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Computational Evaluation and *in vitro* Antiproliferative Study of Novel Substituted Rhodanine Derivatives

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Abstract

Aims: The present study aims to carryout molecular modelling with 3 different anticancer targets and *in vitro* evaluation of a series of 8 novel rhodanine derivatives. **Methods:** Various softwares like Chemsketch, Molinspiration, docking module LibDock using Discovery Studio 2021 were used for computational evaluation of the novel rhodanine derivatives. *In vitro* anticancer activity was evaluated against breast cancer (MDAMB 231) and cervical cancer (HeLa) cell lines. The cytotoxicity was evaluated by MTT assay method and IC_{50} was calculated. **Results:** Out of the 8 derivatives, compound 2h and 2e showed potent cytotoxicity. They also showed good binding affinity with different anticancer targets. Compound 2h showed IC_{50} < 62.5 µg/ml for both cell lines. The anticancer potential was confirmed by its good docking score and binding affinity of different anticancer targets were carried out by LibDock using Discovery Studio 2021. **Conclusion:** Novel synthesized rhodanine derivatives which could be developed into potent anticancer agents in future.

Keywords

Cytotoxicity, MTT assay, Molecular docking, Rhodanine

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INTRODUCTION:

Cancer is considered to be one among the most fatal health problems in the world. In the last few years chemotherapy dominates as an important choice for cancer treatment apart from the use of radiotherapy and surgery. Thus, considerable efforts have been focused on the research for novel anticancer agents. Moreover, since resistance to therapy is an important problem faced in cancer treatment, the search for newer compounds with anticancer effects is the need of the hour.

Heterocyclic structures such as thiazolidinone ring play a major role in developing novel class of anticancer agents with a broad spectrum of cytotoxicity against many human cancer cells [1-6]. Substituted 2-thioxo-4-thiazolidinones as rhodanine derivatives are known to exhibit wide range of pharmacological activities. Their anticancer effects

have been associated with many targets, such as inhibitors of translation initiation, inhibitors of interaction between BH_3 domain and Bcl-XL, inhibitors of phosphatase of regenerating liver 3 (PRL-3), inhibitors of JNK-stimulating phosphatase-1 (JSP-1), and inhibitors of sphingosine kinase (SK) [7]. Recently, substituted rhodanine derivatives were investigated for tumor aggregation inhibitor [8-10]. For example, GSK1059615 is a novel, ATP-competitive, and thiazolidinedione inhibitor of PI3Kα [11]. BA Rao et al, has reported the synthesis and anticancer activity of some rhodanine derivatives attached with 2-chloropyridine scaffold [12]. The anti-proliferative potential of 5-substituted imidazolidine-2,4-diones (hydantoins) [13] and 5-arylidene-2,4-imidazolidinediones [14] are reported to be related to the inhibition of EGFR-kinase epidermal growth factor receptor. Rhodanine-3-acetic acid derivatives are also found to be associated with anticancer activity [15-16].

In continuation of our work on synthesis and antimicrobial studies of new derivatives having secondary amino substituted alkyl fragment at N-3 position of rhodanine [17], we report herein an attempt to carry out their antiproliferative activity against breast and cervical cancer cells.

Additionally, molecular docking is used to determine the affinity and the activity of the analogues to three selected protein targets of interest in terms of docking score using Discovery Studio 2021 software. The main aim of docking is to attain the conformation of protein and the ligands and to optimize the absolute orientation between the protein and ligands. In this paper, we have made docking studies for eight new chemical entity (NCE), 3-(dialkylamino) alkyl -2-thioxothiazolidin-4-one against the proteins **ERα** (Estrogen receptor), **PR** (Progesterone receptor) and **Aurora Kinase**.

Molecules with better docking score were subjected to analysis for cytotoxic activity by *in vitro* by MTT assay on breast cancer cell lines (MDAMB 231) and cervical cancer (HeLa) cell lines. Breast cancer chemotherapy is marked by targeting the function of receptors such as ERα, PR and EGFR (epidermal growth factor receptor). Predominantly ERα, PR and Aurora Kinase has been implicated in breast cancer tumorigenesis and progression. Aurora Kinase A overexpression is reported to be crucial for survival of HPV transformed cervical cancer cells. Therefore, the role of these target receptors i.e., **Erα, PR** and **Aurora Kinase** are studied by *in silico* analysis.

MATERIALS AND METHODS:

Chemistry

A new chemical entity (NCE), 3-(dialkylamino) alkyl-2-thioxothiazolidin-4-one (Rhodanine derivatives) (Figure 1) were prepared previously [17] by the reaction of substituted primary amine (I), carbon disulphide and ethylbromoacetate in acetonitrile. Compounds were characterized by melting points, IR and NMR Spectra and were purified by column chromatography to get excellent yield [17].

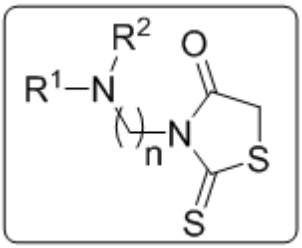


Figure 1: 3-(dialkylamino) alkyl -2-thioxothiazolidin-4-one (Rhodanine derivatives)

Computational evaluation

Computational techniques provide outstanding tools for contributing experimental findings and understanding the reactivity of various molecular structures. The structures of all the synthesized rhodanine derivatives were modelled using various softwares such as Chemsketch, Molinspiration and Discovery Studio 2021

Molecular docking studies

The PDBIDs of proteins under each protein group were considered as targets for the molecular docking study; Progesterone receptor - 1A28, Estrogen receptor α - 3ERT and Aurora Kinase-4ZTR.Docking study has been carried out using software and Target proteins (1A28, 3ERTand 4ZTR) were downloaded from protein data bank.

Preparation of ligands

Structures of the biochemical compounds were obtained from PubChem A [18] 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 were 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 were retrieved from Research Collaboratory for Structural Bioinformatics (RCSB)

Protein Data Bank [19]. 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 complexed ligands were manually removed. The Protein Prepare protocol in Discovery studio v.21 was used to insert the missing atoms, missing loop regions, deleting alternate conformations, removing waters, standardizing atom names and protonating 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 'define and edit binding site' option in the software based on the PDB site records. For the molecular interaction study LibDock protocol [20], 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 [21], which are essential for drug-like pharmacokinetic profile in rational drug design [22]. The druggability of the ligand molecules was also predicted by ADMET analysis which compute the absorption, distribution, metabolism, excretion, and toxicity potential of a pharmaceutical compound within an organism [23]. The 2D structures of the molecules were subject to analysis of solubility, intestinal absorption, hepatotoxicity, plasma protein binding ability, blood - brain barrier (BBB) penetration, cytochrome P450 inhibition and AMES mutagenicity using ADMET descriptors in Discovery studio v.21.

***In vitro* anticancer studies**

Reagents and Cell Line: 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) were used for the study. MDAMB-231 (breast carcinoma) and HeLa (human cervical cancer) were procured from National Centre for Cell Sciences, Pune, India. The cells were cultured and maintained in DMEM medium containing 10% fetal bovine serum at 37°C, 5% CO_2 and saturated humidity.

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

$$\% \text{ of Cytotoxicity} = 100 - \frac{\text{Treated cells OD}}{\text{Control cells OD}} \times 100$$

$IC_{50}$was calculated using Easyplot software.

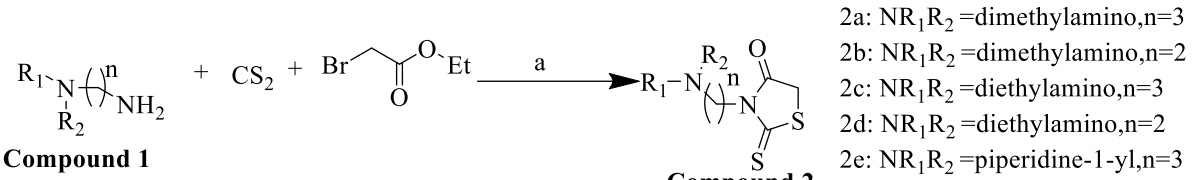
Microscopy: MDAMB-231and HeLa cells were treated with the compounds at different concentrations for 48 and 72 hours and assessed for morphological changes using phase-contrast microscopy (Magnus, Magcam, DC5). The morphology of the untreated and treated cells was captured and compared for cytotoxic effects.

Statistical analysis: Data was expressed as mean of three independent experiments for the cytotoxicity studies.

RESULTS AND DISCUSSION

Chemistry

Synthesis of a series of new derivatives having secondary amino substituted alkyl fragment at N-3 position of rhodanine (Scheme 1) were published previously [17]. Different Rhodanine derivatives (2a-2h) synthesized are given in Table 1.

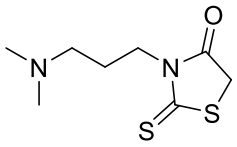
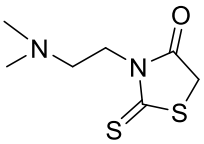
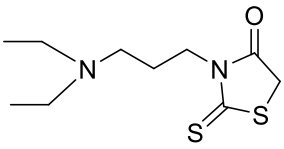
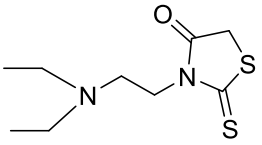
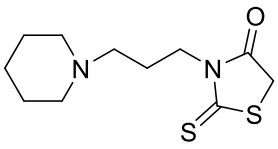
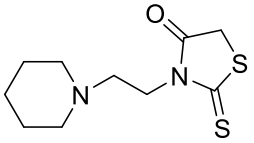
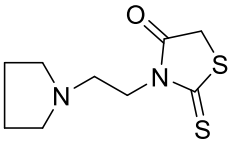


2a: NR_1R_2 =dimethylamino,n=3
2b: NR_1R_2 =dimethylamino,n=2
2c: NR_1R_2 =diethylamino,n=3
2d: NR_1R_2 =diethylamino,n=2
2e: NR_1R_2 =piperidine-1-yl,n=3
2f: NR_1R_2 =piperidine-1-yl,n=2
2g: NR_1R_2 =pyrrolidine-1-yl,n=2
2h: NR_1R_2 =azepan-1-yl,n=3

Scheme 1: (a) = Acetonitrile, room temperature

Computational evaluation

Molecular descriptor analysis and drug likeness of Rhodanine derivatives (2a-2h) synthesized were shown in Table 2, 3 and 4. Molar volume was calculated based on group contributors and it also analyses the transport characteristics of a molecule mainly gastrointestinal absorption (HIA) or Blood brain barrier (BBB). It was found that the calculated values of molar volumes were within the range (Table 2). 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 molecular weight less than 500 Daltons and Log P value ranges from 0.49-2.17 (Table 3).

Compound code	Structure	Chemical Name	Molecular formula
2a		**3-(3-dimethylamino) propyl)-2-thioxothiazolidin-4-one**	$C_8H_{14}N_2OS_2$
2b		**3-(2-(dimethylamino) ethyl)-2-thioxothiazolidin-4-one**	$C_7H_{12}N_2OS_2$
2c		**3-(3-diethylamino) propyl)-2-thioxothiazolidin-4-one**	$C_{10}H_{18}N_2OS_2$
2d		**3-(2-(diethylamino)ethyl)-2-thioxothiazolidin-4-one**	$C_9H_{16}N_2OS_2$
2e		**3-(3-(piperidin-1-yl) propyl)-2-thioxothiazolidin-4-one**	$C_{11}H_{18}N_2OS_2$
2f		**3-(2-(piperidine-1-yl) ethyl)-2-thioxothiazolidin-4-one**	$C_{10}H_{16}N_2OS_2$
2g		**3-(2-(pyrrolidine-1-yl) ethyl)-2-thioxothiazolidin-4-one**	$C_9H_{14}N_2OS_2$

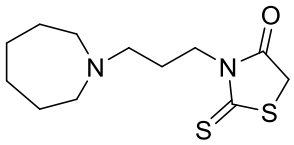
2h		**3-(3-(azepan-1-yl) propyl)-2-thioxothiazolidin-4-one**	$C_{12}H_{20}N_2OS_2$

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

Compound Code	Molar Refractivity	Molar volume cm^3	Parachor (cm^3)	Surface Tension(dyne/cm)
2 a	60.04±0.4	172.0±5.0	477.7±6.0	59.4±5.0
2 b	55.41±0.4	155.5±5.0	437.6±6.0	62.5±5.0
2 c	69.30±0.4	204.8±5.0	557.8±6.0	55.0±5.0
2 d	64.67±0.4	188.4±5.0	517.7±6.0	57.0±5.0
2 e	71.90±0.4	202.3±5.0	568.4±6.0	62.2±5.0
2 f	67.27±0.4	186.0±5.0	528.3±6.0	65.1±5.0
2 g	62.63±0.4	169.6±5.0	488.3±6.0	68.6±5.0
2 h	76.53±0.4	218.6±5.0	608.5±6.0	59.9±5.0

Table 2: Molecular descriptor analysis by ACD Lab Chemsketch 12.0

Compound Code	mi Log P	Molecular weight	nON	nOHNH	N rotb	n violation
2a	0.76	218.35	3	0	4	0
2b	0.49	204.32	3	0	3	0
2c	1.51	246.40	3	0	6	0
2d	1.24	232.37	3	0	5	0
2e	1.67	258.41	3	0	4	0
2f	1.39	244.38	3	0	3	0
2g	0.89	230.36	3	0	3	0
2h	2.17	272.44	3	0	4	0

Table 3: Analysis of Lipinski rule of five using molinspiration

Compound code	GPCR Ligand	Ion exchange modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
2a	-1.09	-0.92	-2.02	-2.26	-1.73	-0.77
2b	-1.32	-1.10	-2.27	-2.51	-1.97	-0.92
2c	-0.82	-0.76	-1.70	-1.79	-1.40	-0.60
2d	-1.01	-0.90	-1.90	-1.98	-1.60	-0.72
2e	-0.66	-0.63	-1.49	-1.56	-1.16	-0.46
2f	-0.78	-0.71	-1.66	-1.72	-1.30	-0.54
2g	-0.89	-0.79	-1.79	-1.85	-1.36	-0.67
2h	-0.56	-0.57	-1.34	-1.40	-1.02	-0.30

Table 4: Analysis of drug-likeness of derivatives

Molecular Docking studies

Eight compounds were selected for the study and the 3 D structures of standard drug 5-Flurouracil was downloaded from PubChem database in .sdf format. These ligands were prepared to generate possible conformers and tautomers. 3D structure of proteins was procured from PDB. The protein structures were cleaned (water molecules and other heteroatoms removed), prepared and minimized before docking. Docking module LibDock using Discovery Studio 2021 was used to study interaction between the Protein and ligand molecules. The binding site of the protein defined, and the docking performed. The binding energy, LibDock scores, nature of bonding and bond length of the docked ligands were estimated.

Molecular Docking with Anti-cancer Targets

Docking with Progesterone receptor (PDB ID: 1A28)

The three-dimensional structure of human progesterone receptor ligand-binding domain was downloaded from PDB database with PDB ID: 1A28 with crystallographic resolution 1.80 A^0 (Figure 2). The protein consists of two polypeptide chain A and B. The protein chain consists of total 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.

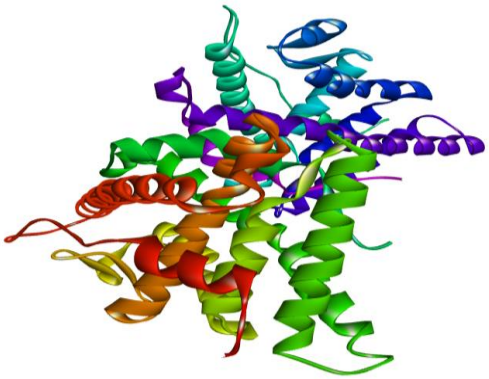


Figure 2: Three-dimensional structure of 1A28

90 poses of each selected ligands in the docked complexes were generated. The interacting molecular complexes among these having high LibDock score, and maximum number of hydrogen bonds and active residues were selected. All the 8 compounds were showed good interaction with progesterone receptor comparison with the standard drug 5-Fluorouracil (PubChem ID: 3385). Table 5 shows the Libdock score of best conformers of the ligands.

Sl. No.	PubChem ID	LibDock Score
1.	2a	71.5281
2.	2b	66.4112
3.	2c	75.2353
4.	2d	73.6294
5.	2e	80.7950
6.	2f	81.9398
7.	2g	72.6232
8.	2h	87.7934
9.	5-Fluorouracil	59.6716

Table 5: Libdock score of Ligands against human progesterone receptor (PDB ID: 1A28)

The ligands 2e and 2h showed top binding affinity compared with standard drug molecule 5-Fluorouracil with Libdock score 80.7951, 87.7934 and 1 and 5 hydrogen bond interactions respectively. The docked complex of progesterone receptor (PDB ID: 1A28) with top score ligands and Standard ligand as shown in Figure 3 was analysed to study non-bond interactions between the target and the ligand molecule. The interacting residues, nature of interacting bond and the bond distance are given in Table 6. The results revealed that the two ligands bind the same residues as those which bind with the standard drug. Standard drug 5-Fluorouracil showed a LibDock score of 59.6716 with six hydrogen bonds (GLN725, ARG766, VAL760 and PHE778). 2e and 2h also showed good hydrogen bond interactions with same interacting amino acid residues.

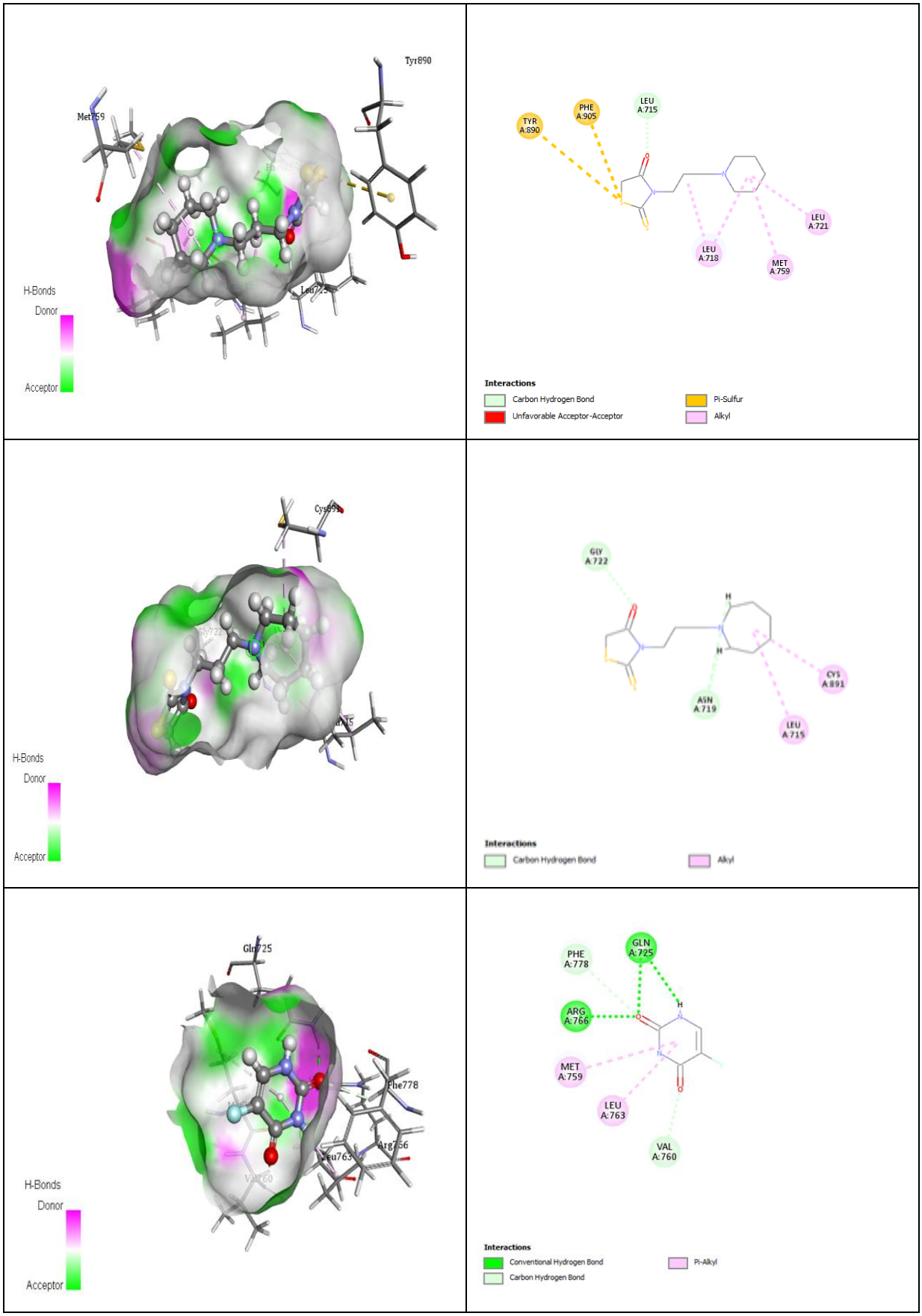


Figure 3: Surface view and 2D diagram of human progesterone receptor with ligands 2e, 2h and 5-Fluorouracil

Sl. No.	PubChem ID	LibDock Score	Interacting Residues	Bond Distance	Nature of Bonding
1.	2e	80.795	A:LEU715:HA - 2e:O7	3.05751	Hydrogen Bond
			2e:S5 - A:TYR890	4.99611	Other
			2e:S5 - A:PHE905	5.43846	Other
			A:LEU718 - 2e	5.13846	Hydrophobic
			A:LEU721 - 2e	5.15679	Hydrophobic
			A:MET759 - 2e	5.34987	Hydrophobic
			2e - A:LEU718	4.05067	Hydrophobic
2.	2h	87.7934	A:GLY722:HA2 - 2h:O7	3.07547	Hydrogen Bond
			2h:H35 - A:ASN719:OD1	1.87911	Hydrogen Bond
			2h:H37 - A:ASN719:OD1	2.96042	Hydrogen Bond
			A:LEU715 - 2h	5.29091	Hydrophobic
			A:CYS891 - 2h	5.13289	Hydrophobic
3.	5-Fluorouracil	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

Table 6: Interactions between human progesterone receptor and ligands

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^0 (Figure 4). 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 ligands in the docked complexes were generated. Ligands 2e, 2f and 2h showed top binding affinity. Table 7 shows the Libdock score of best conformers of the ligands.

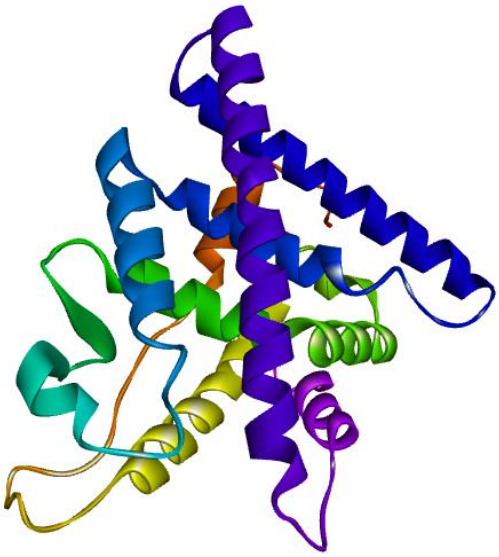


Figure 4: Crystallographic structure of 3ERT

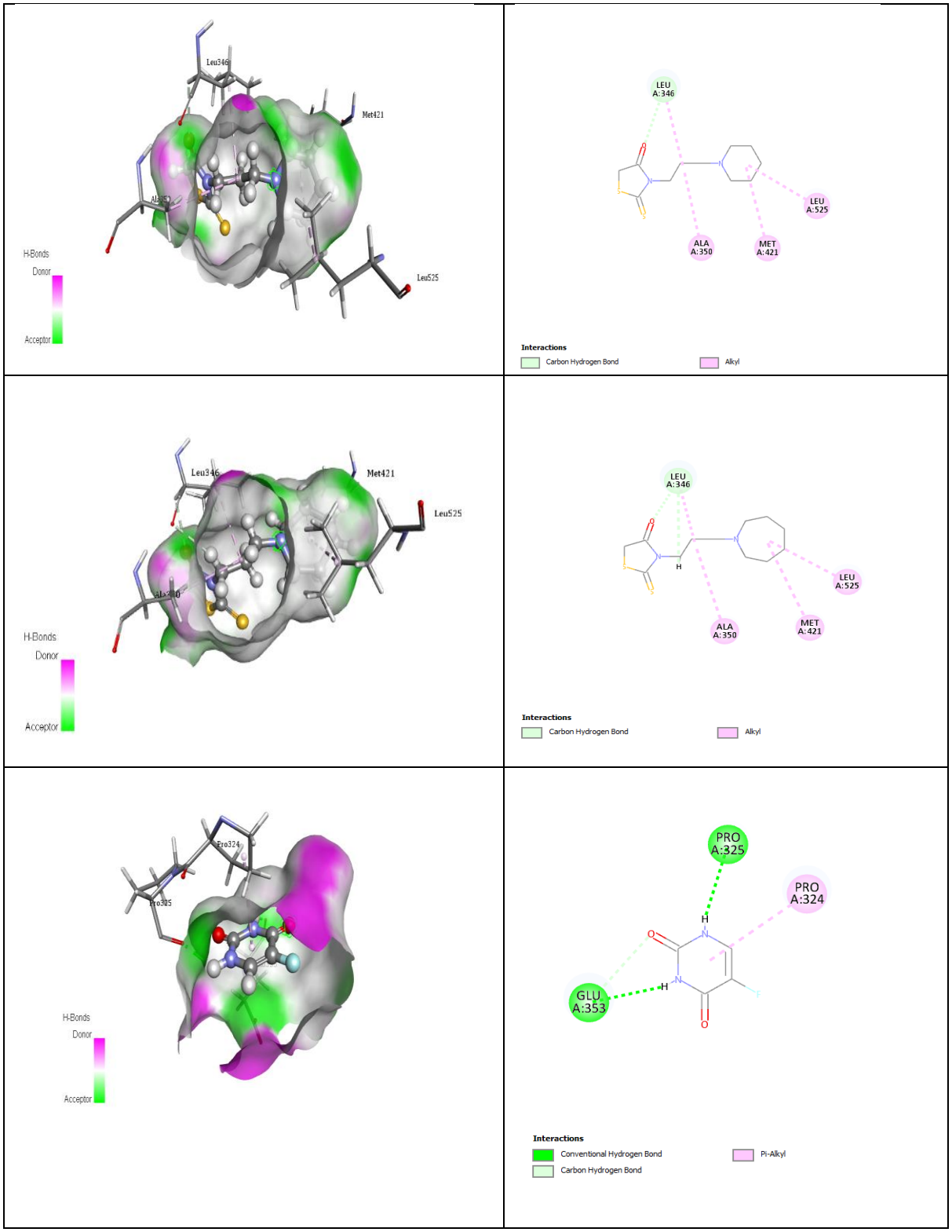


Figure 5: Surface view and 2D diagram of Estrogen receptor alpha (PDB ID: 3ERT) with ligands 2e, 2h and 5-Fluorouracil

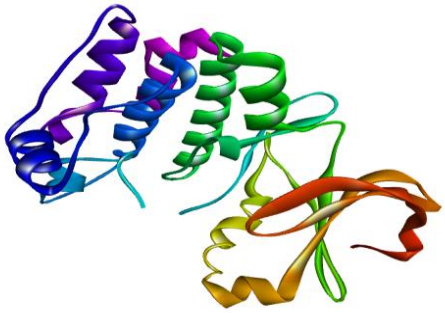


Figure 6: Crystallographic structure of 4ZTR

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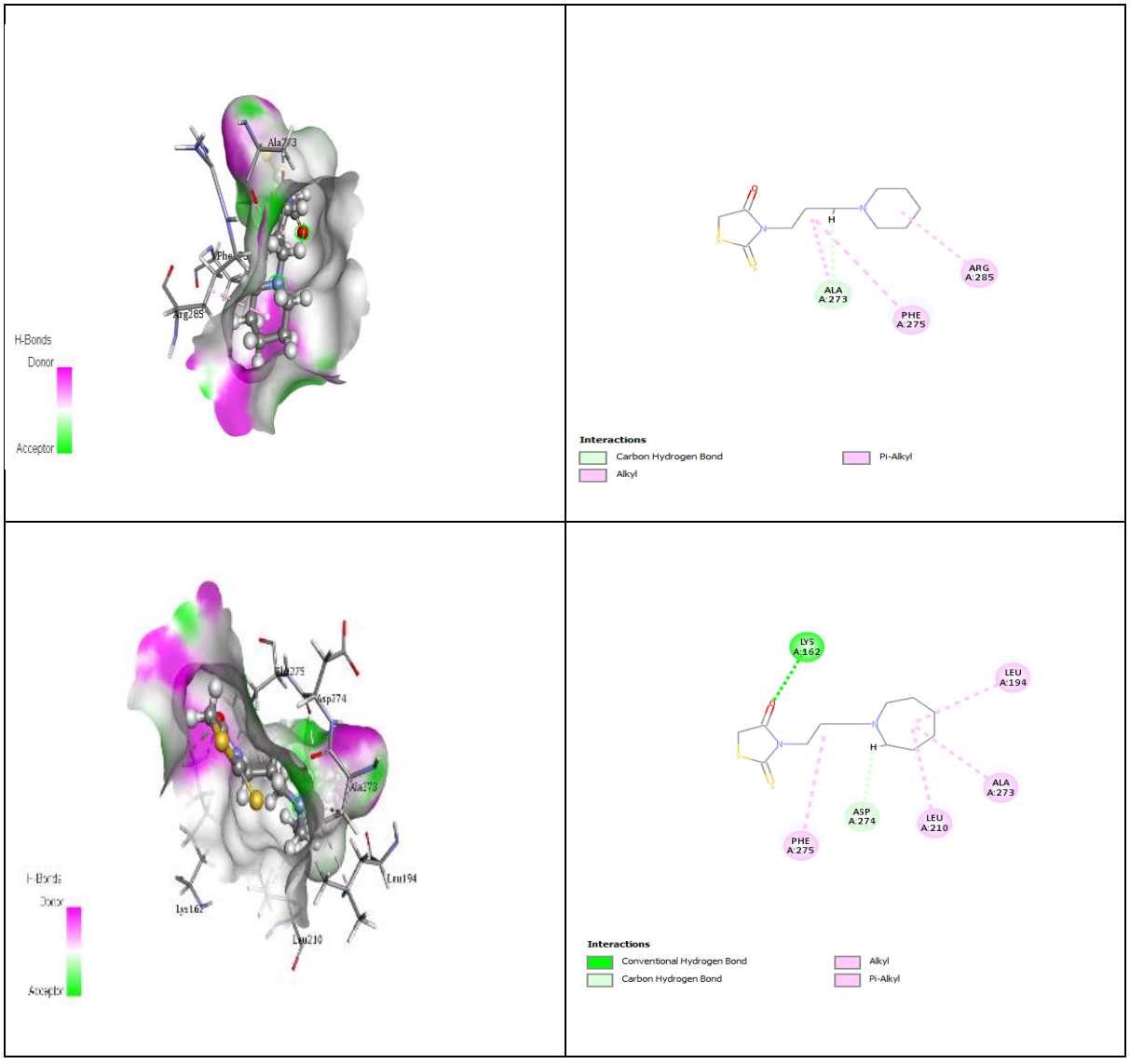

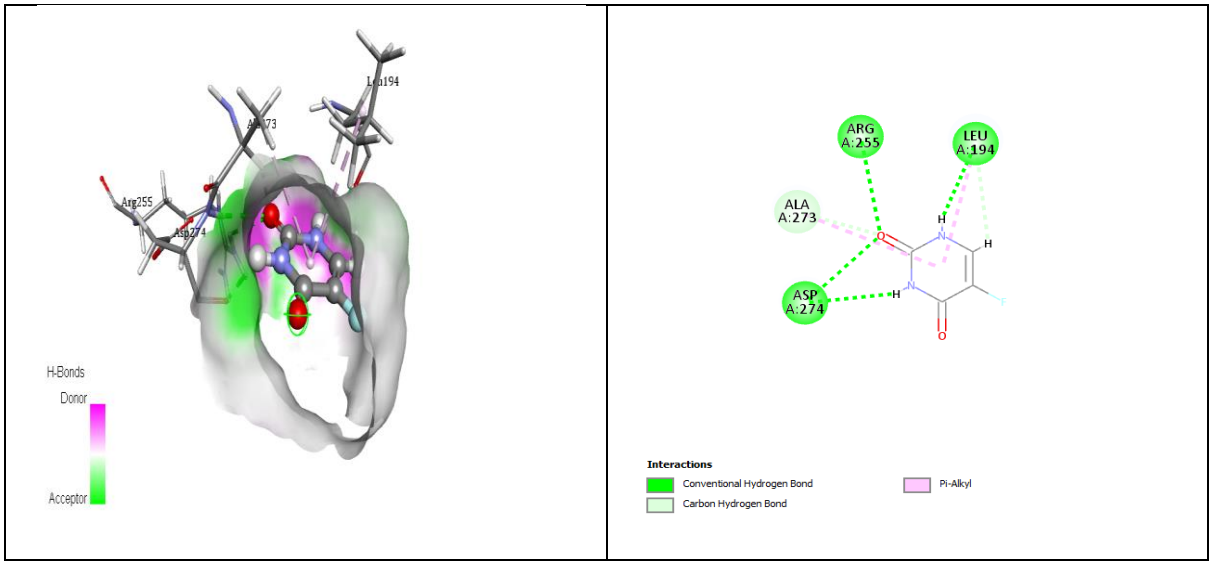

Figure 7: Surface view and 2D diagram of Aurora Kinase from (PDB ID: 4ZTR) with ligands 2e, 2h and 5-Fluorouracil

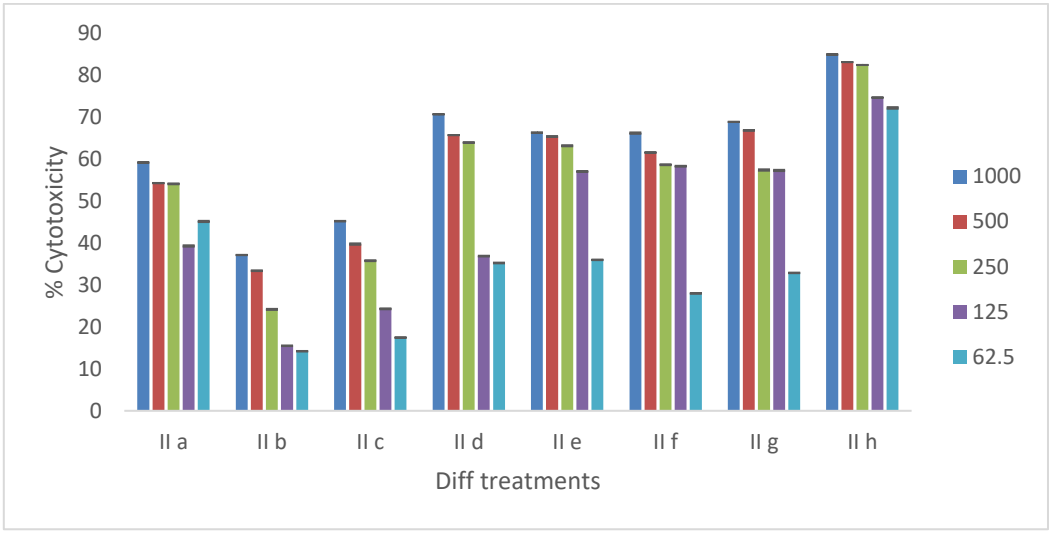

Figure 8: Percentage (%) of cytotoxicity induced by the compounds (2a-2h) after 48 hours on HeLa cells at different concentration.

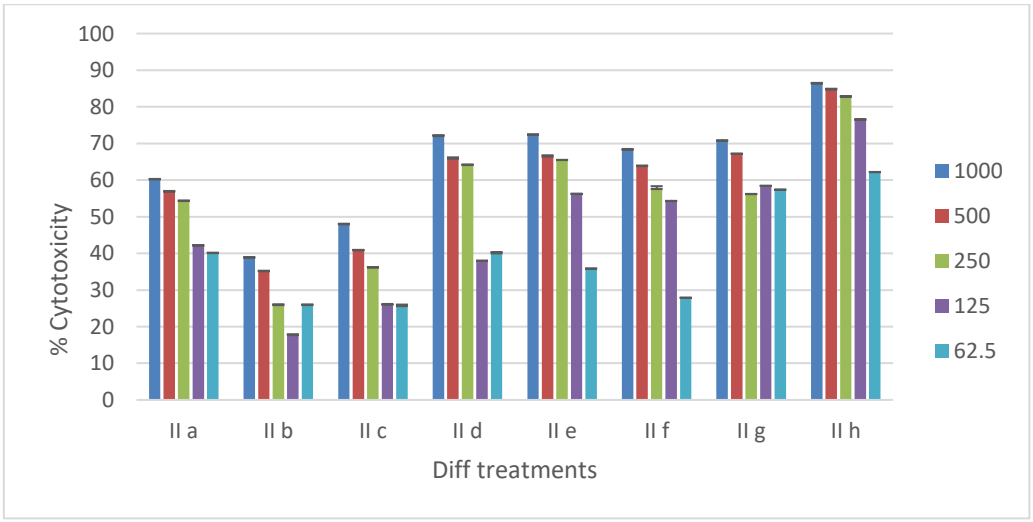

Figure 9: Percentage (%) of cytotoxicity induced by the compounds (2a-2h) after 72 hours on HeLa cells at different concentration.

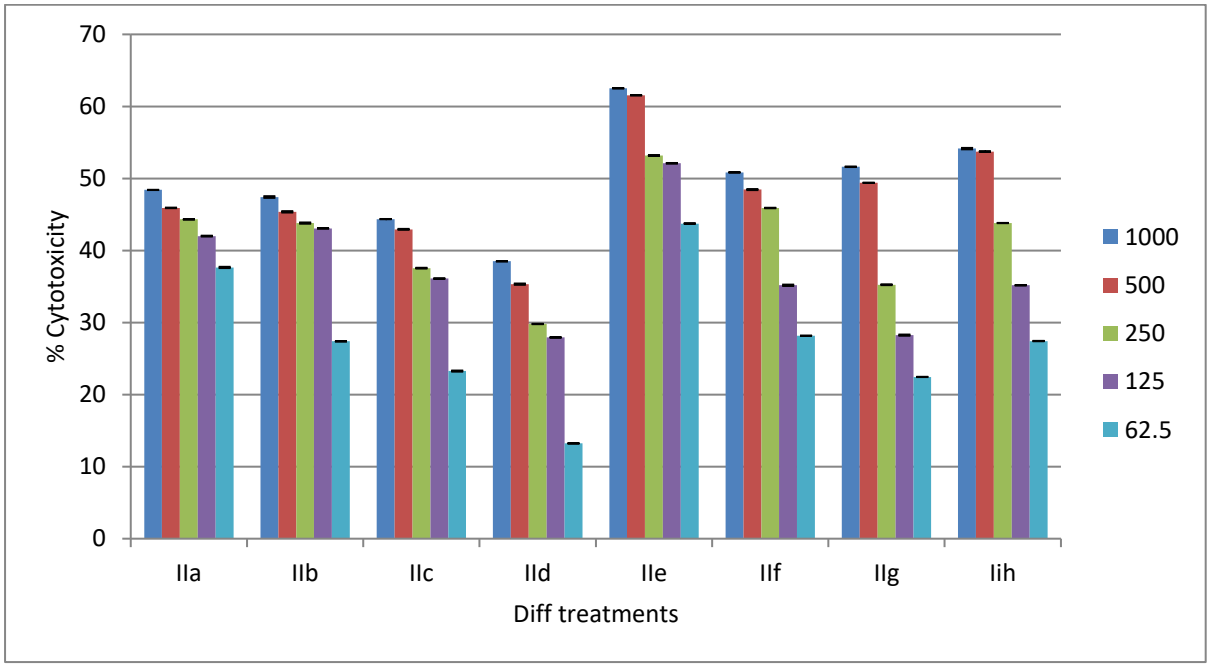

Figure 10: Percentage (%) of cytotoxicity induced by the compounds (2a-2h) after 48 hours on MDAMB-231 cells at different concentration.

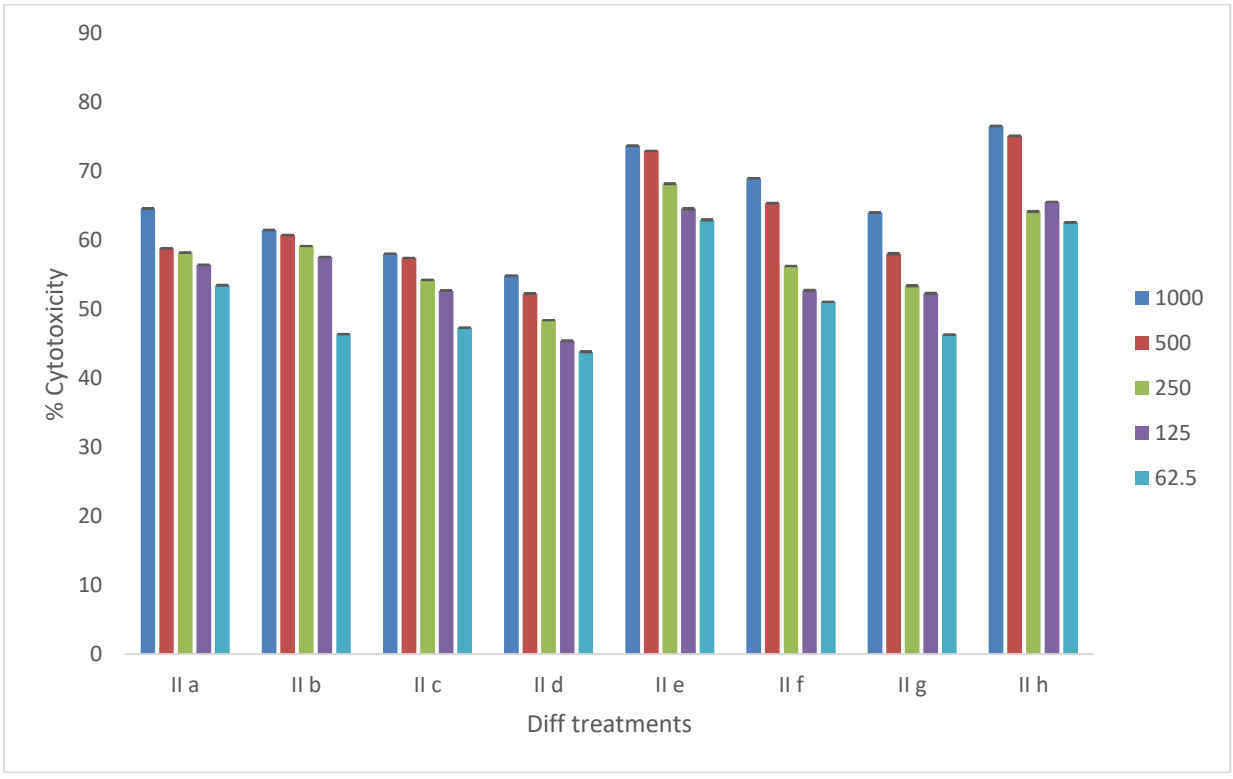

Figure 11: Percentage (%) of cytotoxicity induced by the compounds (2a-2h) after 72 hours on MDAMB-231 cells at different concentration.

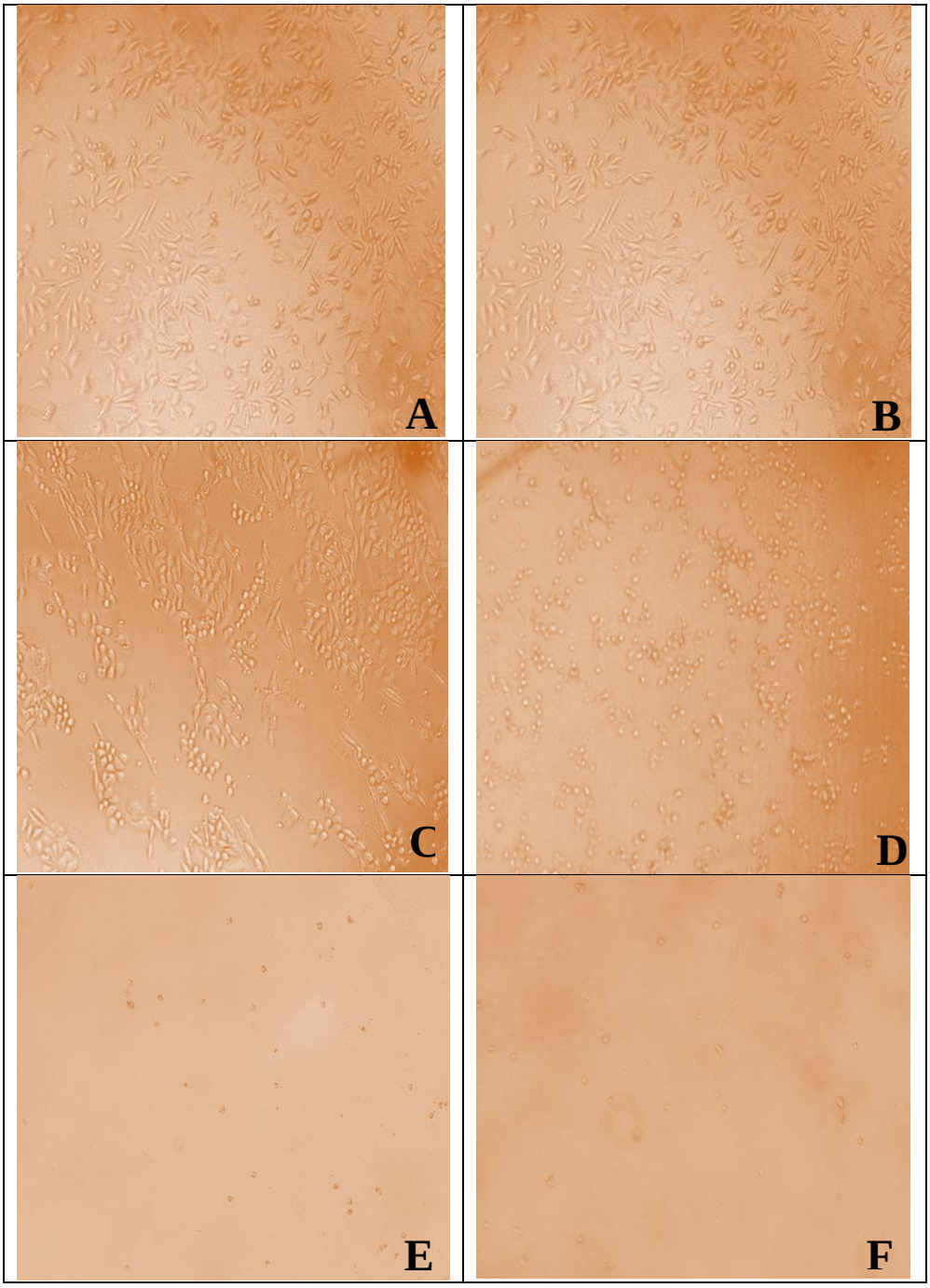

Figure 12: Morphology of (A) Untreated MDAMB – 231 cells (B) Treated MDAMB – 231 cells with compound 2h (C) Untreated HeLa cells with compound 2h (B) Treated HeLa cells (E) MDAMB – 231 cells treated with Doxorubicin (F) HeLa cells treated with Cisplatin

Sl. No.	PubChem ID	LibDock Score
1.	2a	78.3945
2.	2b	73.6186
3.	2c	78.4313
4.	2d	78.5955
5.	2e	78.5103
6.	2f	79.2683
7.	2g	77.4720

8.	2h	84.4972
9.	5-Fluorouracil	61.5565

Table 7: Libdock score of Ligands against Estrogen receptor alpha

The docked complex of Estrogen receptor (PDB ID: 3ERT) with top score ligands and Standard ligands were analysed to study non-bond interactions between the target and the ligand molecule (Figure 5). The interacting residues, nature of interacting bond and the bond distance are given in Table 8.

Sl. No.	PubChem ID	LibDock Score	Interacting Residues	Bond Distance	Nature of Bonding
1.	2e	78.5103	A:LEU346:HA - 2e:O7	2.85582	Hydrogen Bond
			A:ALA350 - 2e	5.0435	Hydrophobic
			A:MET421 - 2e	5.37602	Hydrophobic
			A:LEU525 - 2e	4.67943	Hydrophobic
			2e - A:LEU346	4.33377	Hydrophobic
2.	2f	79.2683	A:MET357:HA - 2f:O7	1.91041	Hydrogen Bond
			2f:H18 - A:GLU353:O	2.42585	Hydrogen Bond
			2f:H19 - A:GLU353:OE1	2.75914	Hydrogen Bond
			2f:H24 - A:GLU353:OE1	2.69633	Hydrogen Bond
			2f:H25 - A:PRO325:O	1.50455	Hydrogen Bond
			A:PRO324 - 2f	5.08328	Hydrophobic
			A:ILE326 – 2f	4.67315	Hydrophobic
3.	2h	84.4972	A:LEU346:HA - 2h:O7	2.81705	Hydrogen Bond
			2h:H21 - A:LEU346:O	2.74123	Hydrogen Bond
			A:ALA350 - 2h	4.71429	Hydrophobic
			A:MET421 - 2h	5.32784	Hydrophobic
			A:LEU525 - 2h	4.78233	Hydrophobic
			2h - A:LEU346	4.30564	Hydrophobic
4.	5-Fluorouracil	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

Table 8: Interactions between Estrogen receptor alpha and Ligands

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 2.85 A^0 (Figure 6). 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 standardised ligand molecules was selected as the binding site.

90 poses of each selected ligands in the docked complexes were generated. Ligands 2e, and 2h showed top binding affinity. Table 9 shows the Libdock score of best conformers of the ligands.

The docked complex of Aurora Kinase (PDB ID: 4ZTR) with top score ligands and standard ligands as shown in Figure 7 was analysed to study non-bond interactions between the target and the ligand molecule. The interacting residues, nature of interacting bond and the bond distance are given in Table 10.

Sl. No.	PubChem ID	LibDock Score
1.	2a	73.5186
2.	2b	69.3723
3.	2c	83.5832
4.	2d	75.9956
5.	2e	89.5905
6.	2f	83.1887
7.	2g	80.7101
8.	2h	92.1274
9.	5-Fluorouracil	61.7531

Table 9: Libdock score of Ligands against Aurora Kinase

Sl. No.	PubChem ID	LibDock Score	Interacting Residues	Bond Distance	Nature of Bonding
1.	2e	89.5905	2e:H23 - A:ALA273:O	2.41346	Hydrogen Bond
			A:ALA273 - 2e	4.64472	Hydrophobic
			A:ARG285 - 2e	4.00193	Hydrophobic
			A:PHE275 - 2e	5.45348	Hydrophobic
2.	2h	92.1274	A:LYS162:HZ2 - 2h:O7	2.56919	Hydrogen Bond
			A:LYS162:HZ3 - 2h:O7	2.26212	Hydrogen Bond
			2h:H35 - A:ASP274:O	2.29204	Hydrogen Bond
			A:LEU194 - 2h	4.73122	Hydrophobic
			A:LEU210 - 2h	5.11239	Hydrophobic
			A:ALA273 - 2h	5.20478	Hydrophobic
			A:PHE275 - 2h	4.69426	Hydrophobic
3.	5-Fluorouracil	61.7531	A:ARG255:HH21 - 3385:O3	2.56561	Hydrogen Bond
			A:ASP274:HN - 3385:O3		
			3385:H10 - A:ASP274:O	2.42453	Hydrogen Bond
			3385:H11 - A:LEU194:O	2.17877	Hydrogen Bond
			A:ALA273:HA - 3385:O3	2.20125	Hydrogen Bond
			3385:H12 - A:LEU194:O	2.20819	Hydrogen Bond
			3385 - A:LEU194	2.71202	Hydrogen Bond
			3385 - A:ALA273	4.95219	Hydrophobic
				4.94942	Hydrophobic

Table 10: Interactions between Aurora Kinase and ligands

ADME and Toxicity Prediction

The drug likeness studies of the ligands were calculated by ADMET descriptors in Discovery studio 2021.The results of ADMET screening (Table 11) 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.

Sl No	Comp-ound	Solu-bility	BBB	CYP 2D6	Hepato-toxic	Absor-ption	PBB	ALogP	PSA	AMES Mutagenicity
1	2a	4	2	False	False	0	False	1.351	24.005	Non-Mutagen
2	2b	4	2	False	False	0	False	1.289	24.005	Non-Mutagen
3	2c	3	1	False	False	0	False	2.049	24.005	Non-Mutagen
4	2d	3	1	False	False	0	False	1.986	24.005	Non-Mutagen
5	2e	3	1	False	False	0	False	2.268	24.005	Non-Mutagen
6	2f	3	1	False	False	0	False	2.206	24.005	Non-Mutagen
7	2g	3	1	False	False	0	False	1.750	24.005	Non-Mutagen
8	2h	3	1	False	False	0	True	2.662	24.005	Non-Mutagen

Table 11: ADMET prediction of the ligands

	IC 50(µg/ml)			
Compound	MDAMB-231		HeLa cells	
	48 hrs	**72hrs**	**48 hrs**	**72hrs**
2a	>1000	<62.5	215	220
2b	>1000	81	>1000	>1000
2c	>1000	89	>1000	>1000
2d	>1000	355	187	185
2e	110	<62.5	104	106
2f	>1000	<62.5	108	115
2g	627	99	106	<62.5
2h	407	<62.5	<62.5	<62.5

Table 12: IC_{50} concentrations of the different compounds at 48 and 72 hrs on MDAMB-231 and HeLa cells

***In vitro* anticancer studies**

The synthesized Rhodanine derivatives were screened for their anticancer activity using MTT assay against MDAMB-231 and HeLa cell lines. Untreated cells served as control. The percentage cell inhibition at different concentration of compounds and IC_{50} value was determined. IC_{50} concentrations of the different compounds at 48 and 72 hrs on MDAMB-231 and HeLa cells are given in table 12. Percentage of cytotoxicity induced by the compounds (2a-2h) after 48 and 72 hrs on MDAMB-231 and HeLa cells are shown in Figure 8,9,10 and 11. There was a dose and time dependent percentage of cytotoxicity induced by the compounds (2a-2h) on MDAMB-231 and HeLa cells. The compounds 2e and 2h were most prominent compounds eliciting cytotoxicity on cells after 48 and 72 hrs of treatment on MDAMB-231 cells and the compounds 2d, 2e, 2f, 2g and 2h were most prominent compounds eliciting cytotoxicity on cells after 48 and 72 hrs of treatment on HeLa cells. The positive control used for the study were Doxorubicin for MDAMB and Cisplatin for HeLa cells. The morphology of MDAMB – 231 and HeLa cells before and after treatment with the extract of compound 2h are shown in figure 12.

On culture with MDAMB-231 cells, 2e showed cytotoxicity inducing 62.51% and 61.54% cell death after 48hrs, 73.75% and 72.99% after 72hrs: 2h induced 54.14% and 53.73% cell death after 48hrs, 76.60% and 75.17% after 72hrs at 1000 and 500 µg/ml concentrations. On treatment with HeLa cells, 2d showed cytotoxicity inducing 70.68% and 65.71% cell death after 48hrs, 72.19% and 66.02% after 72hrs; 2e induced 66.29% and 65.37% cell death after 48hrs, 72.48% and 66.59% after 72hrs; 2h showed cytotoxicity inducing 84.92% and 83.11% cell death after 48hrs, 86.41% and 84.81% after 72hrs at 1000 and 500 µg/ml concentrations.

From the results it is clearly seen that all the 8 compounds have shown moderate to potent cytotoxicity. The cytotoxicity increases with increase in concentration or dose dependent cytotoxicity was seen with the tested compounds. Out of all the 8 compounds, 2h has shown highest cytotoxicity in the range 86.41% to 62.18% for HeLa cell line and 76.60% to 62.61% for MDA MB cell line. Hence compound 2h may be considered to have better cytotoxicity. Further studies are warranted in the compound to develop it further as a potent anti-cancer agent.

CONCLUSION

The present study involves computational modelling and invitro evaluation of a series of 8 novel synthesised rhodanine derivatives. Out of the 8 compounds compound 2h and 2e showed better binding affinity and good anticancer inhibitory activity. Compound 2h (3-(3-(azepan-1-yl) propyl)- 2-thioxothiazolidine-4-one has shown good binding affinity for all the three targets involved in the progression and development of cancer with an IC_{50} value < 62.5 µg/ml for both breast cancer cell line and cervical cancer cell line. The structure activity relationship (SAR) studies show that presence of bulky heterocyclic ring substitution with long chain carbon group at N-position of rhodanine core moiety has showed good binding affinity and potent cytotoxicity. Hence compound 2h with azepine substitution at N position of rhodanine (2-thioxothiazolidine-4-one) can be nominated as a good lead in anticancer category. Other compounds 2a, 2e, 2f and 2g also showed moderate cytotoxicity.

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