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Molecular Docking Studies of a Cyclic Octapeptide-Cyclosaplin from Sandalwood

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Abstract

Natural products from plants such as, chemopreventive agents attract huge attention because of their low toxicity and high specificity. The rational drug design in combination with structure based modeling and rapid screening methods offer significant potential for identifying and developing lead anticancer molecules. Thus, the molecular docking method plays an important role in screening a large set of molecules based on their free binding energies and proposes structural hypotheses of how the molecules can inhibit the target. Several peptide based therapeutics have been developed to combat several health disorders including cancers, metabolic disorders, heart-related, and infectious diseases. Despite the discovery of hundreds of such therapeutic peptides however, only few peptide-based drugs have made it to the market. Moreover, until date the activities of cyclic peptides towards molecular targets such as protein kinases, proteases, and apoptosis related proteins have never been explored. In this study we explore the in silico kinase and protease inhibitor potentials of cyclosaplin as well as study the interactions of cyclosaplin with other cancer-related proteins. Previously, the structure of cyclosaplin was elucidated by molecular modeling associated with dynamics that was used in the current study. Docking studies showed strong affinity of cyclosaplin towards cancer-related proteins. The binding affinity closer to 10 indicated efficient binding. Cyclosaplin showed strong binding affinities towards protein kinases such as EGFR, VEGFR2, PKB and p38 indicating its potential role in protein kinase inhibition. Moreover, it displayed strong binding affinity to apoptosis related proteins and revealed the possible role of cyclosaplin in apoptotic cell death. The protein-ligand

interactions using LigPlot displayed some similar interactions between cyclosaplin and peptide-based ligands especially in case of protein kinases and a few apoptosis related proteins. Thus, the in silico analyses gave an insight of cyclosaplin as a potential apoptosis inducer and protein kinase inhibitor.

Keywords: apoptosis; cyclosaplin; molecular docking; protein kinases; Sandalwood

1. Introduction

Cancer is a well-recognized global health problem responsible for ~7.6 million deaths (~13% of all deaths) worldwide, which is expected to rise to 13.1 million by 2030 (WHO, 2012). Despite the advancement in the field of cancer research, still there is an urgency to discover and develop anti-cancer therapeutics. Natural products are of particular interest as chemopreventive agents because of their low toxicity and potential effectiveness [1]. The conventional drug discovery techniques are time consuming and expensive process [2]. Thus, rational drug design in combination with structure based modeling and rapid screening methods offer significant potential for identifying and developing lead anticancer molecules. The use of molecular docking method addresses in deducing the ligand binding sites with a protein of known three-dimensional structure. One of the computational approaches, such as docking helps in screening a large set of molecules based on their free binding energies and proposes structural hypotheses of how the molecules can inhibit the target. Recently, several in silico based studies have been performed on small molecules including peptides to identify their anti-cancerous properties [3]. Peptide based therapeutics has been effective in combating several health disorders including cancers, metabolic disorders, cardiovascular health, and infectious diseases. Peptides are structurally diverse,

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have wide spectrum of therapeutic action, low absorption in body tissues and high specificity to targets [4]. Several cyclic peptides with diverse biological activities, such as antibacterial activity, immunosuppressive activity, and anticancer activity have been reported [5]. For example, tyrocidine and gramicidin S with antibacterial activity, cyclosporin A displaying immunosuppressive activity, and Cyclo-RGDfV having antiangiogenic activity [5-7]. Apart from their use as cytotoxic agents, peptides can also be used in drug formulations for enhancing biological activity, targeted drug delivery, or transport across cellular membranes. Thus, revival of interest in therapeutic peptides and extensive research has seen peptides entering into clinical trials improved significantly over the decade [8]. Despite the discovery of hundreds of such therapeutic peptides however, only few peptide-based drugs have made it to the market. Moreover, until date the activities of cyclic peptides towards molecular targets such as protein kinases, proteases, and apoptosis related proteins have never been explored. In this study we explore the *in silico* kinase and protease inhibitory potentials of cyclosaplin as well as study the interactions of cyclosaplin with other cancer-related proteins.

2. MATERIALS AND METHODS

2.1 Softwares and tools

ACD/ChemSketch 12.01, AutoDock Vina 1.1.2, Avogadro, CycloPsWeb, GROMACS, LigPlot, Modeller 9.2, MGL tools, Open Babel, Protein Data Bank (PDB), PubChem, PyMOL, and Swiss Target Prediction.

2.2 Molecular modeling of cyclic peptide

In our previous work, we used the Bioinfo Meta Server to find structures similar to cyclosaplin and GROMACS**,** a molecular dynamics tool for predicting energy minimized

structures of cyclosaplin using ab-initio procedures [9]. Briefly, a primary structure was placed up in a cubic box, including water molecules using Modeller 9.2 program [10]. The energy minimization steps were carried out with partial simulation with a step size of 0.002 ps, followed by 100 ps run to attain normal temperature and pressure respectively. A density adjusted simulation box appeared, which was further used to perform complete simulation under temperature and pressure of 300 K and 1 atm, respectively, for 2 ns. All simulations were performed on an Intel Xeon workstation.

2.3 Ligand preparation

The purified cyclic octapeptide (cyclosaplin) and various peptides for specific proteins (positive control) were used as ligands for docking studies. The ligand molecules were drawn in either ACD/Chem Basic freeware (ACD/ChemSketch 12.01) or using CycloPsWeb and saved as MDL mol file formats. The MDL files were converted to pdb format files using Open Babel. The ligands used in the study are represented in the Table 2.3.

Table 2.3 Ligands used in the study

S.No.	Ligand	References
1	CVRACGAD (Cyclic)	Vila et al., 2010
2	Cilengitide (Cyclic)	Alghisi et al., 2009
3	RPRTSSF (Cyclic)	Tal-Gan et al., 2011
4	FWCS (Linear)	Gill et al., 2014
5	YSV (Linear)	Zhu et al., 2006
6	CTTHWGFTLC (Cyclic)	Koivunen et al., 1999
7	CRRHWGFEFC (Cyclic)	Koivunen et al., 1999
8	RGDS (Linear)	Aguzzi et al., 2004
9	CKVILTHRC (Cyclic)	Heins and Quax, 2010

10	AYACNTSTL (Linear)	Hirohashi et al., 2002
11	Cyclosaplin (Cyclic)	Mishra et al., 2014

The ligand structures were also optimized using AutoDock prior to docking [11].

2.4 Lipinski rule for ligands

The peptide based-ligand molecules selected for docking experiments were screened for Lipinski rule of five. Lipinski's rule of five [12] states that a drug molecule generally does not violate more than one of the following five rules

- Molecular mass less than 500 Da
- High lipophilicity (expressed as LogP less than 5)
- Less than 5 hydrogen bond donors
- Less than 10 hydrogen bond acceptors
- Molar refractivity between 40-130

Lipinski's rule of five was also checked in Supercomputing Facility for Bioinformatics & Computational Biology, IIT Delhi where PDB structures of the molecules were uploaded to the online server (http://www.scfbio-iitd.res.in/utility/LipinskiFilters.jsp).

2.5 Protein preparation

The Swiss Target Prediction was used to for predicting the potential targets of cyclosaplin [13]. The protein structures were obtained from Protein Data Bank (PDB) [14]. The proteins selected for the study were Epidermal Growth Factor Receptor kinase domain (EGFR), Vascular Endothelial Growth Factor r2 Receptor kinase (VEGFR2), Matrix metalloproteases (MMP-2, MMP-9), and Apoptosis related proteins (Procaspase 3, Procaspase 7, Caspase 9, TRAIL, SURVIVIN). EGFR Kinase and Procaspase 3 were previously used in our study and were used as a control in this study [9]. The files in pdb

format for each receptor were converted to respective PDBQT format using MGL tools. The polar hydrogen atoms were added to the receptor molecules prior to docking studies. Three dimensional affinity grids were created at the geometric centre of the target protein.

2.6 Docking studies using AutoDock Vina

The energy-minimized structures of cyclic octapeptide and peptide inhibitors or inducers (positive control) were docked with target proteins using AutoDock Vina 1.1.2 [15]. The receptor and ligand files were represented in PDBQT file format, a modified pdb format containing atomic charges, atom type definitions for ligand and topological information (rotatable bonds). For docking, the entire receptor was enclosed inside a grid box, with a grid spacing of 1Å, keeping receptor rigid, and ligand as a flexible molecule. After defining the binding site and receptor–ligand preparation, docking runs were launched from the command prompt. The interaction energy between the ligand and the receptor was calculated for the entire binding site and expressed as affinity (Kcal/mol).

2.7 Protein-Ligand interactions

LigPlot was used to study protein-ligand interactions for a given pdb file encrypting the docking [16]. The LigPlot program self generated schematic 2D representations of the interfaces of protein-ligand complexes from standard pdb file input. The output was in the form of informative representation of the intermolecular interactions and their strengths, including hydrogen bonds, hydrophobic contacts, and atom accessibilities.

3. RESULTS

3.1 Molecular modeling of cyclic peptide

Previously, the tertiary structure of cyclic octapeptide was elucidated by molecular modeling associated with dynamics by our group. The energy-minimized structure of

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cyclosaplin is shown in Fig. 3.1. The cyclosaplin is positively charged and 25% hydrophobic in nature, as shown by antimicrobial peptide database (APD).

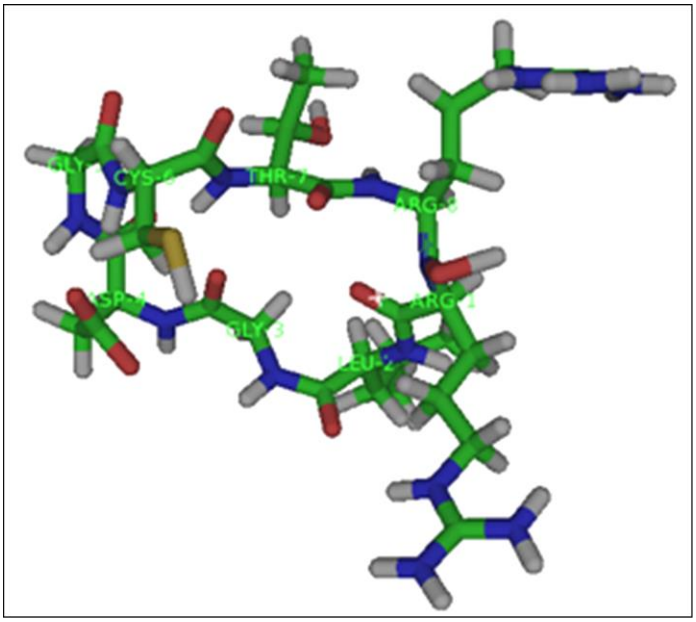


Fig 3.1 The energy minimized structure of cyclosaplin

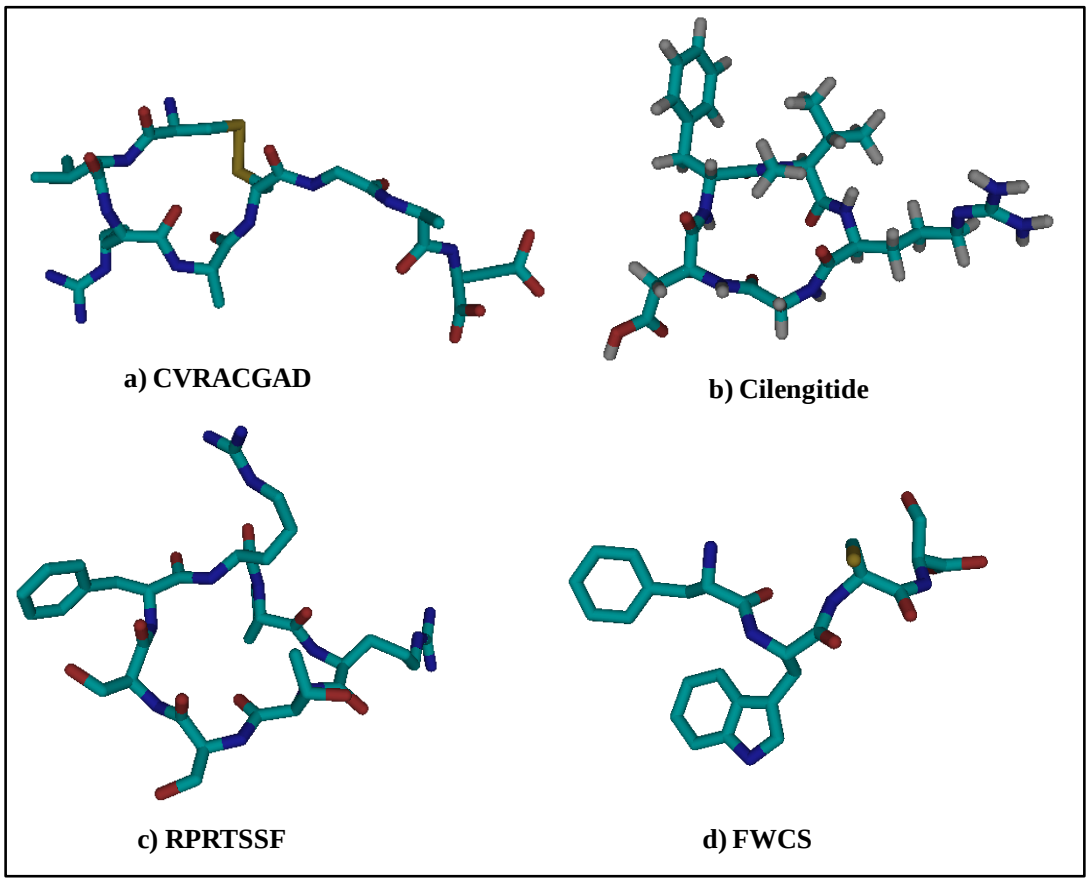
3.2 Ligand preparation

The ligand structures were drawn in CycloPsWeb or downloaded from PubChem and converted to pdb format using Open Babel whereas modeled structure was used in case of cyclosaplin (Table 3.2). The structures were energy minimized and saved in PDBQT format by MGL tools (Fig. 3.2).

Table 3.2: Molecular weight and molecular formula of the ligands

S.No.	Ligand	Molecular weight (Da)	Molecular formula
1	CVRACGAD (Cyclic)	791.9	$C_{29}H_{49}N_{11}O_{11}S_2$
2	Cilengitide (Cyclic)	588.6	$C_{27}H_{40}N_8O_7$
3	RPRTSSF (Cyclic)	875.0	$C_{39}H_{66}N_{14}O_9$
4	FWCS (Linear)	541.6	$C_{26}H_{31}N_5O_6S_1$
5	YSV (Linear)	367.4	$C_{17}H_{25}N_3O_6$
6	CTTHWGFTLC (Cyclic)	1166.3	$C_{52}H_{71}N_{13}O_{14}S_2$

7	CRRHWGFEFC (Cyclic)	1338.5	$C_{60}H_{79}N_{19}O_{13}S_2$
8	RGDS (Linear)	433.4	$C_{15}H_{27}N_7O_8$
9	CKVILTHRC (Cyclic)	1070.3	$C_{45}H_{79}N_{15}O_{11}S_2$
10	AYACNTSTL (Linear)	943.0	$C_{39}H_{62}N_{10}O_{15}S_1$
11	Cyclosaplin (Cyclic)	858.9	$C_{33}H_{60}N_{14}O_{12}S_1$



a) CVRACGAD

b) Cilengitide

c) RPRTSSF

d) FWCS

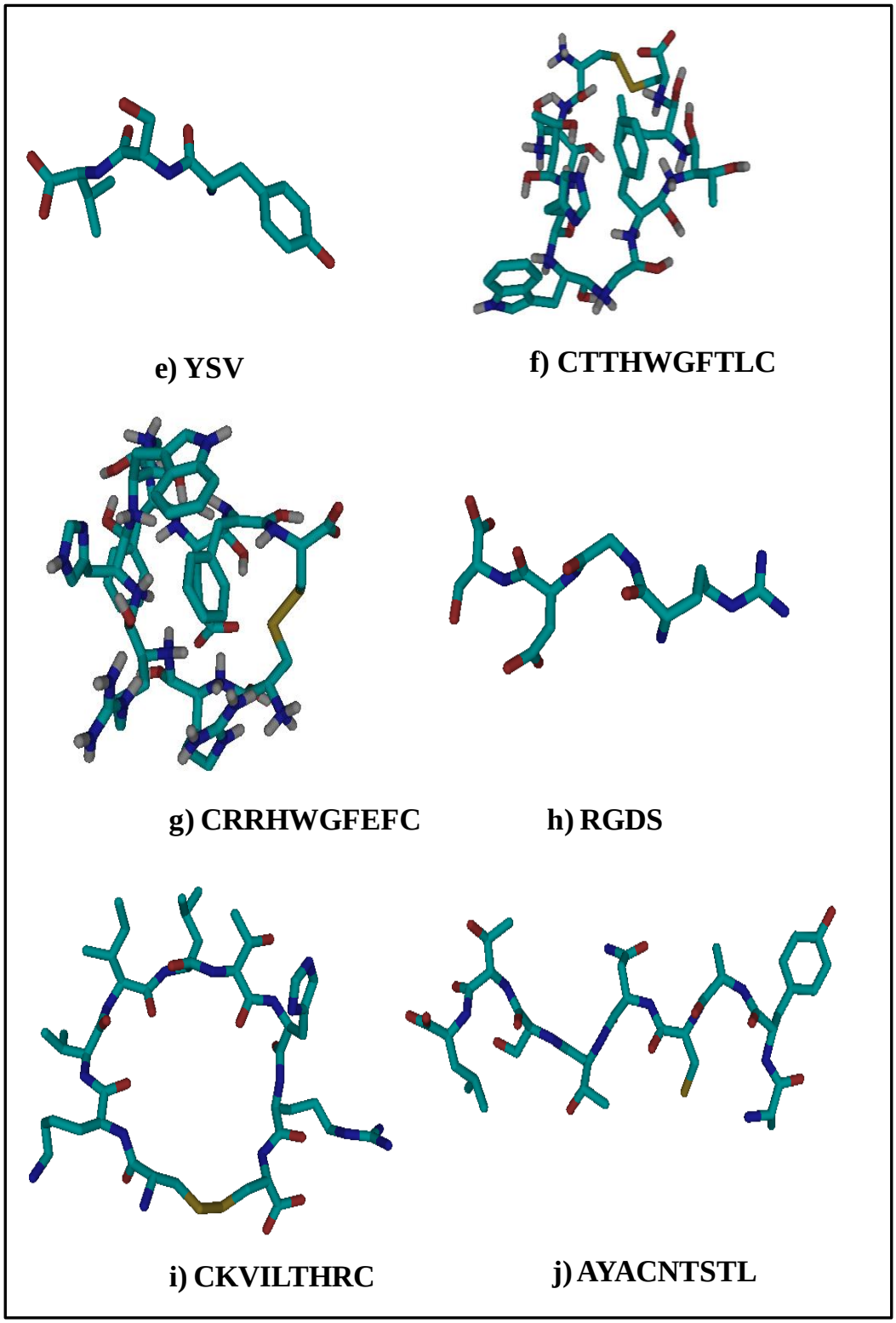


Fig 3.2 The different peptide based ligands for target cancer-related proteins used in docking studies. Cyan blue = Carbon, Grey = Hydrogen, Deep blue = Nitrogen, Red = Oxygen and Yellow = Sulfur.

3.3 Lipinski Rule

The ligands prepared for docking were screened for Lipinski's rule of five. The commercially available or reported peptide inhibitors/inducers (positive control) were also tested against the target proteins respectively (Table 3.3).

Table 3.3: Physiochemical parameters of ligand molecules screened for Lipinski's Rule

Ligand	Molecular weight (Da)	Hydrogen Bond Donor	Hydrogen Bond Acceptor	LogP	Molar Refractivity
CVRACGAD	791.9	13	13	-4.7	206.6
Cilengitide	588.6	7	8	-1.4	170.9
RPRTSSF	875.0	15	12	-5.9	236.7
FWCS	541.6	8	7	-0.7	143.2
YSV	367.4	6	6	-1.0	93.14
CTTHWGFTLC	1166.3	16	17	-4.0	331.1
CRRHWGFEFC	1338.5	20	17	-3.6	381.6
RGDS	433.4	10	8	-4.7	99.7
CKVILTHRC	1070.3	16	16	-3.3	306.4
AYACNTSTL	943.0	16	16	-6.1	230.0
Cyclosaplin	858.9	17	13	-6.5	243.0

3.4 Protein preparation

The Swiss Target Prediction was used to predict the possible targets of cyclosaplin (Fig. 3.4a). The cancer related proteins were downloaded from RCSB protein data bank and converted to PDBQT format using AutoDock tools (Fig. 3.4b).

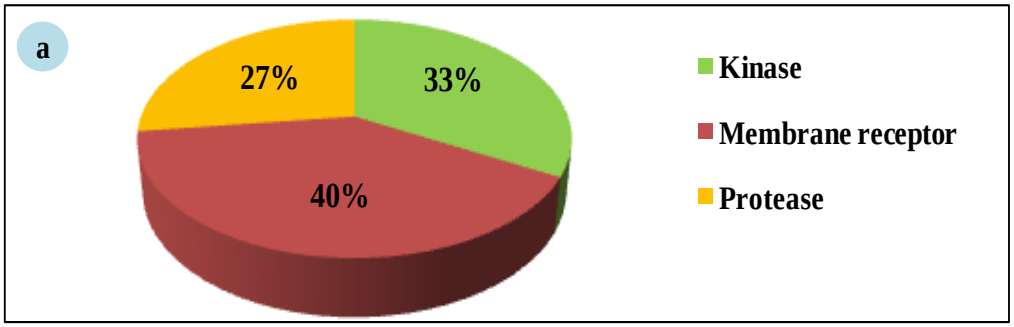


Fig 3.4a The possible targets of cyclosaplin as predicted by Swiss Target Prediction.

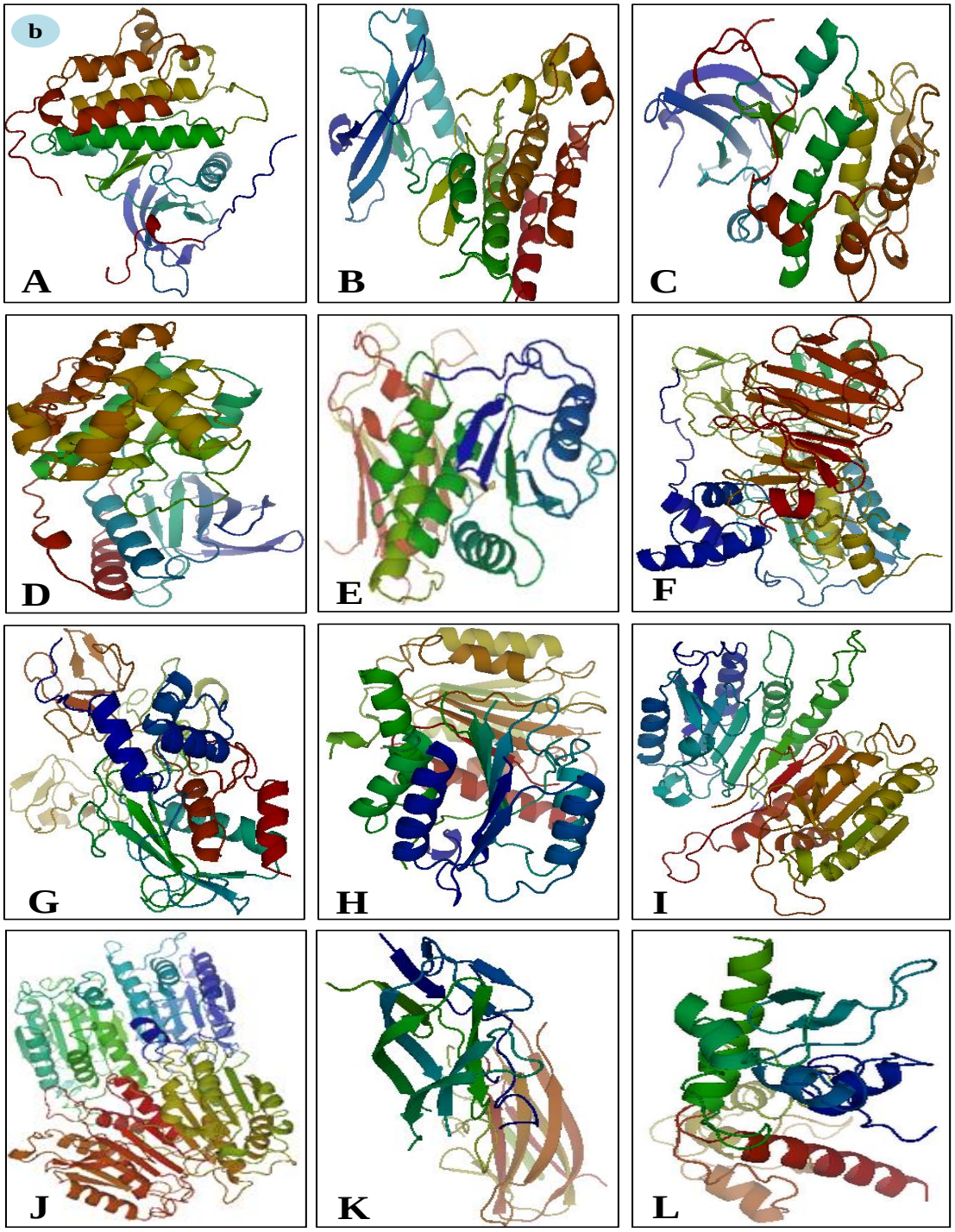


Fig 3.4b Different energy minimized proteins (rainbow spectrum) used in docking studies. A) EGFR kinase B) VEGFR2 kinase C) PKB D) p38 E) PTEN F) MMP-2 G) MMP-9 H) Procaspase 3 I) Procaspase 7 J) Caspase 9 K) TRAIL L) SURVIVIN.

3.5 Docking studies using AutoDock Vina

The docking process was carried out using AutoDock Vina. The docking scores are graphically represented in Fig. 3.5a and the binding affinities of ligands are represented as Kcal/mol (Table 3.4). The affinity value of less than 5 depicts negligible binding whereas values closer to 10 indicate efficient binding. The protein ligand docking studies are represented in Fig. 3.5a-c.

Table 3.4: Comparative binding affinity of different ligands with receptors

S.No.	Receptor	Ligand	Binding affinity (Kcal/mol)
1.	Epidermal Growth Factor Receptor Kinase (Previous study; Mishra et al., 2014)	CVRACGAD Cyclosaplin	-7.7 -6.8
2.	Vascular Endothelial Growth Factor r 2 Receptor Kinase	Cilengitide Cyclosaplin	-8.1 -7.8
3.	Protein Kinase B	RPRTSSF Cyclosaplin	-7.5 -8.1
4.	p38 (Mitogen Activated Protein Kinase)	FWCS Cyclosaplin	-8.9 -8.3
5.	PTEN	YSV Cyclosaplin	-7.8 -6.3
6.	Matrix metalloproteinase-2 (MMP-2)	CTTHWGFTLC Cyclosaplin	-7.8 -8.2
7.	Matrix metalloproteinase-9 (MMP-9)	CRRHWGFEFC Cyclosaplin	-8.4 -7.3
8.	Procaspase 3 (Previous study; Mishra et al., 2014)	Cilengitide Cyclosaplin	-8.1 -7.8

9.	Procaspase 7	RGDS	-6.8
		Cyclosaplin	-8.7
10.	Caspase 9	RGDS	-6.7
		Cyclosaplin	-8.9
11.	TRAIL	CKVILTHRC	-6.4
		Cyclosaplin	-8.2
12.	SURVIVIN	AYACNTSTL	-7.2
		Cyclosaplin	-7.4

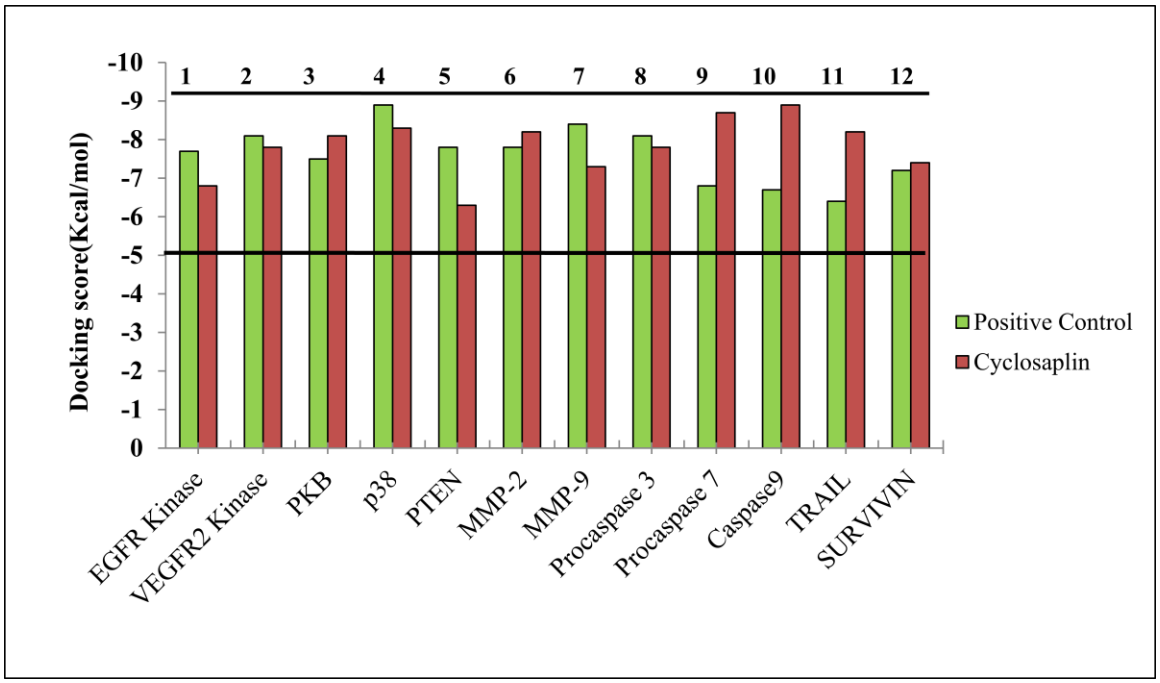


Fig. 3.5a Docking scores in Kcal/mol for various cancer-related proteins. The binding affinities closer to 10 indicate efficient binding.

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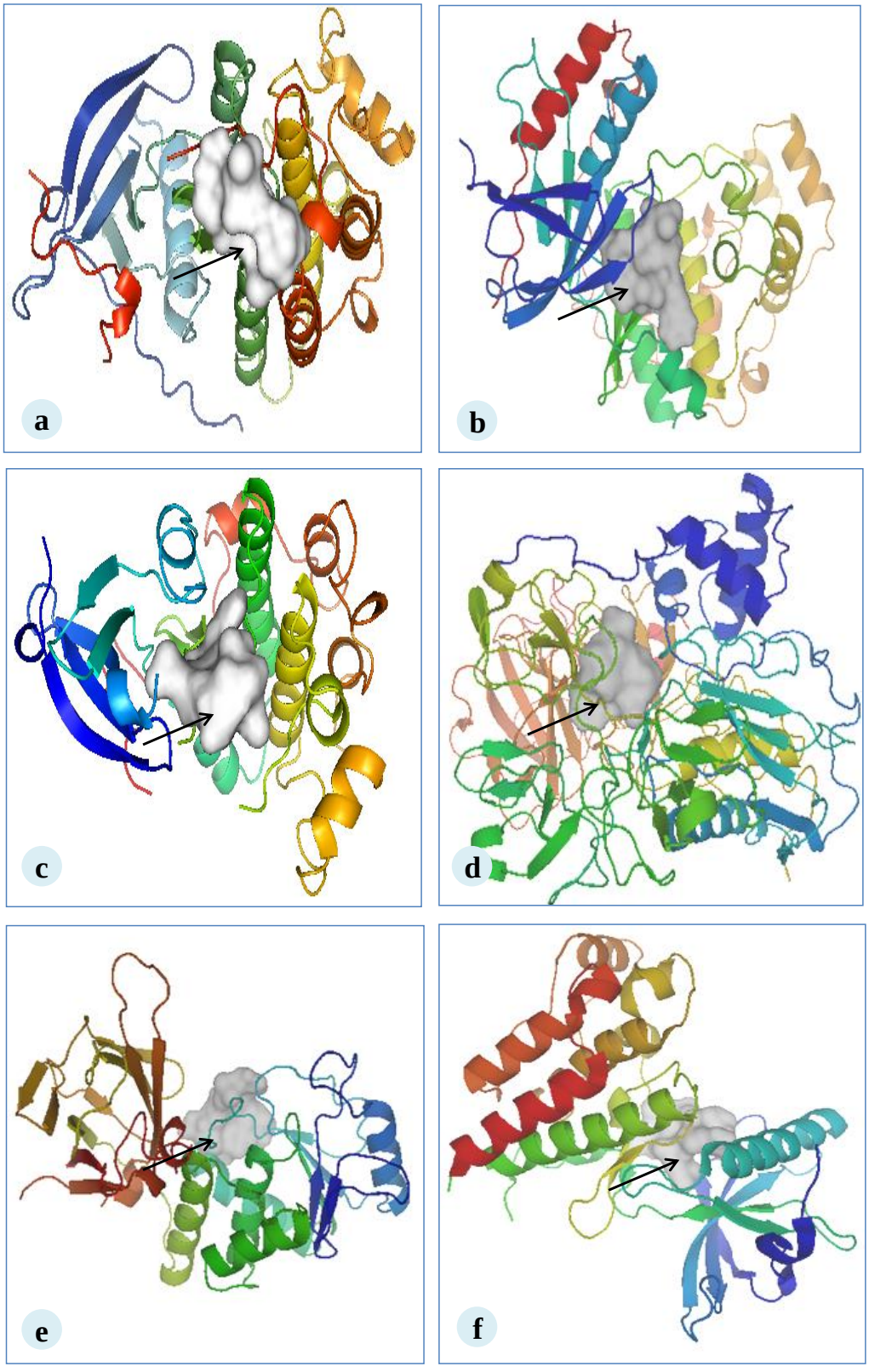

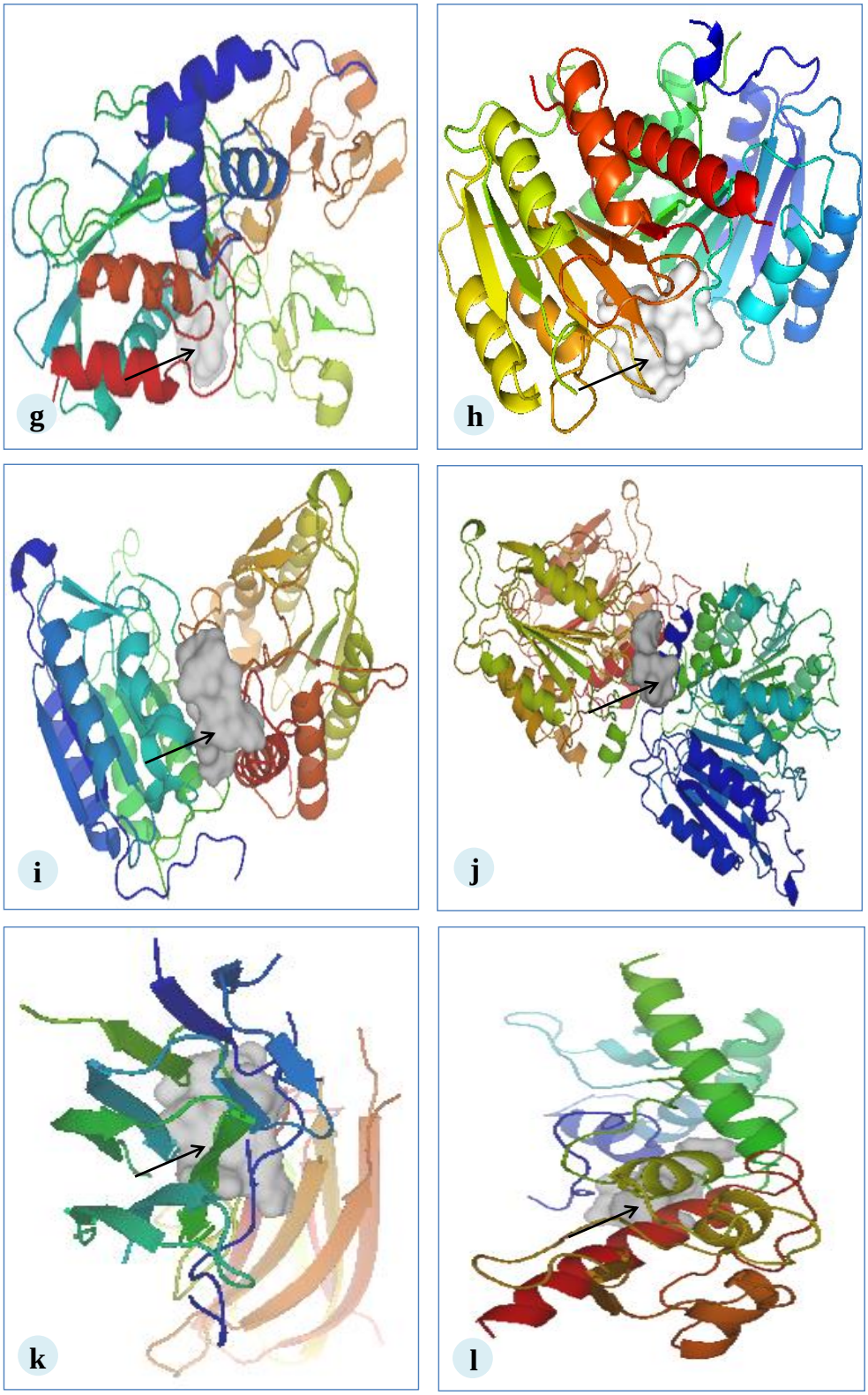

Fig. 3.5b Cyclosaplin bound to different receptors. Cyclosaplin is shown in white surface indicated by arrow and proteins are depicted in rainbow spectrum. a) EGFR kinase (Previous study; Mishra et

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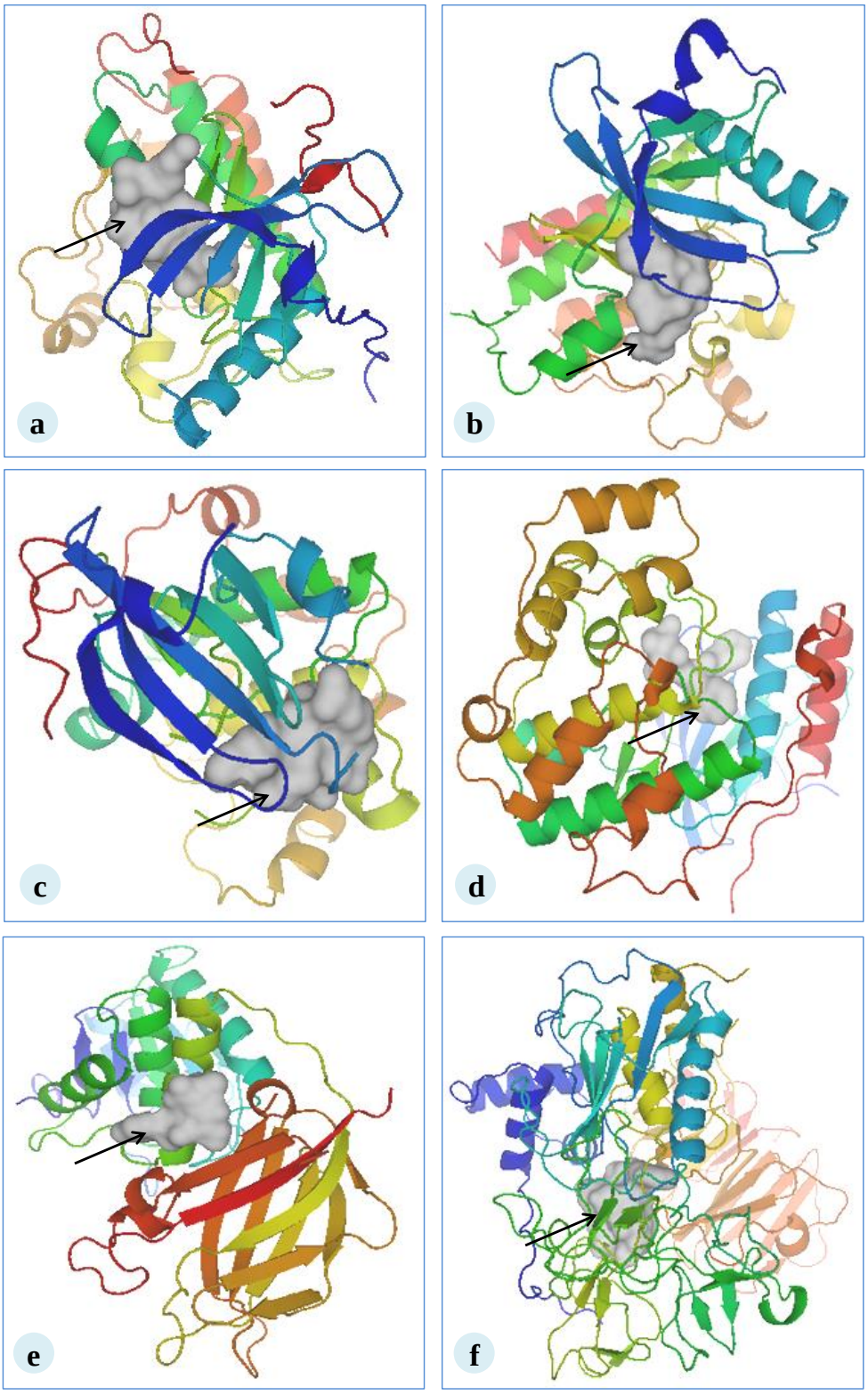
al., 2014) b) VEGFR2 kinase c) PKB d) p38 e) PTEN f) MMP-2 g) MMP-9 h) Procaspase 3 (Previous study; Mishra et al., 2014) i) Procaspase 7 j) Caspase 9 k) TRAIL l) SURVIVIN.



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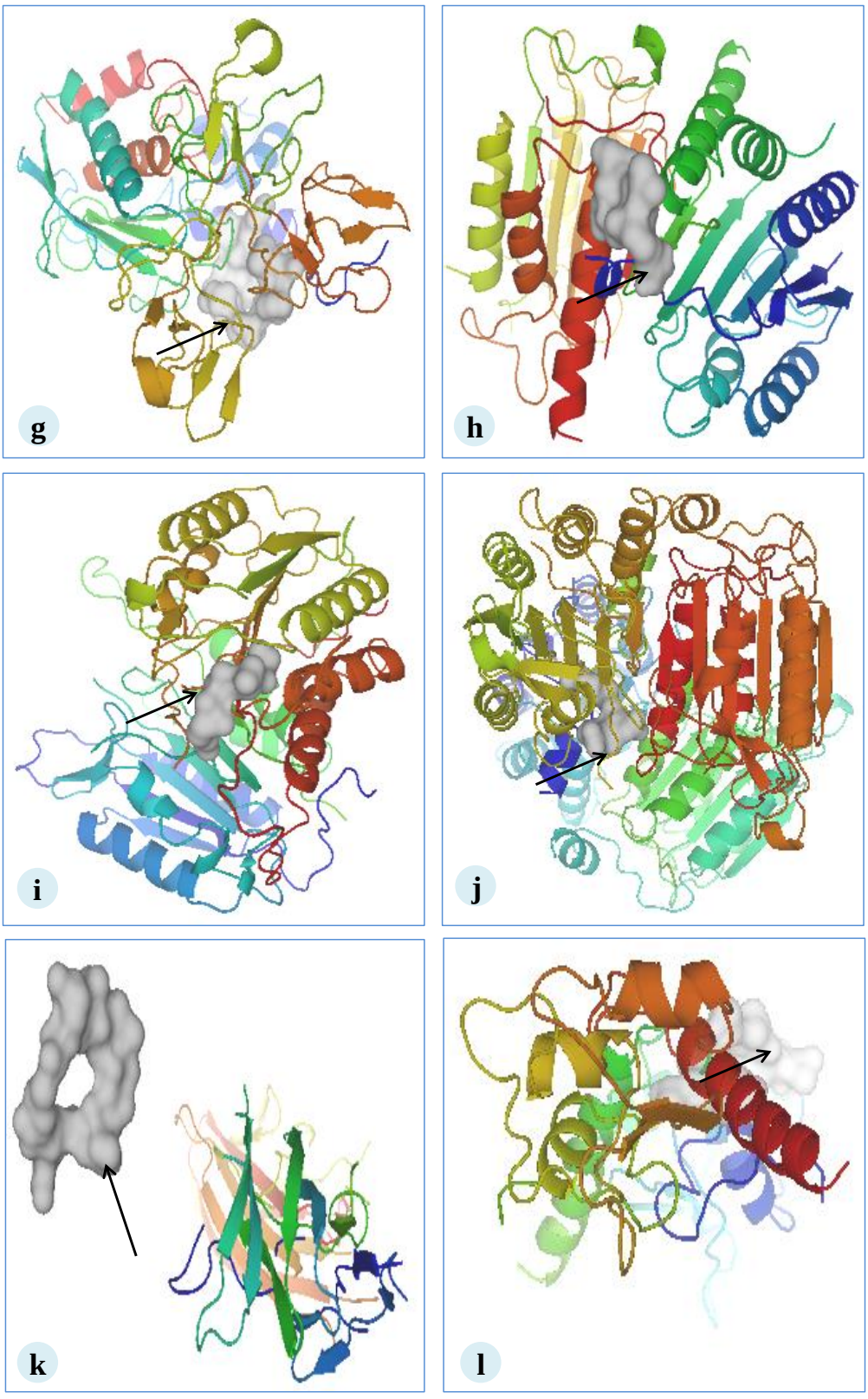

Fig. 3.5c Peptide based ligands bound to specific proteins. Ligands are shown in white surface indicated by arrow and proteins are depicted in rainbow spectrum.

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a) CVRACGAD bound to EGFR kinase, b) Cilengitide bound to VEGFR2 kinase, c) RPRTSSF bound to PKB, d) FWCS bound to p38, e) YSV bound to PTEN, f) CTTHWGFTLC bound to MMP-2, g) CRRHWGFEFC bound to MMP-9, h) Cilengitide bound to Procaspase 3, i) RGDS bound to Procaspase 7, j) RGDS bound to Caspase 9, k) CKVILTHRC unbound to TRAIL, and l) AYACNTSTL bound to SURVIVIN.

3.6 Protein-Ligand interactions

The protein-ligand interaction study is performed using LigPlot. The interactions of the ligands cyclosaplin and various peptide-based ligands with amino acids residues of the target proteins are shown in Table 3.5. The H-bonds and hydrophobic contacts between the docked complexes are shown in Fig. 3.6a-c, and Fig. 3.7a-c.

Table 3.5: Molecular interactions of ligands with amino acids of protein (Amino acids showing similar interactions are marked in bold; Black = cis similarity; Red = trans similarity).

S. No.	Protein	Ligand	Hydrophilic interactions	Hydrophobic contacts	No. of H-bonds
1	EGFR Kinase	CVRACGAD	Lys721, Thr766	Ala719, Asp831, Gly695, Gly772 Leu694, Leu768	2
		Cyclosaplin	Glu961,Asp960 (4)	Arg962, Asp950, Gln788,Gln952, Gly786,Met963, Pro951, Ser787, Tyr789	5
2	VEGFR 2 Kinase	Cilengitide	**Asn923**,**Asp 1046** **Thr926**	Ala866, Arg1032, Arg1066,**Asp1064** Cys919, Cys1045, Gly841, **Leu840**, Phe918, Ser925, **Val848**	3
		Cyclosaplin	Arg842, Arg842 Glu885	Ala1065, **Asn923**, **Asp1046**,**Asp1064** Gly843,Gly846, Gln847,**Leu840**, Leu1035,Lys868, **Thr926**,**Val848**	3
3	Protein Kinase B	RPRTSSF	**Arg274,** Glu315, **Lys181**, Lys277, Tyr327	**Asp275**, Glu200, Gly295, Leu317, Lys160, **Phe163**,	5

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				Thr162, **Thr199**, Val198	
		Cyclosaplin	**Arg274**, Arg274, **Asp275**, Asp293, Val272, Val272	Ile188, Leu183, **Lys181**,**Phe163**, **Thr199**,**Tyr273**	6
4	p38 (Mitogen Activated Protein Kinase)	FWCS	**Arg173**, Arg 67	**Asp168**,Glu71, Glu178,His64, Leu74,Leu75, Leu171,**Ly53**, **Phe169**,Thr68, **Tyr35**	2
		Cyclosaplin	Asp112,Asp112, Asp150,Asn115, **Lys53**,**Phe169**, **Phe169**, Ser154	**Arg173**,Asn114, Asn155,**Asp168**, **Gly170**, Leu167, Met109, **Tyr35**, Val38	8
5	PTEN	YSV	Arg172, Arg173	Asp324, Leu318, Leu320,Phe279, Tyr176, Tyr177, Tyr180, Val275	2
		Cyclosaplin	Ala72,Ala72, Gln87, Gln97	Glu91,Glu99, Leu100, Pro89, Tyr88	4
6	MMP-2	CTTHWGFTLC	Arg98, **Gly371**, **Gly394**, **Thr 511**, Thr547	**Gln393**,**Gly216**, Lys99, Lys372, Met373,Ser365, **Ser546**,Tyr395, **Tyr425**,**Tyr427**	6
		Cyclosaplin	**Gly394**, **Tyr425**	Asp392,Glu515, **Gln393**,**Gly216**, **Gly371**,Phe512, Pro100,Pro514, Leu 548, **Ser546**, Thr426, **Tyr427**, **Thr511**,Tyr277, Tyr395	2
7	MMP-9	CRRHWGFEFC	Leu371, Arg2, Cys1	Arg370, Arg424, Glu427, Gln391, Gly392, Lys92, Phe425, Pro97, Pro233, Ser240, Ser242, Thr426 Tyr393, Tyr423	3
		Cyclosaplin	Arg221, Thr331	Arg279, Asp226,	4

				Asp284, Gly227, Gly285,Pro219, Pro272, Thr220,	
8	Procaspase 3	Cilengitide	Ala33, Arg238, Asn32, Ser36 (2), Tyr37, Tyr274, Tyr276	Asn35, Glu272, Leu230, Lys38, His234	8
		Cyclosaplin	Gln261, Glu124 Lys186	Arg164, Gly125, Ile126,Ile187, Leu136,Lys186, Pro188,Tyr197, Val189	3
9	Procaspase 7	RGDS	Arg87, Asn88, Gly228, Gln184 Thr189, Tyr229	Arg187, Gly188, **Gln287**, His144, Lys285,Pro227, Ser239,Thr90	6
		Cyclosaplin	Arg170, Glu176, Glu284, Gly168, Phe282, Phe282, Ser277	Ala169,Ala217, Arg167,Asp204, Gln276,Lys286, Leu175,**Gln287**, Ile288,Val215, Glu216, His283	7
10	Caspase 9	RGDS	Gly269, Ser339	Ala149,Arg408, Asp150, Asp340, **Gly276**, Gly277 Gln399,Ile154, Ile396, Lys398, Met400, Thr337	2
		Cyclosaplin	Pro273, Ser144, Ser144	Arg146, Asp228, Glu143,Gly147, Gly225,**Gly276**, Ile154,Leu155, Lys278,Lys414, Ser274	3
11	SURVIVIN	AYACNTSTL	Arg18, Arg37, Cys31, Gly30	Glu29,Glu36, Glu40, **Gln92**, Ile74, **Leu14**, **Leu96**, Leu104, **Lys15**,Lys90, Met1,**Phe13**, **Phe93**, Thr34	4
		Cyclosaplin	**Gln92**,Glu94, Lys91, **Phe13**	Asp16,Gly2, **Leu14**,Leu96, Leu102,**Lys15**, **Phe 93**, Pro4	4

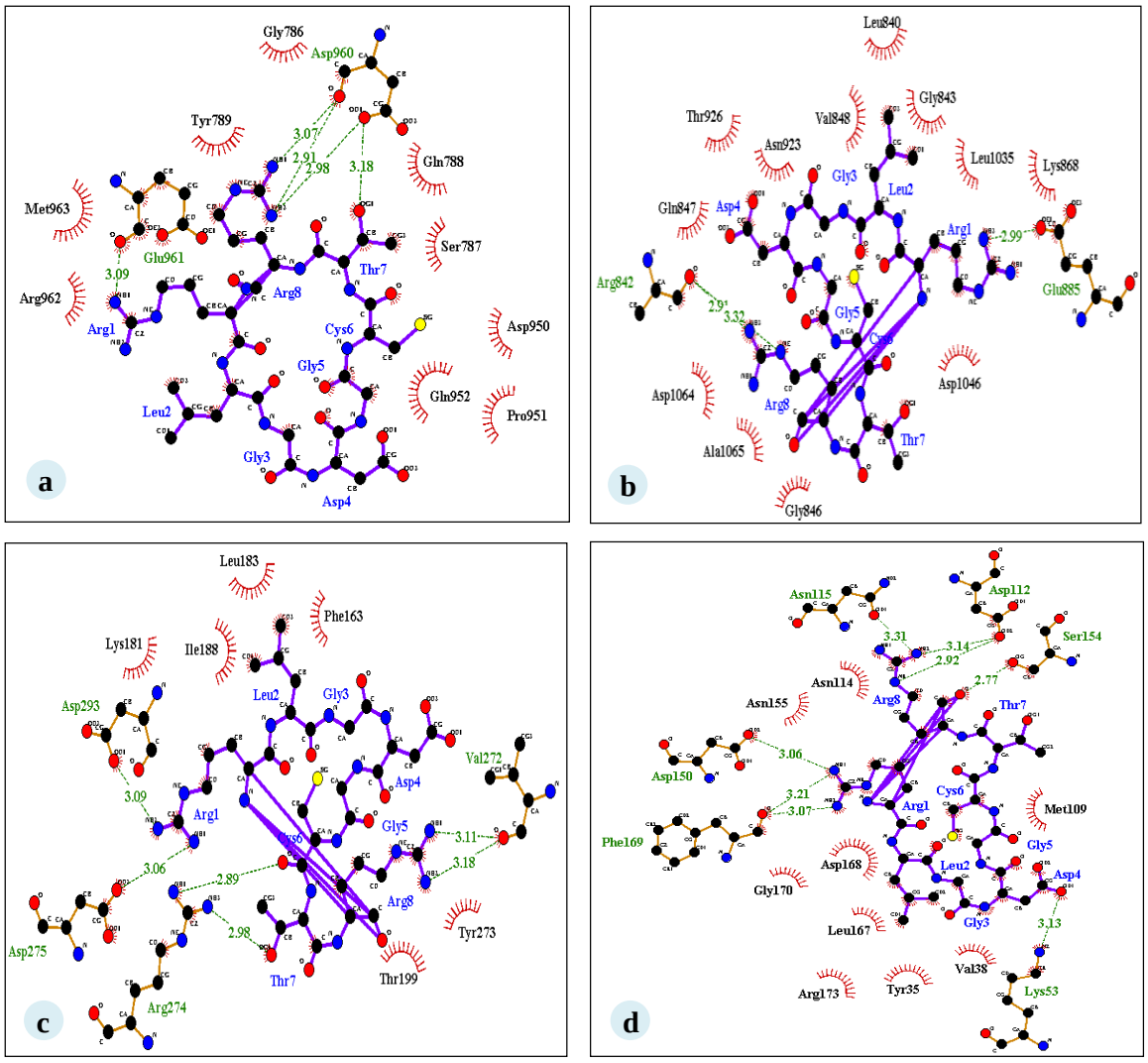

Fig. 3.6a Interaction of cyclosaplin with various cancer-related proteins a) EGFR Kinase b) VEGFR2 Kinase c) PKB d) p38

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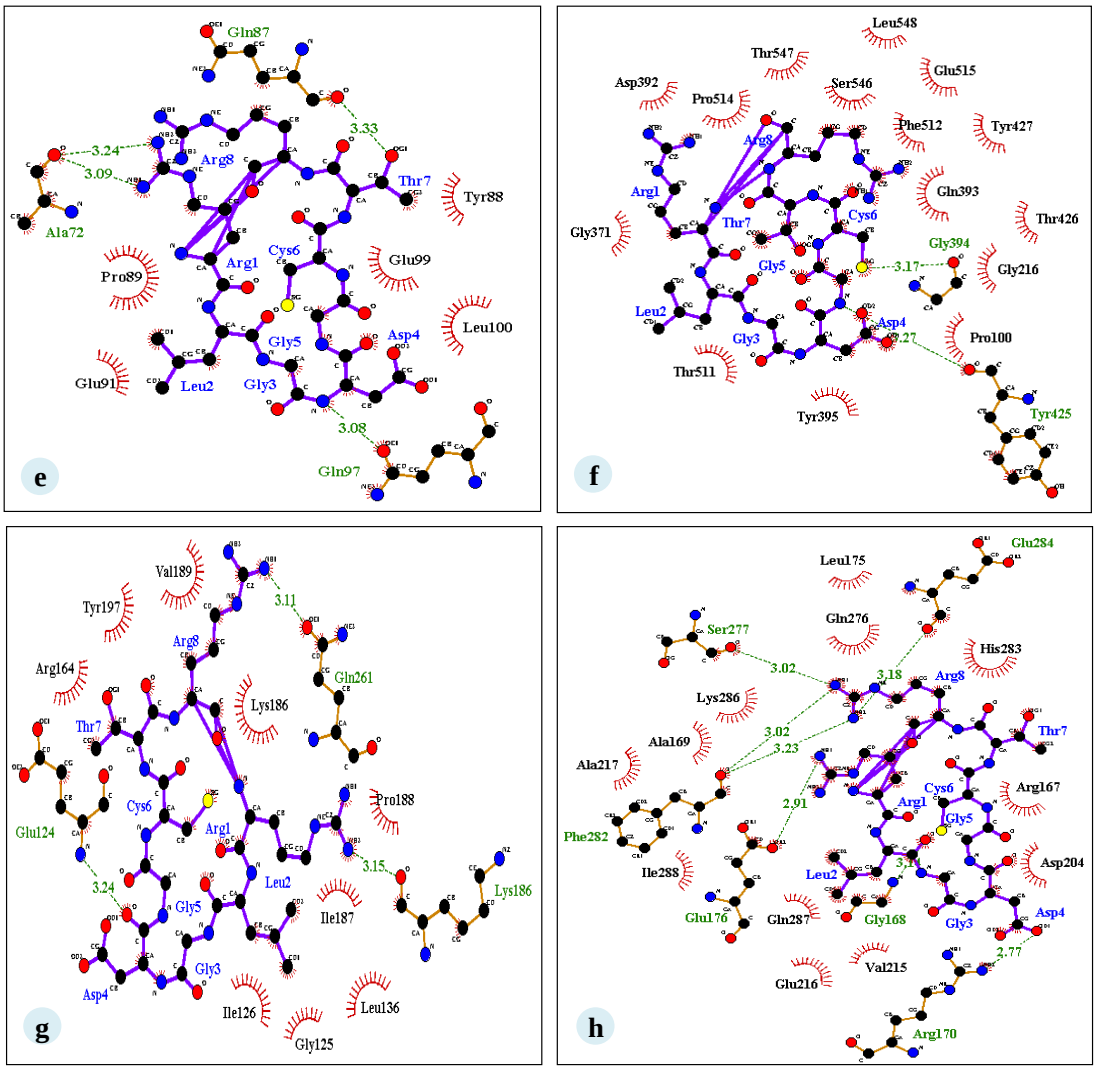
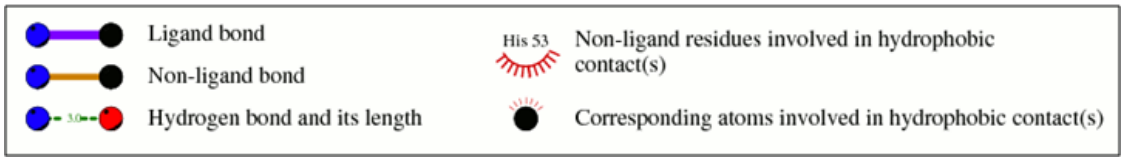


Fig. 3.6b Interaction of cyclosaplin with various cancer-related proteins. e) PTEN f) MMP-2 g) Procaspase 3 h) Procaspase 7



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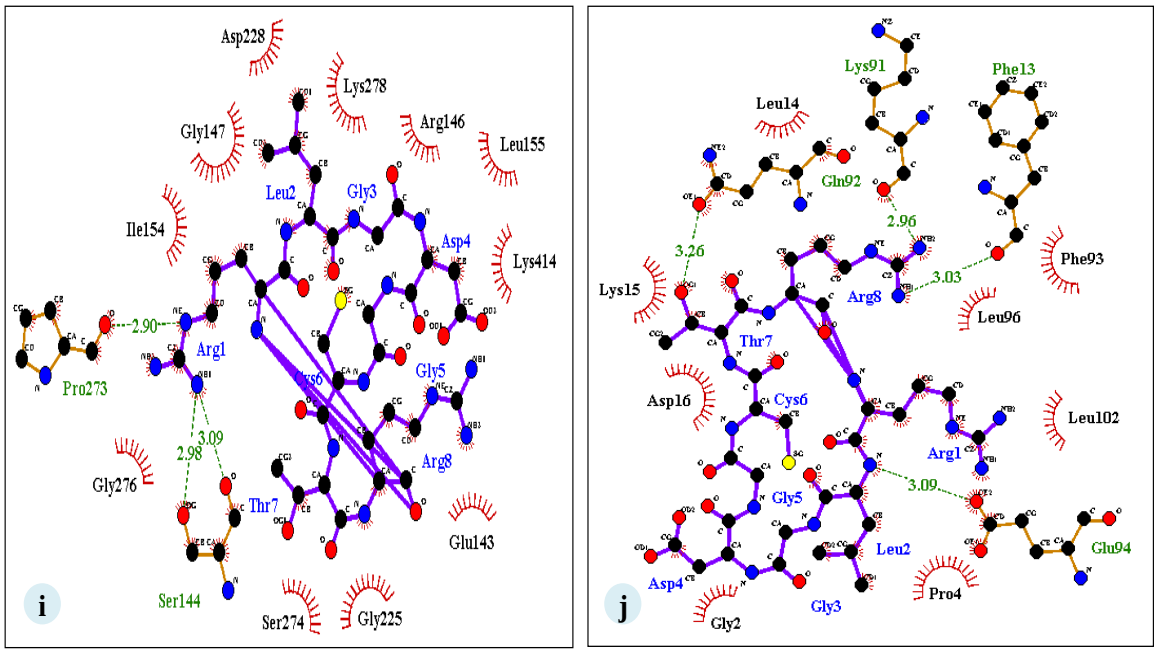

Fig. 3.6c Interaction of cyclosaplin with various cancer-related proteins i) Caspase 9 j) SURVIVIN

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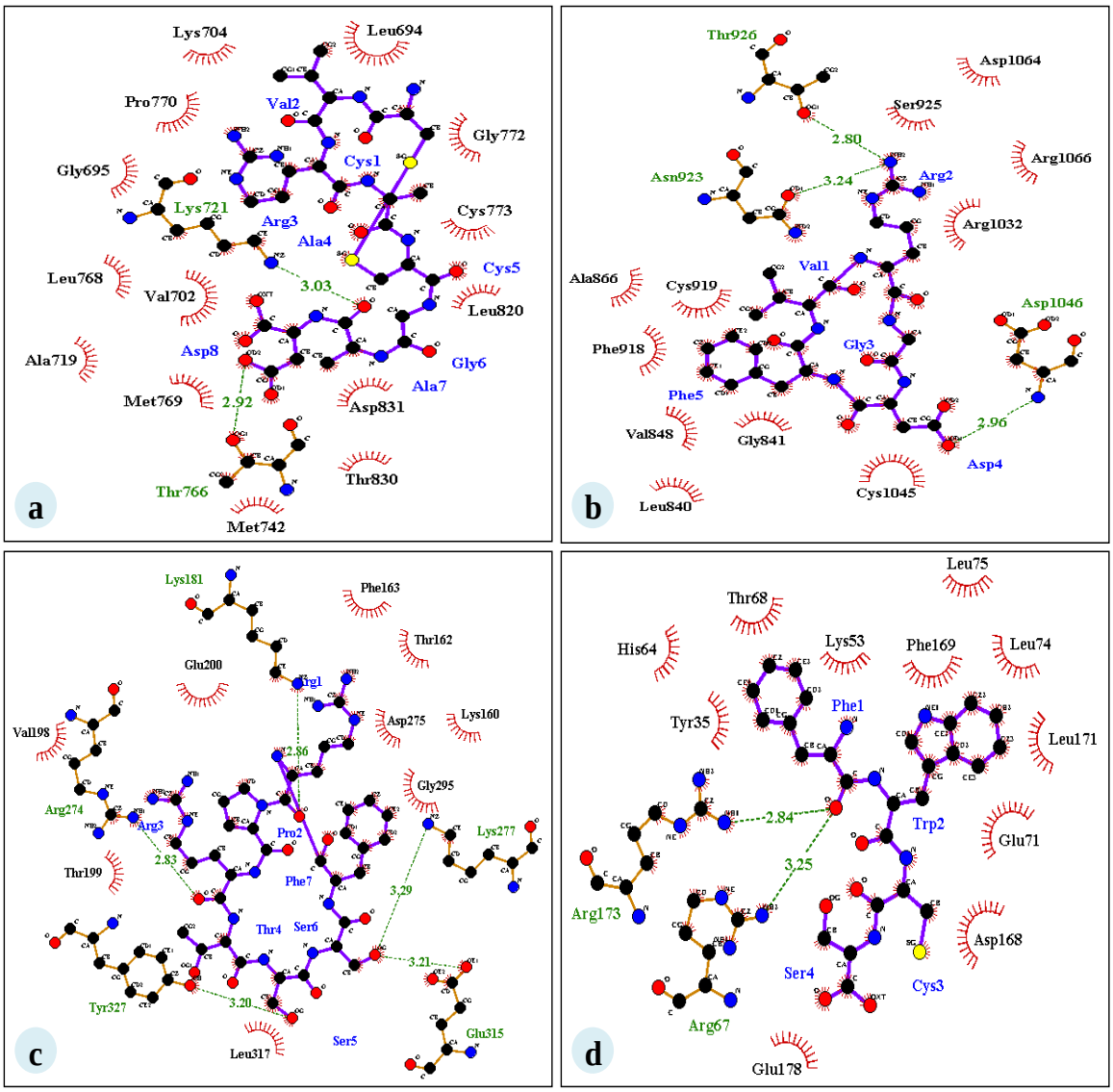

Fig. 3.7a Protein-ligand interactions using LigPlot. a) CVRACGAD and EGFR kinase b) Cilengitide and VEGFR2 kinase c) RPRTSSF and PKB d) FWCS and p38.

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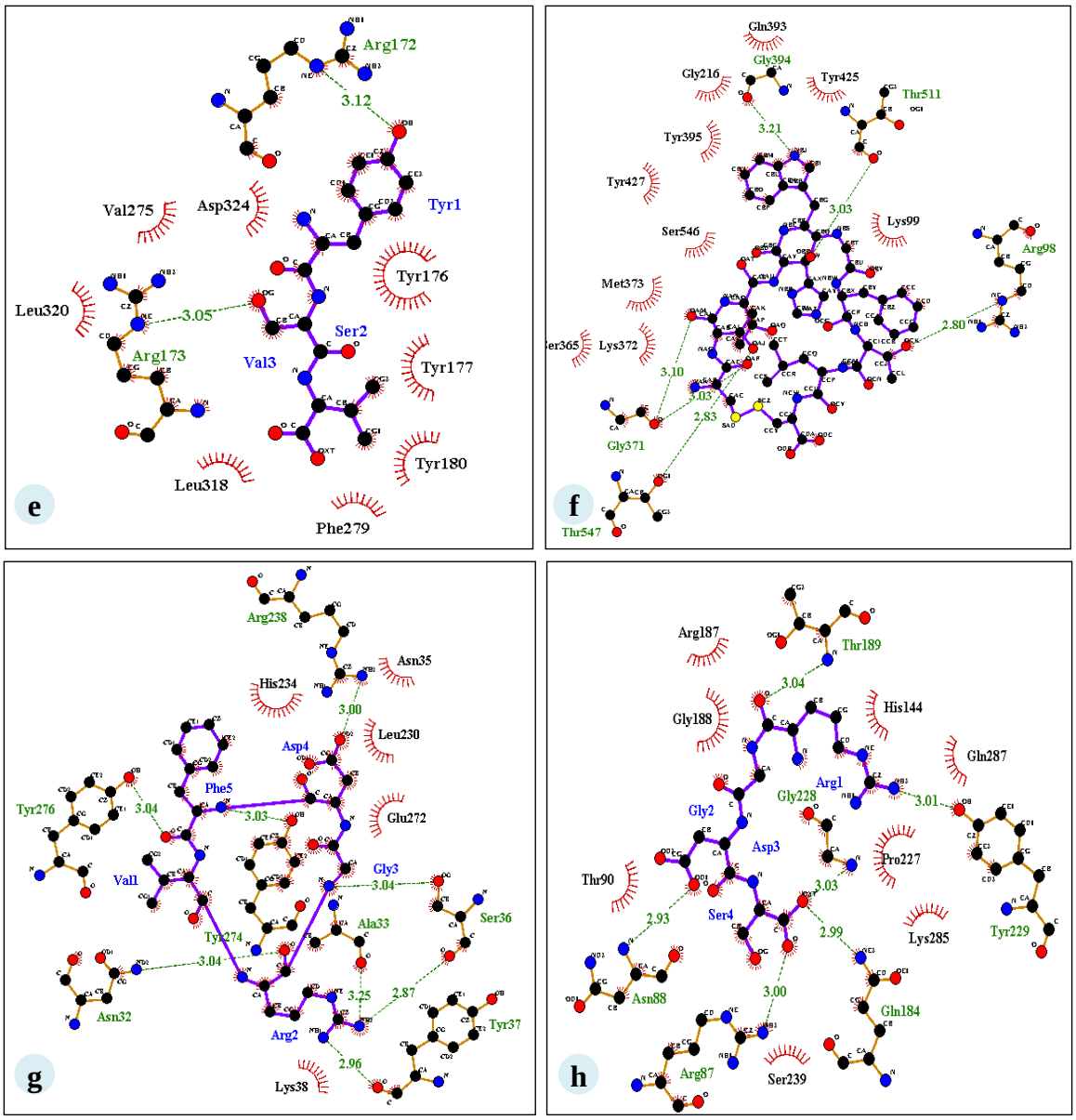

Fig. 3.7b Protein-ligand interactions using LigPlot. e) YSV and PTEN f) CTTHWGFTLC and MMP-2 g) Cilengitide and Procaspase 3 h) RGDS and Procaspase 7.

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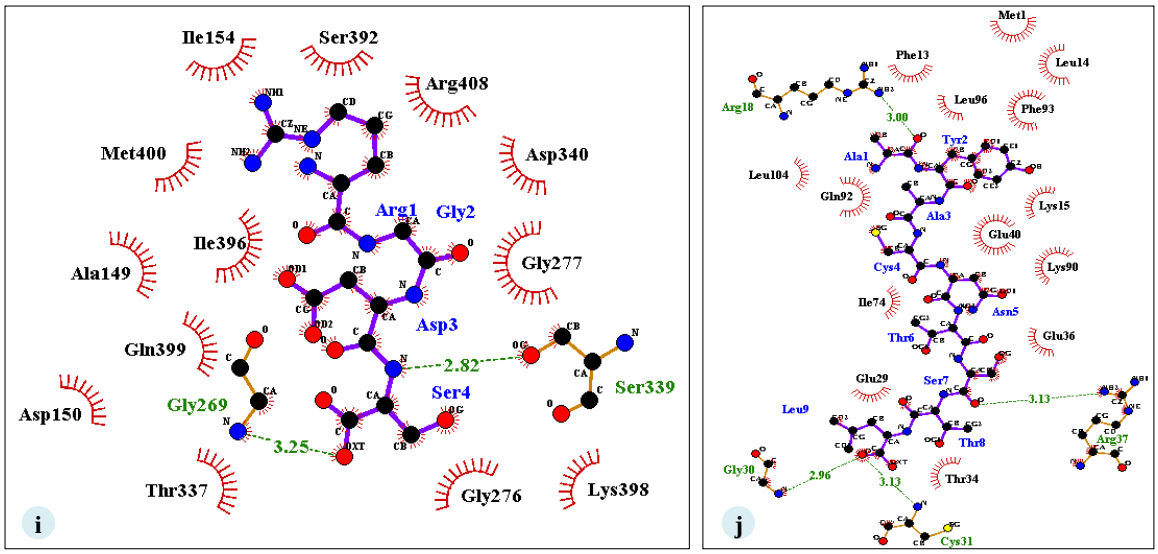
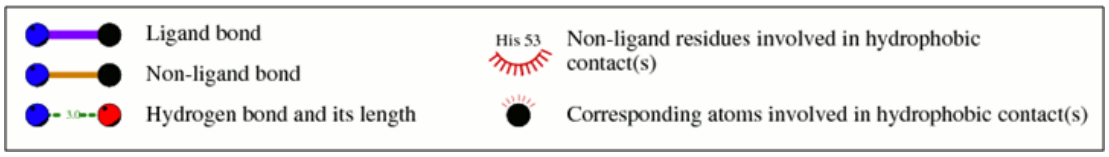


Fig. 3.7c Protein-ligand interactions using LigPlot. i) RGDS and Caspase 9 j) AYACNTSTL and SURVIVIN.



4. DISCUSSION

Peptides are effective receptor-binding ligands; many other classes of ligands sharing this binding trait include small molecules, endogenous proteins, and antibodies [17]. Cyclic peptides have built-in stable pharmacokinetic characteristics, including enzyme stability, conformational rigidity, improved receptor site selectivity and pharmacological specificity. In addition, cyclic peptides are reported to be potent protein kinase inhibitors, protease inhibitors (MMP-2 and MMP-9), angiogenesis blocker, and apoptosis inducers [18-21]. In comparison to small molecules, cyclic peptides can be more selective whereas, the size of molecule can be smaller than protein molecules such as antibodies and growth factors. So, in the present study an attempt was made to investigate the potential of cyclosaplin and other reported peptide-based ligands against specific cancer-related proteins.

In our previous study, cyclosaplin was isolated, purified, and characterized from Santalum album L. [9]. The cyclosaplin was molecularly modeled and the energy minimized structure was further used for docking studies (Fig. 3.1). The ligands were energy minimized prior to docking studies (Table 3.2, Fig. 3.2). All the peptide-based ligands along with cyclosaplin were screened for Lipinski's rule of five (Table 3.3). Some of these peptides violated the rule yet displayed drug like properties in the experimental studies *in vitro*. Cyclic peptides tend to have properties (e.g., MW, number of polar atoms, total polar surface area) that put them outside conventional predictors of "drug-likeness" such as Lipinski's rule of five [12]. Inspite of this, many compounds exhibit drug like properties, including the potential to penetrate cellular membranes. The potential targets of cyclosaplin were predicted by Swiss Target Prediction [13] (Fig. 3.4a) and the proteins used in docking studies were energy minimized and represented in Fig 3.4b. Relative binding affinities were scored for the cyclosaplin and peptide-based ligands, represented as Kcal/mol (Table 3.4). The affinity value of less than 5 depicts negligible binding whereas values closer to 10 indicate efficient binding. In addition, the docking scores for various cancers related proteins was represented graphically as shown in Fig. 3.5a. Docking studies revealed the strong binding affinities of cyclosaplin towards apoptosis related proteins procaspase 3 (-7.8 Kcal/mol; Mishra et al., 2014), procaspase 7 (-8.7 Kcal/mol), caspase 9 (-8.9 Kcal/mol), TRAIL (-8.2 Kcal/mol), SURVIVIN (-7.4 Kcal/mol), and protease MMP-2 (-8.2 Kcal/mol) (Fig. 3.5a-b). Cyclosaplin also demonstrated effective binding affinities towards other cancer-related proteins such as EGFR (-6.8 Kcal/mol); [9], VEGFR2 (-7.8 Kcal/mol), PKB (-8.1 Kcal/mol), p38 (-8.3 Kcal/mol), PTEN-tumor suppressor (-6.3 Kcal/mol), and MMP-9 (-7.3 Kcal/mol) (Table 3.4, Fig. 3.5a-c). The peptide-based ligands (positive control) reported in the literature or under clinical studies showed strong binding affinities with the specific proteins except for TRAIL (Fig. 3.5c). In case of TRAIL, the

ligand remained unbound to the protein with a low score (-6.4 Kcal/mol). The result indicated the possible role of cyclosaplin in mediating apoptotic cell death. In contrast to most small molecule drugs, peptides have high affinity, strong specificity for targets, and low toxicity, whereas in contrast to chemotherapeutics antibodies, they have good penetration of tissues because of their small size [22-25]. Cyclization is also thought to minimize conformational entropy losses upon target binding, although some studies have shown the impact of cyclization on binding entropy to be more complex [26]. The interaction of the cyclosaplin and other peptide-based ligands with amino acids of various cancer-related proteins were also determined (Table 3.5). We previously showed the structure-activity relationship for EGFR kinase with cyclosaplin [9] but in the present study, we demonstrate the possible interactions between Protein-ligand with key amino acid residues involved in such interactions. In case of EGFR kinase, the peptide inhibitor CVRACGAD (cyclic) showed no similar interactions with cyclosaplin for amino acid residues of the protein (Table 3.5). The cyclosaplin interacted with Asp 960/Glu961, Ser787/Tyr789 forming H-bonds and hydrophobic contacts respectively (Table 3.5, Fig.3.6A, SB.1). Asp-960/Glu-961 facilitates in the movement of the C-terminal tail of the EGF receptor to regulate asymmetric dimmers formation [27]. The side chain of Asp-960 interacts with that of Ser787 and mutation at this site enhanced protein kinase activity [27] whereas Tyr789 is the site for phosphorylation, new potential binding site from the catalytic domain of EGFR [28]. The positive control CVRACGAD (Fig. 3.7A, SB.2) forms H-bond with Lys 721 whose side chains interact with the ATP forming salt-bridges in activated kinases [29]. In addition, it interacts with glycine-rich nucleotide phosphate-binding loop (Gly695-Gly700) and DFG motif (Asp831-Gly833) within the A-loop [29]. The interaction between cyclosaplin and EGFR kinase occurs on Asp960, Glu961, Ser787, and Tyr789 with significant binding affinity. The residues mentioned above played a key role in dimer

formation and the site for phosphorylation respectively; highlighting that cyclosaplin could inhibit EGFR kinase by interacting with C-terminal region of EGFR (Table 3.5, Fig. 3.6A). Interestingly, certain common amino acid residues of most of the proteins shared trans-similarity for example, residues involved in H-bond formation in cyclosaplin matched with residues forming hydrophobic contacts in peptide-based ligands. It is not necessary for hydrophobic interactions to occur between the amino acids only with hydrophobic side chains. It can occur between all the amino acid residues depending on their total hydrophobicity [30]. The architecture of VEGFR-2 involves several important loop domains including glycine-rich loop (also refers to nucleotide binding loop) at residues 841–846, the catalytic loop at residues 1026–1033, and the activation loop at residues 1046–1075 [31]. The active sites around the ATP-binding domain of VEGFR-2 consist of three hydrophobic regions (region 1–3) as well as one polar region (region 4). Between the region 1 and the region 2, Lys866, Glu883, as well as Asp1044 are crucial for receptor activation [31]. Region 3 contains only a few residues including Leu838 and Phe916. The unique polar region involves several residues such as Asn921, Cys1043, Arg1030 and Asn1031 [32]. The interaction between cyclosaplin and VEGFR2 occurs on Glu 885, Asn923, Asp1046, Cys919, and Lys868 with strong binding affinity indicating that cyclosaplin could inhibit VEGFR-2 activity by interacting with the ATP-binding site of VEGFR-2 (Table 3.5, Fig. 3.6A, and Fig. 3.7A, Appendix SB.1). Similar residual interaction occurred in the case of antiangiogenic peptide, cilengitide and VEGFR2 kinase. It is envisaged that a fixed geometry ascertained due to cyclization in peptides could help it bind to receptors more effectively. The RGD peptide or RGD-like peptides are good examples of cyclic peptides as receptor binding molecule (SB.3). The binding affinity of cyclosaplin towards α5β3 was closer to 10 (-9.5 Kcal/mol), indicating strong binding (SB.4). Some common amino acid residues like Arg274, Asp275, Lys181, Phe163, and Thr199 of PKB interacted with both

cyclosaplin (RLGDGCTR) and RPRTSSF (Table 3.5, Fig. 3.6A, and Fig.3.7A, Appendix S1-S2). Mutational analysis of Arg274 in Akt2 is essential for shielding Ther308 in the activation loop against dephosphorylation [33]. The α-helix at C-terminal (αC helix) of the N lobe plays a vital role in regulating the catalytic functions in all the protein kinases [34]. In the inactive state of PKB, His 196 and Glu 200 of the αC helix (are disordered, and contacts between Glu 200 and Lys 181, and those between His 196 and pThr 309 are not formed [34]. The interaction between cyclosaplin and PKB occurs on Arg274, Lys181, Phe163, Thr199, Tyr273, and Leu183 with strong binding, indicating its possible role as PKB inhibitor (Table 3.5, Fig. 3.6A). Moreover, the above interacting amino acid residues are also common to RPRTSSF, the positive control used in this study. In p38, both the peptide based ligands (FWCS and cyclosaplin) had interaction with common amino acid residues involved in phosphate and ATP binding sites (Table 3.5, Fig. 3.6A, and Fig. 3.7A, Appendix S.1-S.2). Among all of the MAP kinases, the phosphorylation sites (Thr-180 and Tyr-182), and the putative phosphate binding ligands (Arg67, Arg70, Arg149, Arg173, Arg186, and Arg189) are conserved in homologous positions, and thus may interact similarly in different active MAP kinases [35]. The available structural data revealed that most of the small molecule inhibitors of protein kinases bind in the ATP binding pocket [35,36]. ATP binding sites of p38 are the residues corresponding to Glu71, Lys53, and Asp168 [37]. Some of the amino acids of MMP-2 interacted with both the peptide ligands (CTTHWGFTLC and cyclosaplin) forming H-bonds and hydrophobic contacts (Fig. 3.6B, Fig. 3.7B). Similarly, in the case of procaspase 7 (Fig. 3.6B, Fig. 3.7B), caspase 9 and survivin (Fig. 3.6C, Fig. 3.7C) a few amino acids shared similar interactions with both the peptide-based ligands (Positive control; cyclosaplin). In caspase 9, Gly276 interacted with both RGDS and cyclosaplin forming hydrophobic contact whereas in survivin Gln92, Phe13, Phe93, Leu14, Leu96, and Lys15 formed interactions with both the peptide

based ligands. No common interactions were observed in case of PTEN, MMP-9, and Procaspase 3 (Fig. 3.6B, Fig. 3.7B) whereas the positive control failed to interact with TRAIL. Arg1 and Arg8 of cyclosaplin well interacted with amino acid residues of cancer-related proteins. This could be possible because Arg side chains provide positive charges as well as hydrogen bonding capabilities to attract the peptide to the negative surface charges of the protein. Earlier, the binding affinity of cyclic peptide TYY along with its interaction with EphA4 receptor tyrosine kinase by using AutoDock 4 and LigPlot have been reported [38]. Previously, in 3D cell culture the efficacy of cyclosaplin has been shown as an anticancer agent [39]. Apart from anticancer activity, the other biological activities such as antimicrobial activity, antiviral activity, and immunomodulatory function needs to be investigated for cyclosaplin. In this context several analogs of cyclosaplin can be designed and screened *in silico* for above mentioned biological activities prior to *in vitro* studies. Thus, the *in silico* experiments gave a clear insight of cyclosaplin potential as an apoptosis inducer and a potential protein kinase inhibitor.

5. Conclusion

The structure of cyclic octapeptide was elucidated previously by molecular modeling associated with dynamics and was used in the docking studies. Docking studies showed strong affinity of cyclosaplin towards cancer-related proteins especially protein kinases and apoptosis related proteins. Thus, the in silico analyses revealed potential of cyclosaplin as an apoptosis inducer and a protein kinase inhibitor. Based on these studies, appropriate *in vitro* and *in vivo* experiments can be designed rationally to validate its biological activity.

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Conflict of interest

The authors declare no conflict of interest

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