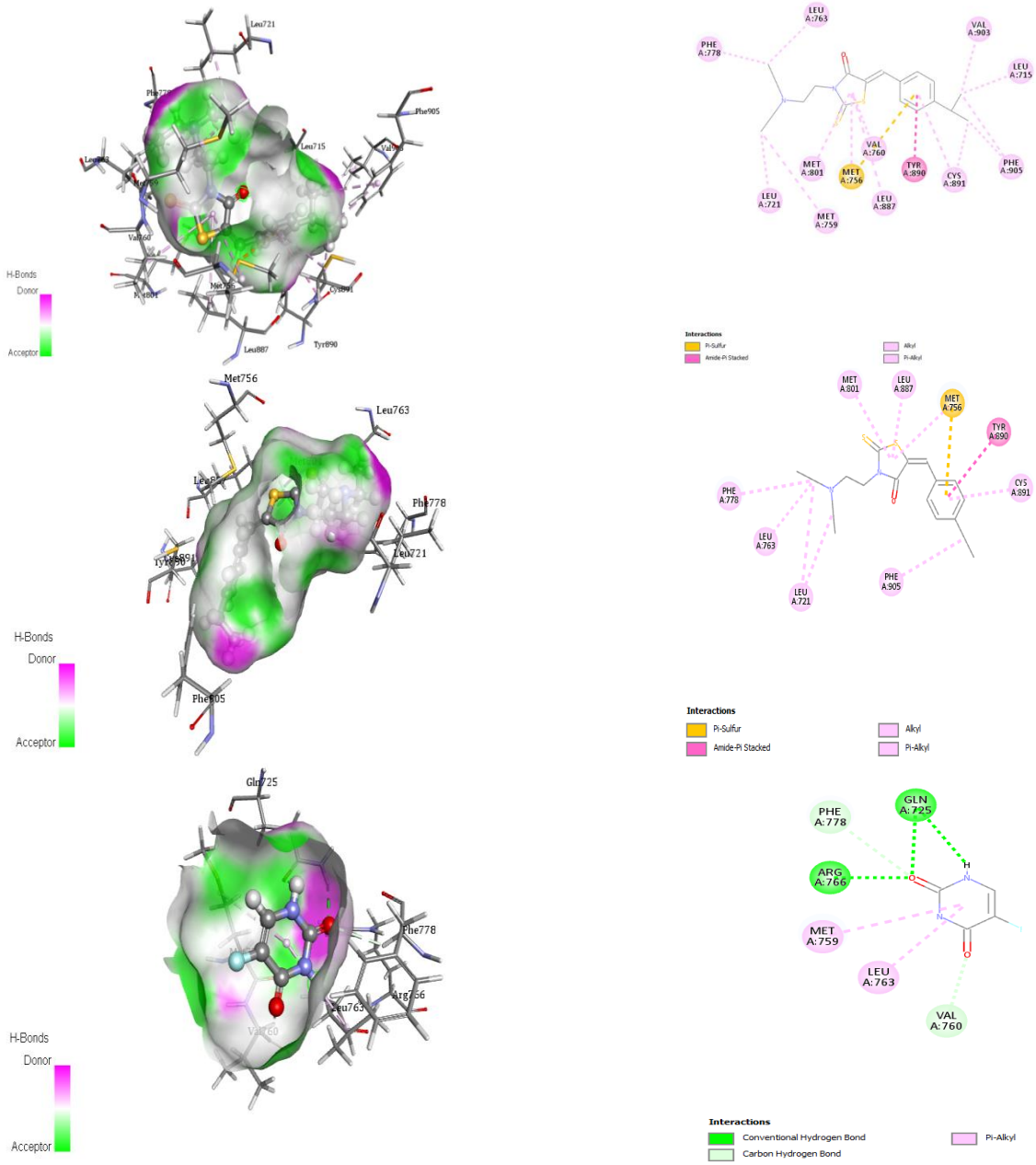


Fig. 8: Surface view and 2D diagram of human progesterone receptor (PDB ID: 1A28) withligands 3j, 3d, and 5-Fluorouracil

The result showed that ligands such as 3j and 3dhave high binding affinity. The docking score 98.6231 corresponded to 3j showed a high binding affinity with the receptor compared with standard drug molecule 5-Fluorouracil. The Standard drug 5-Fluorouracil showedthe LibDock score of 59.6716 with six hydrogen bonds (GLN725, ARG766, VAL760 and PHE778).

Docking with Estrogen receptor alpha (PDB ID: 3ERT)

The three-dimensional structure of Human estrogen receptor alpha ligand binding domain in complex with 4-hydroxytamoxifen was downloaded from PDB database with PDB ID: 3ERT with crystallographic resolution 1.90 A ° (fig. 9). The protein consists of one polypeptide chain A. The protein chain consists of 247 amino acids and has a molecular weight of 27596.2 Daltons. In the present study, the active site of protein interacting with the standardized ligand molecules was selected as the binding site.

90 poses of each selected ligand in the docked complexes were generated. The ligands 3j, 3d and 3i showed top binding affinity (fig. 10). Table 9 shows the Libdock score of best conformers of the ligands and table 10 shows Interactions between Estrogen receptor alpha (PDB ID: 3ERT) and Ligands.

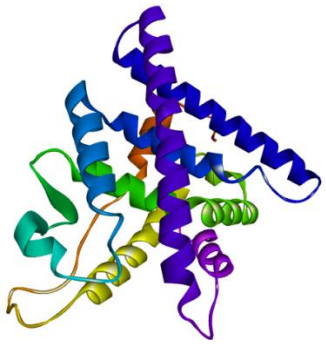


Fig. 9: Crystallographic structure of Estrogen receptor alpha (PDB ID: 3ERT): from PDB database

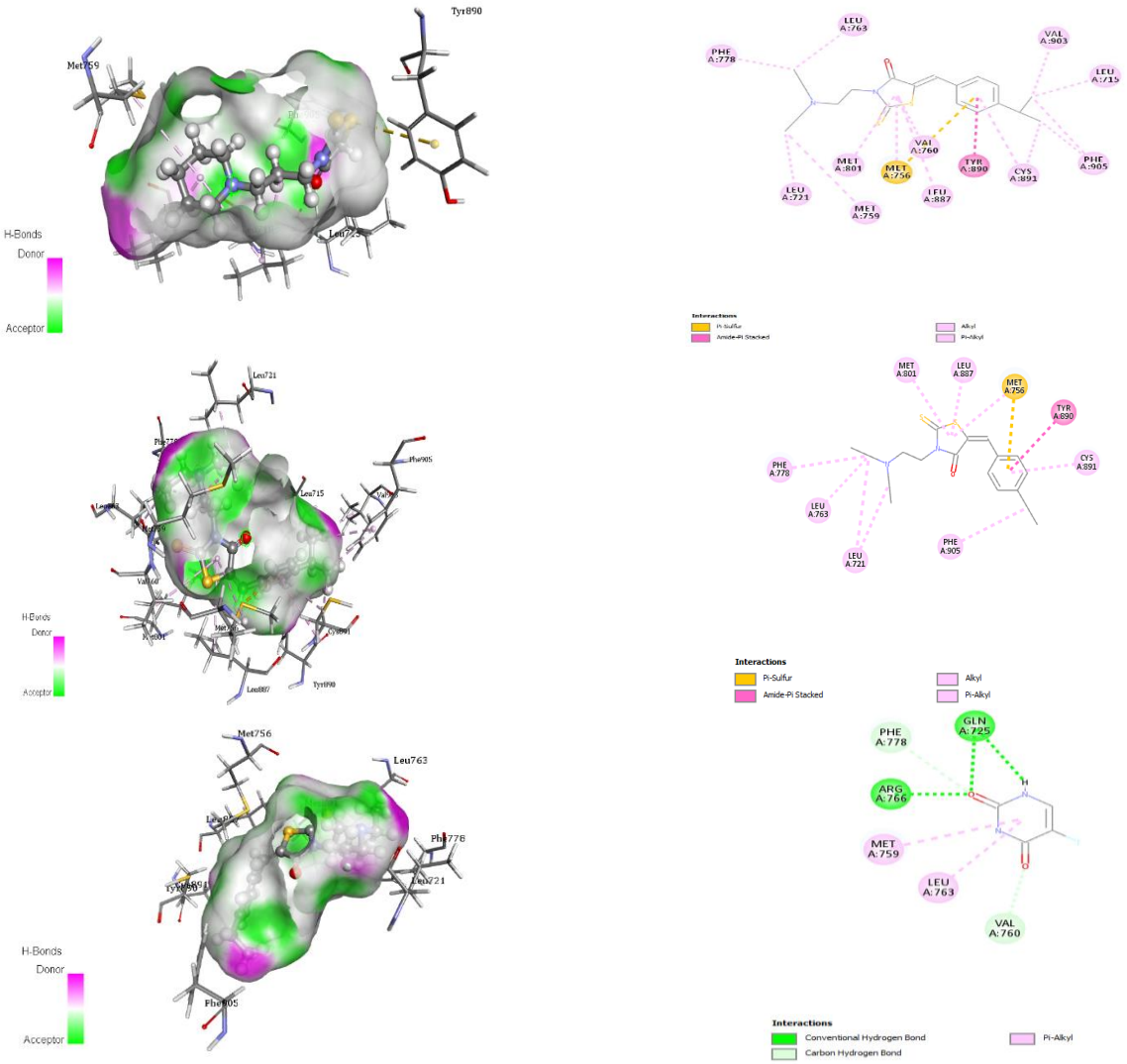


Fig. 10: Surface view and 2D diagram of Estrogen receptor alpha (PDB ID: 3ERT) with ligands 3j, 3d and 5-Fluorouracil

Table 9: Libdock score of Ligands against Estrogen receptor alpha (PDB ID: 3ERT)

S. No.	PubChem ID	LibDock score
1.	3a	77.4554
2.	3b	80.6905
3.	3c	75.6753
4.	3d	88.3553
5.	3e	80.983
6.	3f	83.2322
7.	3g	84.3405
8.	3h	84.3602
9.	3i	88.446
10.	3j	90.6015
11.	5-Fluorouracil (PubChem ID: 3385)	61.5565

Docking with aurora kinase (PDB ID: 4ZTR)

The three-dimensional structure of Human aurora a catalytic domain bound to FK1141 was downloaded from PDB database with PDB ID: 4ZTR with crystallographic resolution 2.85A° (fig. 11). The protein consists of a single polypeptide chain A. The protein chain consists of 249 amino acids and has a molecular weight of 28030.1 Daltons. A. The active site of protein interacting with the standardized ligand molecules was selected as the binding site.

90 poses of each selected ligands in the docked complexes were generated. 3j and 3d showed the top binding affinity. Table 11 shows the Libdock score of best conformers of the ligands.

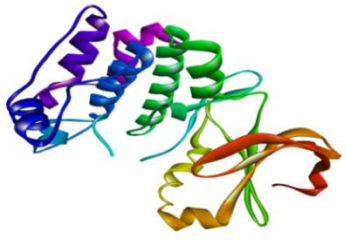


Fig. 11: Crystallographic structure of Aurora Kinase (PDB ID: 4ZTR) from PDB database

Table 10: Interactions between Estrogen receptor alpha (PDB ID: 3ERT) and Ligands

S. No.	PubChem ID	LibDock score	Interacting residues	Bond distance	Nature of bonding
1. 8468	3d	88.3553	3d: H27-A: LEU387:O	2.22204	Hydrogen Bond
			3d: H28–3d: O6	2.81497	Hydrogen Bond
			3d: H29-A: GLU353:OE2	2.4045	Hydrogen Bond
			A: ALA350–3d: C20	4.14062	Hydrophobic
			3d: C11-A: LEU387	3.81854	Hydrophobic
			3d: C12-A: LEU391	3.61711	Hydrophobic
			A: PHE404–3d: C12	5.31126	Hydrophobic
			3d-A: LEU384	5.21945	Hydrophobic
			3d-A: MET388	4.83611	Hydrophobic
			3d-A: ALA350	4.46021	Hydrophobic
			3d-A: LEU525	4.40826	Hydrophobic
2. 78016	3j	90.6015	A: THR347:HG1–3j: S5	3.08446	Hydrogen Bond
			3j: H28-A: ASP351:OD1	2.90345	Hydrogen Bond
			3j: H32-A: TRP383	2.94721	Hydrophobic
			A: MET343:SD–3j	4.48851	Other
			A: PHE404–3j	5.80151	Hydrophobic
			A: LEU346:C,O; THR347:N–3j	4.198	Hydrophobic
			A: ALA350–3j: C11	3.45513	Hydrophobic
			3j: C22-A: LEU391	3.6513	Hydrophobic
			A: TRP383–3j: C11	4.18593	Hydrophobic
			3j-A: ALA350	4.18697	Hydrophobic
			3j-A: LEU525	4.65689	Hydrophobic
			3j-A: LEU346	4.11322	Hydrophobic
			3j-A: ALA350	5.05707	Hydrophobic
3.	5-Fluorouracil (PubChem ID: 3385)	61.5565	3385:H10-A: GLU353:O	1.98323	Hydrogen Bond
			3385:H11-A: PRO325:O	2.04067	Hydrogen Bond
			A: GLU353:HA-3385:O3	2.77189	Hydrogen Bond
			3385-A: PRO324	4.22361	Hydrophobic

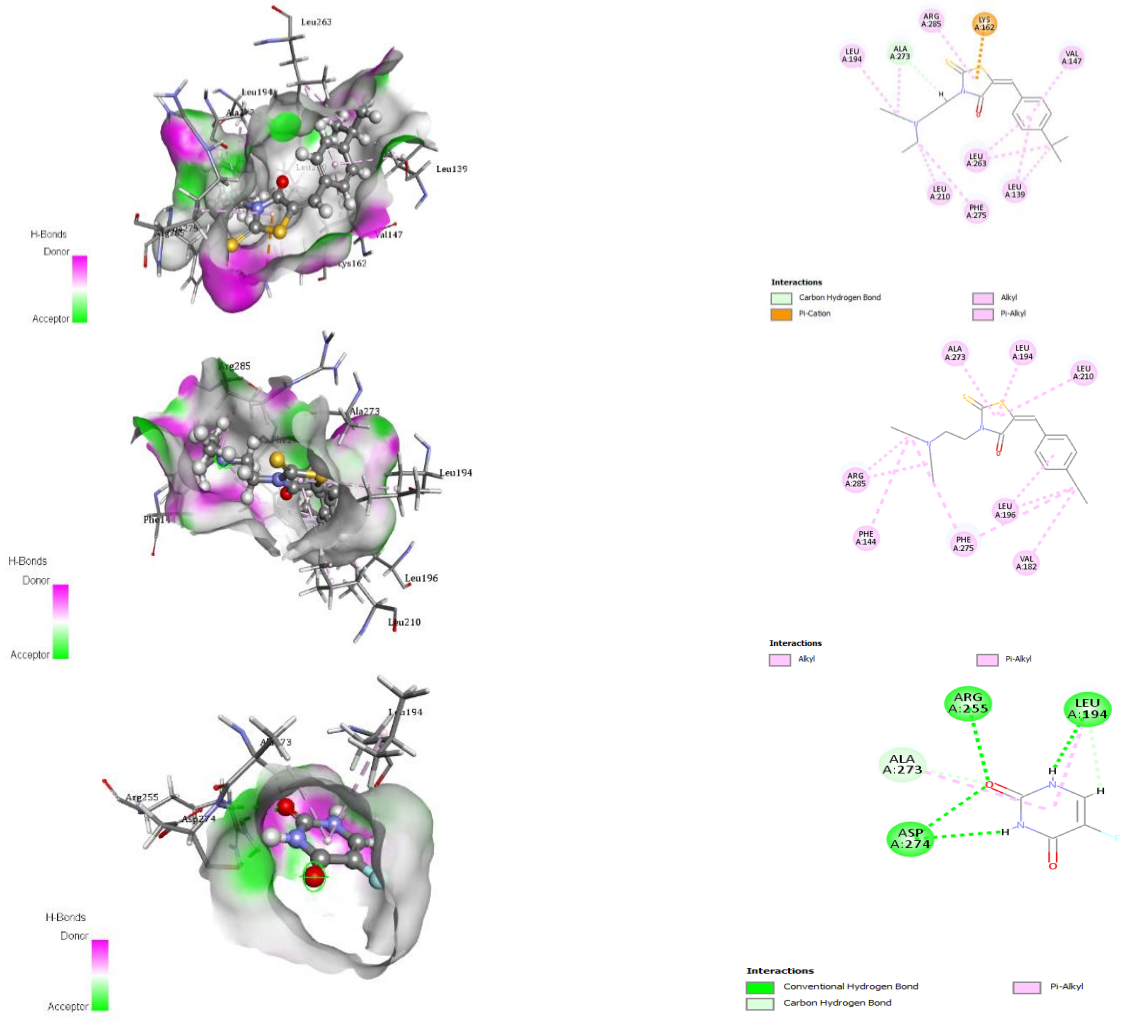


Fig. 12: Surface view and 2D diagram of Aurora Kinase (PDB ID: 4ZTR) with ligands 3j, 3d and 5-Fluorouracil

Table 11: Libdock score of ligands against aurora kinase (PDB ID: 4ZTR)

S. No.	PubChem ID	LibDock score
1.	3a	90.8378
2.	3b	95.2175
3.	3c	91.0061
4.	3d	101.101
5.	3e	95.7262
6.	3f	94.2389
7.	3g	91.4476
8.	3h	94.289
9.	3i	96.9217
10.	3j	100.83
11.	5-Fluorouracil (PubChem ID: 3385)	61.7531

Table 12: Interactions between aurora kinase (PDB ID: 4ZTR) and ligands

S. No.	PubChem ID	Lib dock score	Interacting residues	Bond distance	Nature of bonding
1. 8468	3j	100.83	3j: H26-A: ALA273:O	2.6165	Hydrogen Bond
			3j: H28-3j: O6	2.35338	Hydrogen Bond
			A: LYS162:NZ-3j	4.61104	Electrostatic
			A: ALA273-3j: C11	4.09951	Hydrophobic
			3j: C11-A: LEU194	4.66683	Hydrophobic
			3j: C12-A: LEU210	4.21396	Hydrophobic
			3j: C22-A: LEU139	3.92769	Hydrophobic
			3j: C22-A: LEU263	4.5939	Hydrophobic
			A: PHE275–3j: C12	4.99062	Hydrophobic
			3j-A: ARG285	4.89025	Hydrophobic
			3j-A: LEU139	5.41605	Hydrophobic
			3j-A: VAL147	4.03521	Hydrophobic
			3j-A: LEU263	5.22412	Hydrophobic
2. 78016	3d	101.101	3d: H27–3d: O6	2.49988	Hydrogen Bond
			3d: C11-A: ARG285	3.59196	Hydrophobic
			3d: C12-A: ARG285	3.37677	Hydrophobic
			3d: C20-A: VAL182	4.21815	Hydrophobic
			3d: C20-A: LEU196	4.26219	Hydrophobic
			A: PHE144–3d: C11	5.28838	Hydrophobic
			A: PHE275–3d: C11	5.00919	Hydrophobic
			A: PHE275–3d: C20	4.66244	Hydrophobic
			3d-A: LEU194	5.37504	Hydrophobic
			3d-A: LEU210	5.49187	Hydrophobic
			3d-A: ALA273	3.99519	Hydrophobic
			3d-A: LEU196	4.7084	Hydrophobic
3.	5-Fluorouracil (PubChem ID: 3385)	61.7531	A: ARG255:HH21-3385:O3	2.56561	Hydrogen Bond
			A: ASP274:HN-3385:O3	2.42453	Hydrogen Bond
			3385:H10-A: ASP274:O	2.17877	Hydrogen Bond
			3385:H11-A: LEU194:O	2.20125	Hydrogen Bond
			A: ALA273:HA-3385:O3	2.20819	Hydrogen Bond
			3385:H12-A: LEU194:O	2.71202	Hydrogen Bond
			3385-A: LEU194	4.95219	Hydrophobic
			3385-A: ALA273	4.94942	Hydrophobic

The docked complex of Aurora Kinase (PDB ID: 4ZTR) with top score ligands and standard ligands as shown in fig. 12 wereanalysed to study the interactions between the target and the ligand molecule. The interacting residues, nature of interacting bond and the bond distance are given in table 12.

Docking studies were done with progesterone and estrogen receptors which are involved in the progression and development of breast cancer. Aurora kinase comes under the category of thyrosine kinases. They play an initial role as a regulator of cell growth. Aurora kinase is over expressed in numerous tumours like brain, lung, bladder, colon, breast, head and neck cancers etc.

Rhodanine is a well-known pharmacophore in drug discovery process. A library of compounds was designed and 10 compounds were selected for synthesis. Among the selected 10 derivatives, compounds 3j and 3d showed good docking score for all the 3 targets. Different interactions, bond distance and nature of bonding of 3j and 3d along with standard drug 5-Fluorouracil were studied with 3 different targets.

Docking studies show that all the 10 compounds are energetically favorable in terms of docking score values which ranges from 80.75 to 98.62 for PDBID: IA28 and 77.45 to 90.60 for PDBID: 3ERT and 90.83 to 101.10 for PDBID: 4ZTR

ADME and toxicity prediction

The ligands with comparable scores to other molecules were subjected to predict ADME properties using the toxicity prediction module of the software. The predicted ADME properties are tabulated in table 13.

The drug likeness studies of the ligands were calculated by ADMET descriptors in Discovery studio 2021. The results of ADMET screening showed that all the compounds possess good human intestinal absorption and blood brain barrier (BBB) penetration at 99% confidence levels.

ADMET property of compounds based on the logarithm of the partition coefficient between n-octanol and water (AlogP), polar surface area (PSA), aqueous solubility, plasma protein binding, cytochrome P450 (CYP2D6) binding, blood brain barrier (BBB) penetration, hepatotoxicity, intestinal absorption and ames mutagenicity.

Table 13: ADMET prediction of the ligands

Compounds	Solubility	BBB	CYP2D6	Hepato-toxic	Abs	PBB	A Log P	PSA	Ames mutagenicity
3a	2	1	False	False	0	True	3.186	24.005	Non-Mutagen
3b	2	0	False	True	0	True	4.128	24.005	Non-Mutagen
3c	2	1	False	False	0	True	3.672	24.005	Non-Mutagen
3d	2	0	False	False	0	True	4.826	24.005	Non-Mutagen
3e	2	1	False	True	0	True	3.672	24.005	Non-Mutagen
3f	2	0	False	True	0	True	4.38	24.005	Non-Mutagen
3g	2	1	False	False	0	True	3.883	24.005	Non-Mutagen
3h	2	0	False	False	0	True	4.369	24.005	Non-Mutagen
3i	2	0	False	True	0	True	4.369	24.005	Non-Mutagen
3j	2	0	False	True	0	True	5.077	24.005	Non-Mutagen

Molecular docking helps to find the binding geometry of two interacting molecules with known structures. Docking predicts the preferred orientation of receptor and ligand to each other to form a stable complex. The receptors selected for the *in silico* studies include ER, PR and Aurora kinase. ER and PR receptors are widely aberrant in breast cancers specifically and contribute significantly to the hormonal resistance to therapy.

In the present study, the docking score of 3j and 3d with ER, PR and Aurora kinase are significantly higher than with other compounds. *In vitro* studies reveal that 3j is a good anti-oxidant, but did not exert cytotoxicity in MDA-MB-231 cells. MDA-MB-231 cells are ER and PR negative as well as triple negative, rather kind of resistant and aggressive cancer and this possibly explains between the non-correlations with the *in silico* and *in vitro* data. The compound 3j and 3d may be active in hormone sensitive cell lines like MCF-7 which are ER and PR positive which will be explored in further studies. Compound 3j was moderately active in HeLa cells, but sometimes HeLa cells also follow aurora kinase independent pathway.

Other compounds which showed good cytotoxic responses in the cell lines need to be further investigated with more protein receptors other than ER, PR and Aurora kinase since cancer cells often follow alternate pathways due to mutations and aberrations which occur continuously. Further mechanistic studies are warranted to understand the molecular mechanism of the compounds in the interaction of genes in more sensitive breast and cervical cancer cell lines.

CONCLUSION

Ten novel rhodanine derivatives were evaluated for their antioxidant and anticancer activity. Antioxidant activity of the test compounds was done by DPPH radical scavenging method. The percentage cytotoxic activity and IC_{50} values of all the synthesized compounds were predicted. Compound 3j was found to have potent antioxidant activity with an IC_{50} value of 31.21µg/ml very close to the IC_{50} value of ascorbic acid which was used as a positive control for the study. The anticancer activity of 5-benzylidene substituted rhodanine derivatives in HeLa and MDAMB-231 cell lines was investigated. Out of 10 compounds, 3a, 3b, 3g and 3h showed good anticancer inhibitory activity against MDAMB-231 cells at 1000 and 500 µg/ml concentrations. The compounds 3e, 3f and 3j showed moderate cytotoxic responses in the breast cancer cells.3b,3f, 3g and 3h showed prominent cytotoxicity at 1000 and 500 µg/ml concentrations on HeLa cells. The compounds 3a, 3c, 3d, 3e and 3j showed moderate cytotoxic responses on the cervical cancer cells. The overall biological and docking results reveals that the compound 3j acts as an excellent class of novel anticancer agents that may lead to the development of more potent anticancer drugs in the future.

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Nil

AUTHORS CONTRIBUTIONS

Cici Mathew performed the experimental work and wrote the manuscript. Nandlal helped in drafting and revising the manuscript. Lakshmi S performed the *in vitro* antioxidant and anticancer evaluation and analysed the data statistically. Aswathy T R performed molecular modeling studies. Joyemma Varkey supervised the work and interpreted the results. All authors contributed accordingly and approved the final manuscript.

CONFLICT OF INTERESTS

Declared none

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