

Research Article**Recognition of New Inhibitor of CDK9/Cyclin T1 Complex
as Persuasive Anticancer Agent****Afzal Hussain* and Chandan Kumar Verma**Department of Bioinformatics,
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ABSTRACT:

Cyclin Dependent Kinase regulate the Cell cycle process, which goes from G1, S, G2, M phase. CDK9/Cyclin T1 complex plays a very vital role in the progression of the Cell cycle in the form of the transcription elongation. Inhibition of that target reduces several diseases like Alzheimer's, AIDS, Cardiac Hypertrophy, Cancer and several Inflammations. Structure Based Drug Design is a key approach for finding out the best drug candidate, which reduces the target functionality, to carry out our research we have used virtual screening and docking procedure using the Glide tool of the Schrodinger software and ADMET, MMGBSA and DFT analysis also be done for the same. Results uncovered 12 inhibitors, designated by Maybridge HitFinder ids as MH-12988, MH-11507, MH-12294, MH-9564, MH-3736, MH-10478, MH-6996, MH-7270, MH-12662, MH-14066, MH-13114, and MH-13757 which shows docking scores higher than Flavopiridol (an anti cancer drug). Compound A or (MH-12988), 1-{2-[4 diethylamino) phenyl] hydrazono} -1,2 dihydronaphthalen-2-one was found to be more potent and selective as an inhibitor. Hopefully in the near future the compound (MH-12988) could be used as anticancer agents.

Keywords: 3BLR, CDK9/Cyclin T1, MH, Virtual Screening, Kinase, Anti Cancer.**Abbreviation**

MH	Maybridge HitFinder
CTD	Carboxy Terminal Domain
P-TEFB	Positive Transcription Elongation Factor B
CDK9	Cyclin Dependent Kinase 9
SBVS	Structure Based Virtual Screening
DFT	Density Functional Theory
MW	Molecular Weight
HOMO	High Occupied Molecular Orbital
LUMO	Lowest Unoccupied Molecular Orbital
ATP	Adenosine Tri Phosphate

[I] INTRODUCTION

Cell cycle is the process where the Cell goes through from different phases of the Cell cycle like G1, S, G2 and M. This process is controlled by the CDK family [26]. CDK regulation and deregulation is associated with diverse death leading diseases such as Cancer, Cardiac Hypertrophy and Acquired Immunodeficiency

Syndrome (AIDS) [18]. CDK plays different role like CDK2 play crucial role in apoptosis (Cell Death), CDK5 role in neuronal Cell. CDK7, CDK8 and CDK9 involve in the transcription process [15]. One of the CDK is CDK9 which was first identified in a cDNA screen. It is an active target, engages in the transcription

elongation phase when it interacts with its Cyclin subunit, Cyclin T1. CDK/Cyclin together makes positive transcription elongation factor b (P-TEFb) [18,23,29]. Transcription is a multi step process where it includes production, pre-initiation, initiation, promoter clearance, elongation and termination.

At least 30 kinases identified which are proficient of phosphorylating transcription and splicing [23,36]. CDK9 consist of C- and N- terminal lobe in between the ATP binding site positioned [18] Fig. 1.

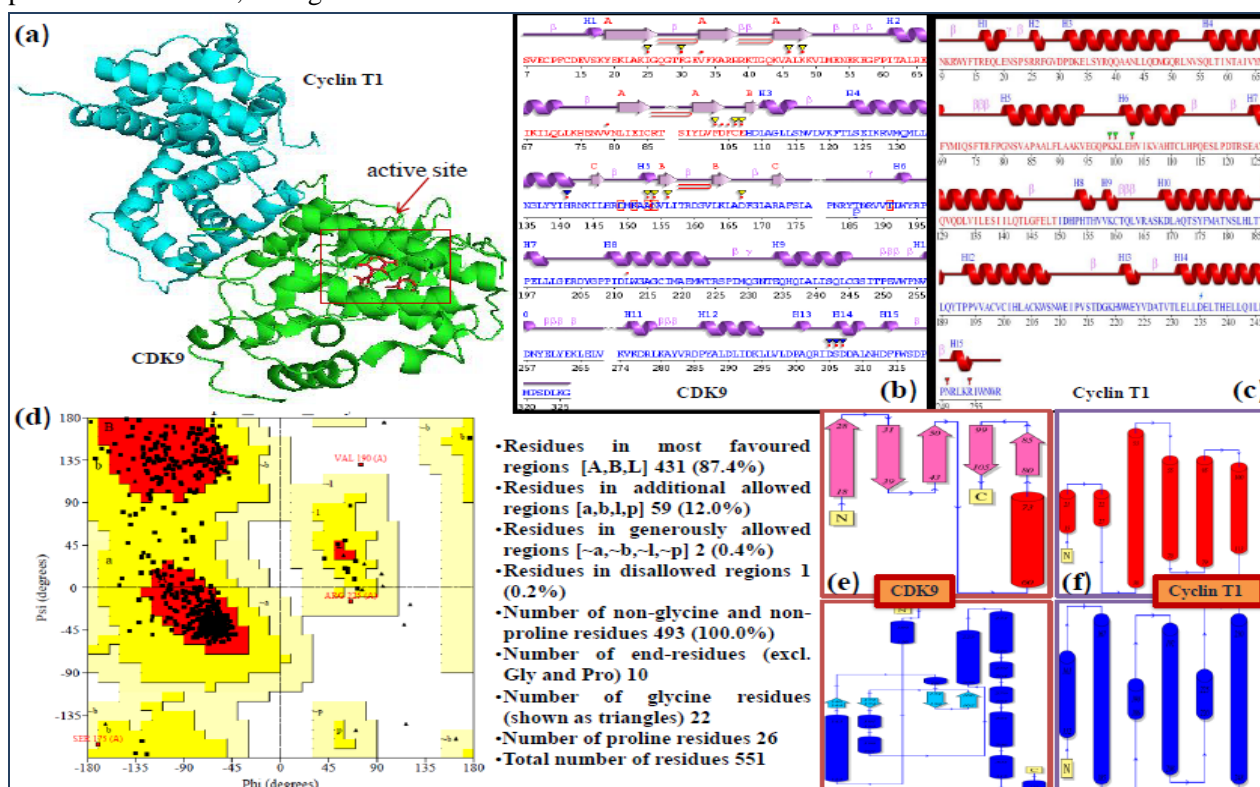


Figure 1. (a) CDK9/Cyclin T1 3D diagram with the active site in red box represented by using PyMOL visualization software. (b) CDK9 secondary structure diagram shows Helix, Sheets and Turns. (c) Cyclin T1 secondary structure diagram shows Helix, Sheets and Turns. (d) Ramachandran plot of CDK9/ Cyclin T1 complex with their statistical data. (e) Domain 1 & Domain 2 of the CDK9. (f) Domain 1 & Domain 2 of the Cyclin T1.

CDK9 action is regulated by the associated Cyclin unit [29]. Different Cyclin unit show interaction with CDK9 like Cyclin T1, Cyclin T2a and Cyclin T2b. Cyclin T1 play major role in the activation rather than T2a, T2b Cyclin subunits [30]. Cyclin K also a regulatory unit of the CDK9 but keeps lesser effectiveness than CDK9/Cyclin T1 complex [12].

Fully activation of the CDK9 kinases requires phosphorylation of its conserved threonine residues [9,18,26,34]. P-TEFb complex phosphorylate the carboxy terminal of the RNA polymerase II. The carboxy terminal

domains (CTD) contain 52 units of heptapeptide. Tyr-Ser-Pro-Thr-Ser-Pro-Ser which is positioned on the RNA pol II subunits [9]. Cell cycle engages in the tumorigenesis, so in the cancer biology CDK inhibitor reduce its characteristics [17]. Cyclin kinases inhibitor (CKI) also plays a very important role for the controlling of the CDK activity at a particular stage [26]. First CDK inhibitor has been found in yeast, which ensures that Cell cycle events do not occur when the first to be completed [28]. Cip and INK4 subunits inhibit the kinases property [29]. A number of small molecule inhibitors have been identified

which bind and cover the ATP binding site of the CDK kinases and disrupt its kinase property. Butyrolactone, Paullones, Flavonoids, Indolinones and other non-specific inhibitor also reduces the kinase action [19,34]. These CDK inhibitor having the antiproliferative property. 5,6 dichloro-1- β -ribofuranosyl benzimidazole (DRB) was the first compound which has been reported, inhibit CDK9 activity [19]. CDK inhibitor evaluated in Restenosis, Alzheimer's disease and Cancer chemotherapy [15]. Flavopiridol is tested in the phase I and phase II clinical trials as potential anti cancer and anti proliferative agent, which shows its negative effect on transcription process [7]. To date 11 kinases inhibitor approved as Cancer treatment by US food and Drug Administration [38]. Some inhibitors shows good inhibiting property and reported like Flavopiridol (**1**) [14,33], DRB (5,6-dichlorobenzimidazole-1- β -D-ribofuranoside) (**2**) [2], CAN-508 [20] (**3**), 2-amino-4-heteroaryl-pyrimidine (**4**), 2 Anilino-4-(thiazol-5-yl) Pyrimidine [35], Roscovitine, CR8 [5,6], LY2857785 [37], dinaciclib [16], KM05283 [21], novel 5-fluoro-*N*,*N* iphenylpyrimidine-2,4diamine[13], derivative of 2-amino-8 hydroxyquinoline [32], phosphonamidate, phosphonate, and phosphinate moieties bearing compounds [27] in the design of novel kinase inhibitors or peptide inhibitors [31] Fig. 2.

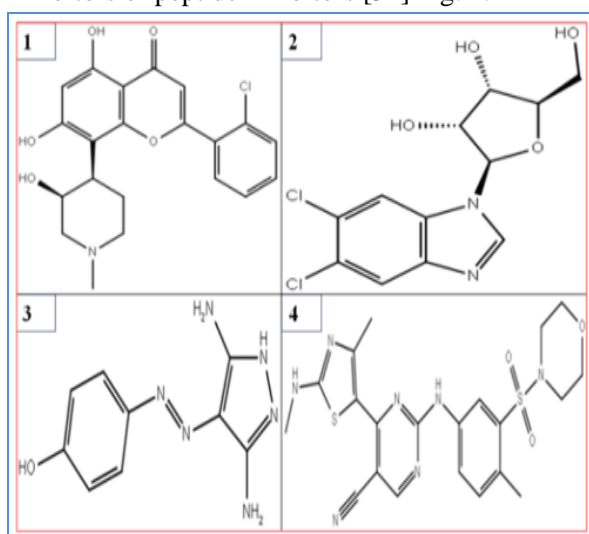


Figure 2. Some Co-crystallized inhibitor with CDK9/Cyclin T1 complex was found in Protein Data Bank.

Structure Based Drug Design is the accurate approach for the development of Cell cycle inhibitor for cancer therapy [8,10]. SBVS technique is very important to find out the drug compound very rapidly from the large library of the compounds in the medicinal chemistry research [25]. CDK9 is the most important therapeutic target for cancer research [18].

A lot of inhibitors have been approved which shows inhibition of cancer progression. Therefore, it is a most important work to find out a selective and novel inhibitor, which can recognize the target active site easily for reducing its infectious properties. In this current study the central aim is to find out a selective and most potent inhibitor for reducing the death leading diseases like Cancer, Cardiac Hypertrophy and Alzheimer's.

[II] MATERIALS AND METHODS

2.1. Protein Preparation

The protein structure has been taken from the Protein Data Bank (PDBID: 3BLR). Prior to docking, protein preparation wizard of the Schrodinger software was used for protein preparation. Following which, all the bond orders were assigned, then hydrogen atoms were added and after that restrained minimization was performed using OPLS force field and RMSD cut off of 0.30\AA . The constraint was kept as default. [3].

2.2. Ligand preparation

A total of 14,400 Ligands in SDF format (Structure Data Format) were retrieved from the Maybridge HitFinder Database. These Ligands were prepared using a LigPrep module of the Schrodinger software. The bond order and the bond angles were assigned after that Ligands minimization was done using OPLS 2005 force field. Epik option was used to keep ligand in the right protonation state in biological conditions [1].

2.3. Grid preparation

The ligand binding site has been selected for generating the Grid of the target molecule. The

Grid module has been selected for that, In which the partial charge cut off was selected as 0.25 and scaling factor was selected as 0.1 [11].

2.4. Reference compound preparation

The reference ligand compound was retrieved from the co-crystallized protein structure which is the FDA approved drug like Flavopiridol, after that reference ligand was prepared by using LigPrep module and this ligand compound kept as reference for the further docking process.

2.5. Virtual screening

The virtual screening has been performed using Maybridge HitFinder database (14,400 compounds). These compounds and the reference compound used for evaluation of the Lipinski filtration and reactive functionality. Glide protocol of the Schrodinger software was used for the virtual screening procedure. The software applies different phases for screening. In the first phase it goes for the HTVS screening (High throughput virtual screening), In the second phase it goes for SP screening (Standard-precision) and in the last and third phase it goes for the XP screening (Extra-precision). The Maybridge HitFinder database contain a huge number of ligand compounds so after performing HTVS screening remaining 10% compound kept for SP screening and after SP screening remaining 10% compound kept for XP screening for eliminating false-positive results. The virtual screening workflow shown here, which gives the best description of the processes. Fig. 3.

2.6. Density functional theory analysis

Electronic effects of Ligand compounds play a vital role in the pharmacological property. Therefore, all the selected inhibitors were subjected to the Jaguar panel of the Schrodinger software using Becke's three-parameter exchange potential and Lee-Yang-Parr correlation function (B3LYP) theory with 6-31G* basis set for finding out the HOMO and LUMO properties. HOMO energy signifies the region where small molecules

donate the electrons during the complex formation while LUMO energy poses showed where the small molecules accept the electron from the target protein. The differences between the HOMO and LUMO molecular orbital energy show the gap energy which indicates the excitation energy and responsible for the stability and reactivity of the compounds [4].

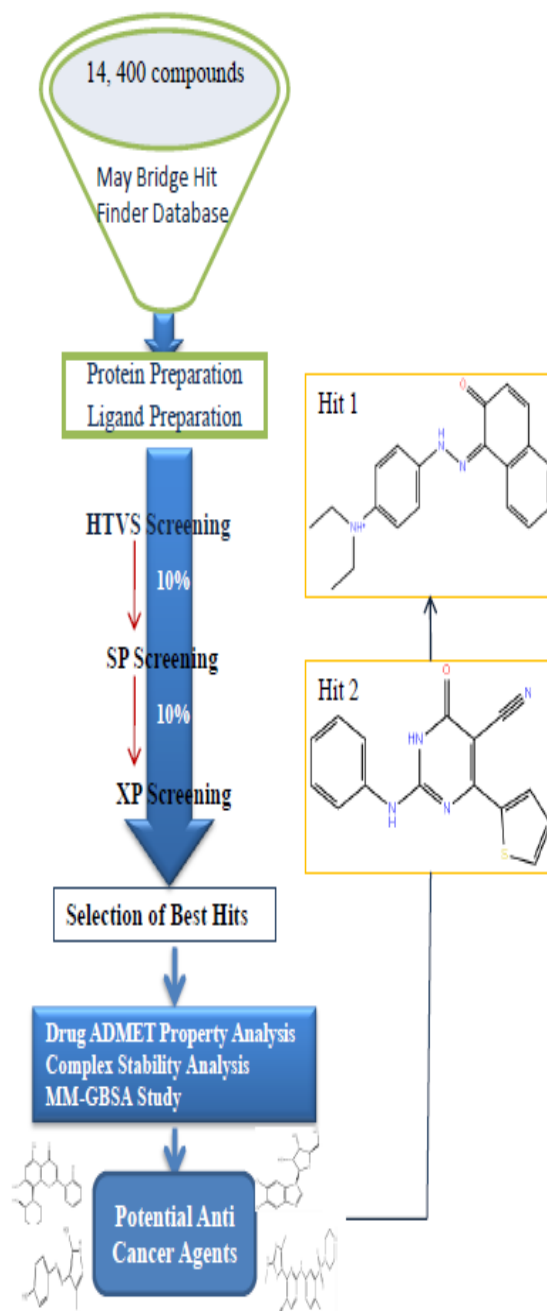


Figure 3. Structure Based Virtual Screening Workflow.

2.7. Drug-likeness prediction

Screened compound has been selected for drug like property analysis. These properties follow the Lipinski rules of five. The properties like Molecular Weight (MW), Hydrogen Bond Acceptor (HBA), Hydrogen Bond Donor (HBD), Predicted Aqueous Solubility (QP log S) and Human Oral Absorption was selected for the prediction [22].

2.8. Drug-Target Binding energy estimation

The MM-GBSA approach was used for drug-target binding energy estimation. It gives the complex (target with drugs) stability [24].

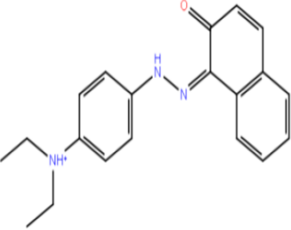
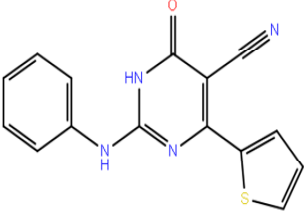
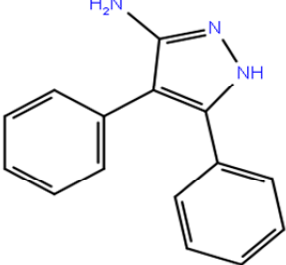
[III] RESULTS

3.1. Virtual screening and docking

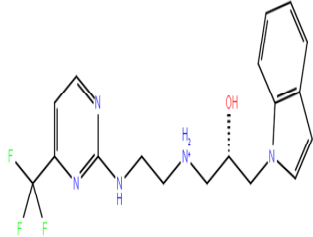
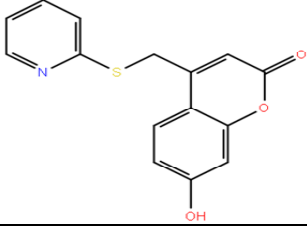
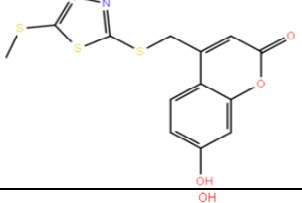
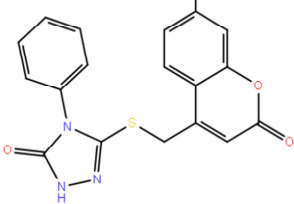
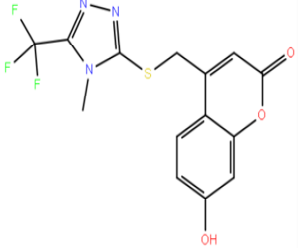
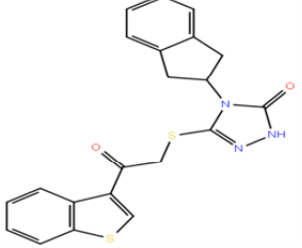
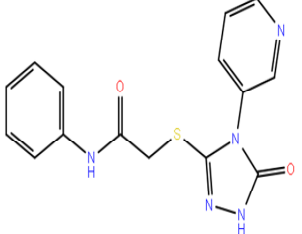
To find out the novel and selective drug compound we have gone through the virtual screening and docking process against Maybridge

HitFinder database. Among 14,400 compounds the twelve best compound has been identified Table 1. Which shows good selectivity and best binding affinity against the target structure. Compound MH-12988 (compound A) is showing good H bonding with the target protein with residues (CYS 106, ASP 109) with docking score -11.963. The docking score is good and the compound MH-12988 cover up the binding site fully. It means it keeps the best binding affinity with the target. Remaining compounds also showing the good docking score, respectively compound MH-11507, MH-12294, MH-9564, MH-3736, MH-10478, MH-6996, MH-7270, MH-12662, MH-14066, MH-13114, MH-13757 having docking score (-11.702, -11.202, -11.195, -11.161, -11.120, -10.999, -10.898, -10.827, -10.826, -10.820, -10.757) The docking and LigPlot interaction diagram of the top twelve ligand with target shown in Fig. 4.

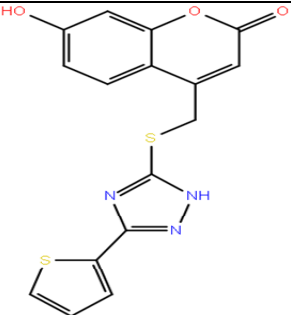
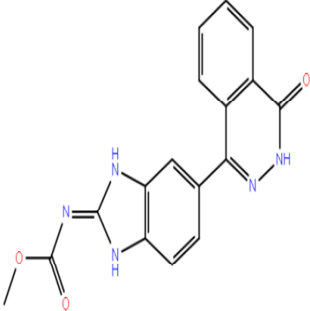
Table 1. 2D structure of the selected inhibitors from Maybridge HitFinder database and reference compounds respectively with their docking scores.

Compound Code	Maybridge HitFinder ID	Structure of Virtual Hits	Mol. Wt.	Mol. Formula	Docking Score
A	MH-12988		319.406	C ₂₀ H ₂₁ N ₃ O	-11.963
B	MH-11507		294.337	C ₁₅ H ₁₀ N ₄ O S	-11.702
C	MH-12294		235.289	C ₁₅ H ₁₃ N ₃	-11.202

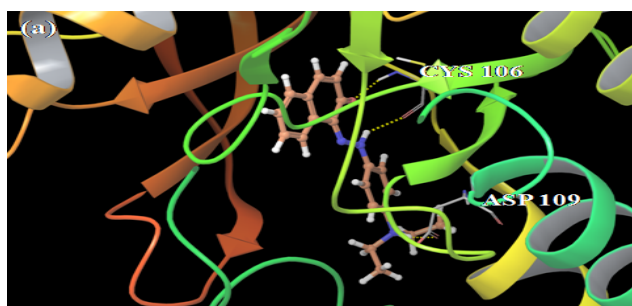
Recognition of New Inhibitor of CDK9/Cyclin T1 Complex as Persuasive Anticancer Agent

D	MH-9564		379.384	$C_{18}H_{20}F_3N_5O$	-11.195
E	MH-3736		285.322	$C_{15}H_{11}NO_3S$	-11.161
F	MH-10478		338.431	$C_{13}H_{10}N_2O_3S_3$	-11.120
G	MH-6996		367.384	$C_{18}H_{13}N_3O_4S$	-10.999
H	MH-7270		357.311	$C_{14}H_{10}F_3N_3O_3S$	-10.898
I	MH-12662		407.516	$C_{21}H_{17}N_3O_2S_2$	-10.827
J	ID -14066		327.367	$C_{15}H_{13}N_5O_2S$	-10.826

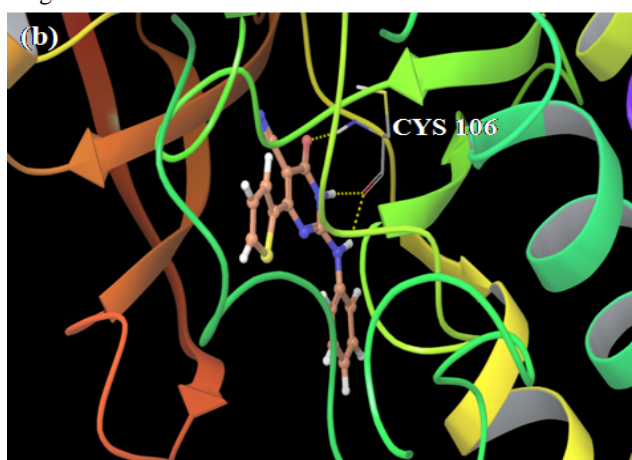
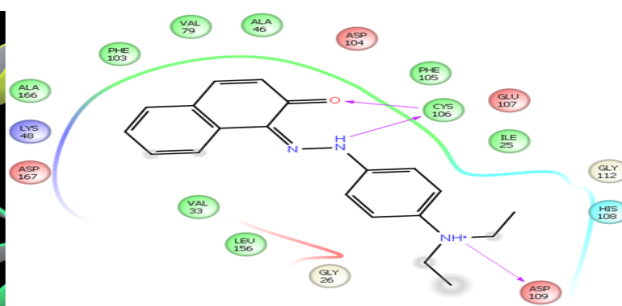
Recognition of New Inhibitor of CDK9/Cyclin T1 Complex as Persuasive Anticancer Agent

K	ID -13114		357.413	$C_{16}H_{11}N_3O_3$ S_2	-10.820
L	ID -13757		335.322	$C_{17}H_{13}N_5O_3$	-10.757
Flavopiridol^r		-	-	-	-10.090

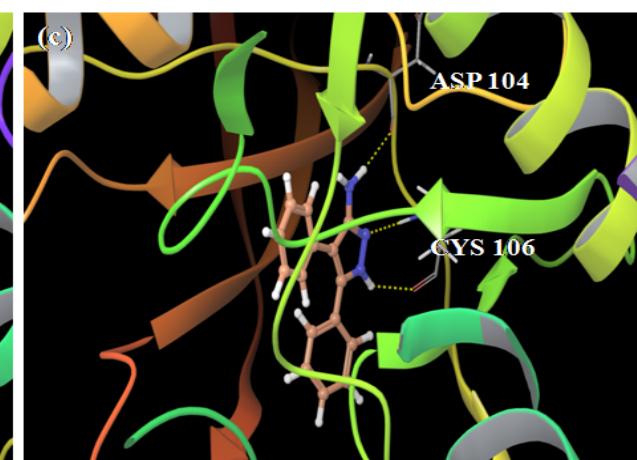
r=Reference compound



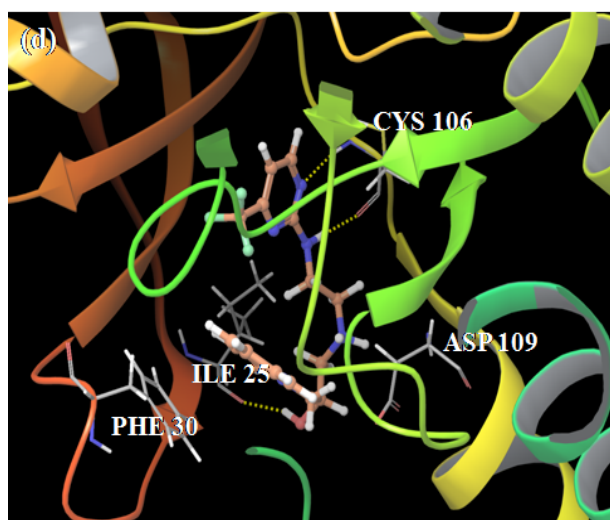
(a) Binding mode of compound A (MH-12988) into the active site of CDK9/Cyclin T1 with Ligplot interaction diagram.



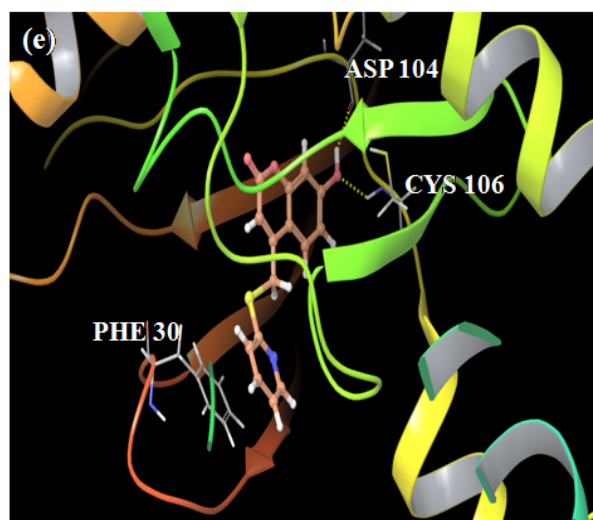
(b) Binding mode of compound B (MH-11507)



(c) Binding mode of compound C (MH-12294)



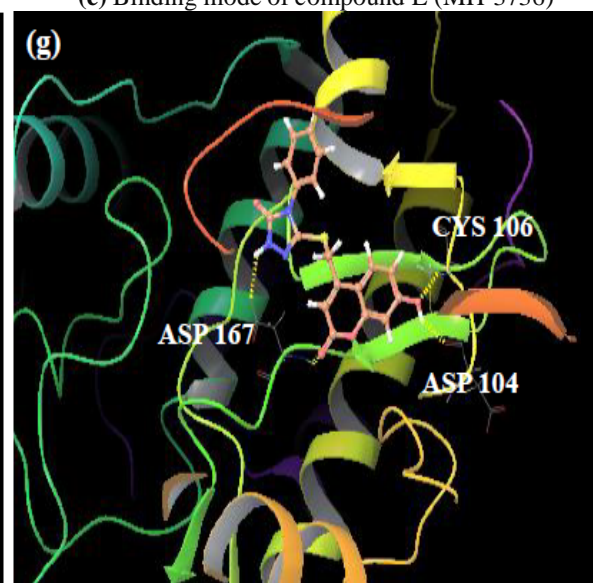
(d) Binding mode of compound D (MH-9564)



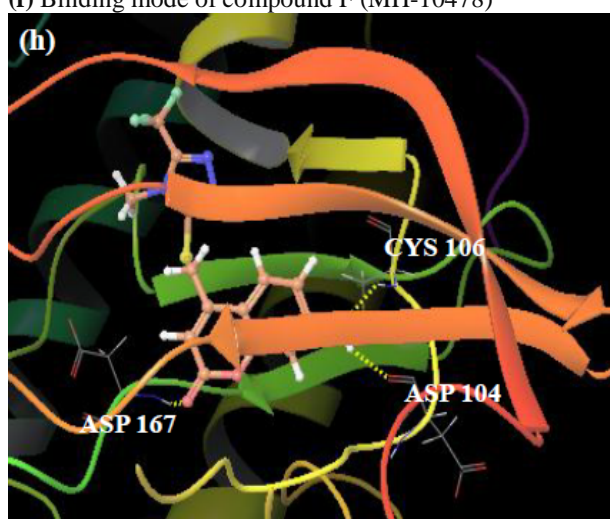
(e) Binding mode of compound E (MH-3736)



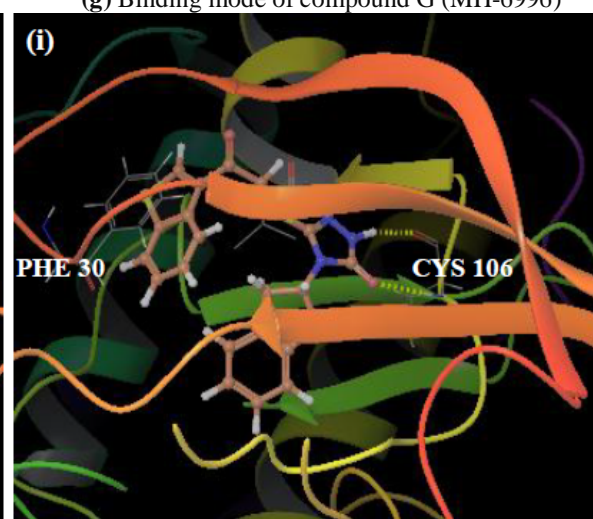
(f) Binding mode of compound F (MH-10478)



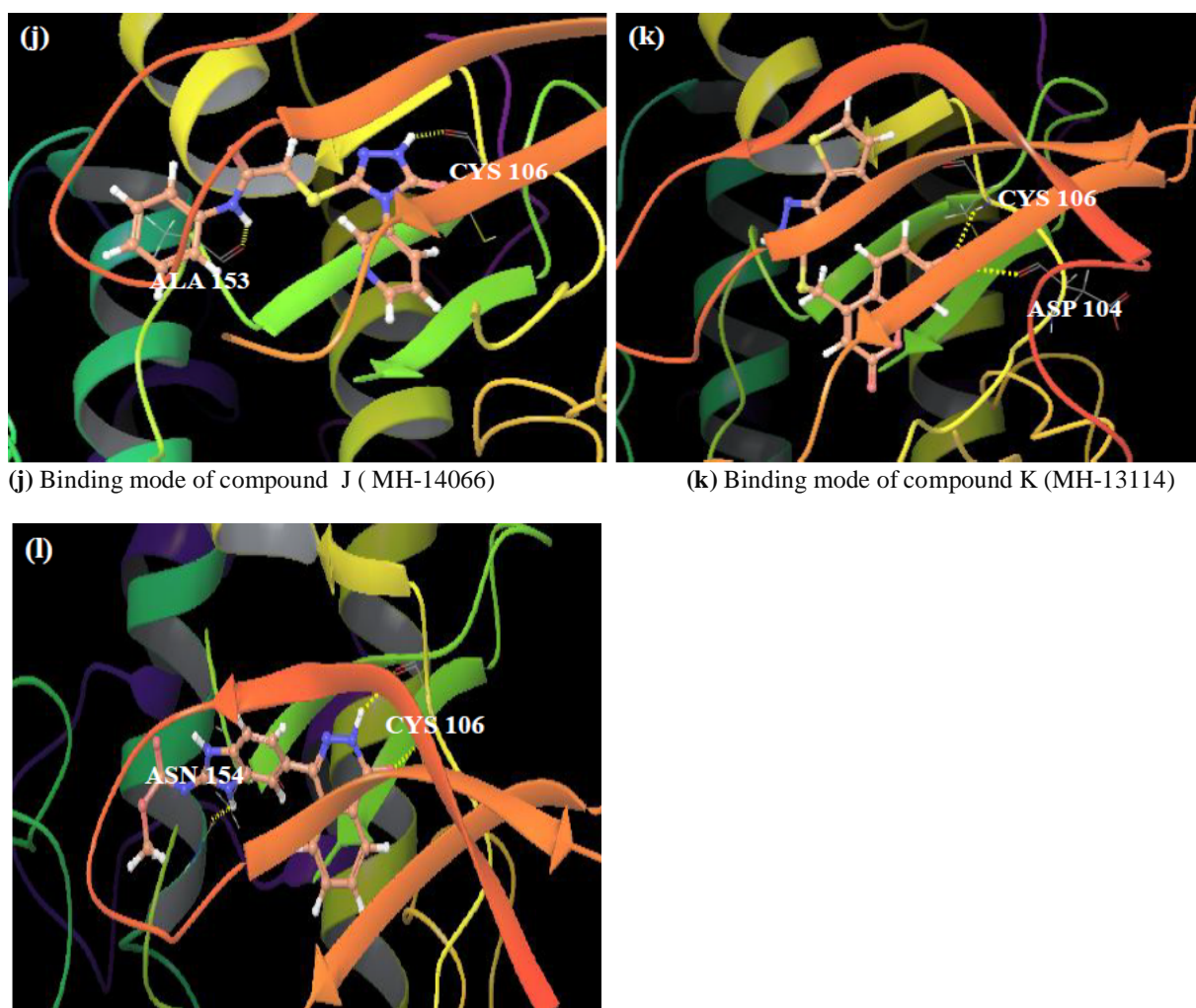
(g) Binding mode of compound G (MH-6996)



(h) Binding mode of compound H (MH-7270)



(i) Binding mode of compound I (MH-12662)



(l) Binding mode of compound L (MH-13757)

Figure 4. Binding mode of the selected top twelve Ligand in the Active site of the CDK9/ Cyclin T1 complex proposed by docking studies. a) Binding mode of compound A. b) Binding mode of compound B. c) Binding mode of compound C. d) Binding mode of compound D. e) Binding mode of compound E. f) Binding mode of compound F. g) Binding mode of compound G. h) Binding mode of compound H. i) Binding mode of compound I. j) Binding mode of compound J. k) Binding mode of compound K. l) Binding mode of compound L. Inhibitors have been shown in stick form and yellow dotted lines indicate the inhibitor protein H-bonding. The critical protein residues have been shown in white color.

3.2. HOMO LUMO stability analysis of the screened compounds

Finding out the stability between drug compounds and the receptor protein, HOMO-LUMO analysis plays a vital role. Hence, the chemical reactivity of the selected inhibitors was analyzed using orbital energy of HOMO and LUMO and the gap energy between them Table 2. HOMO-LUMO diagrams shows the atomic contributions of the orbital Fig. 5. In the HOMO and LUMO diagram, positive

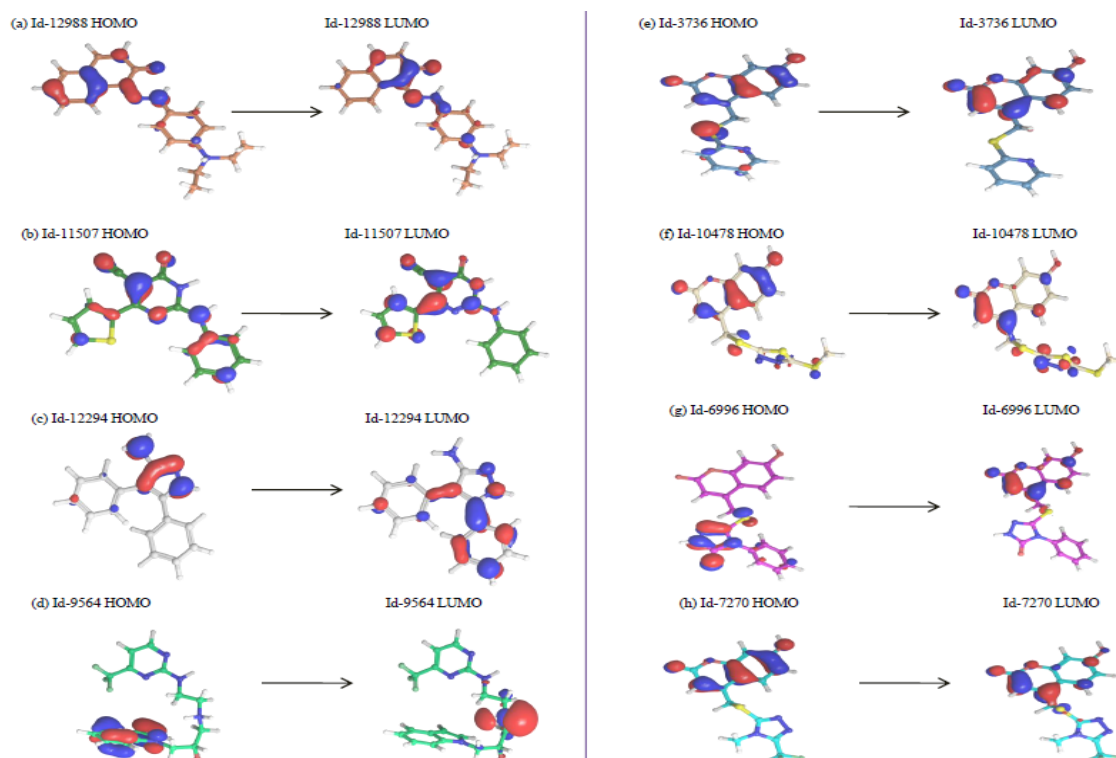
electron density has been represented by red color and the negative electron density has been presented by blue color. The Gap energy between HOMO and LUMO represent the molecular reactivity of the molecules. HOMO LUMO energy of the MH-12988 inhibitor (-0.29735, -0.17827) and remaining inhibitor MH-11507 (-0.22816, -0.08262), MH-12294 (-0.18808, -0.04014), MH-9564 (-0.29471, -0.15031), MH-3736 (-0.22561, -0.05734), MH-10478 (-0.23024, -0.07016),

MH-6996 (-0.22711, -0.06792), MH-7270 (-0.22918, -0.06850), MH-12662 (-0.21635, -0.06810), MH-14066 (-0.22703, -0.04168), MH-13114 (-0.22525, -0.06567), MH-13757 (-0.21423, -0.06152) was found as well as the Gap

energy was found (0.11908, 0.14554, 0.14794, 0.1444, 0.16827, 0.16008, 0.15919, 0.16068, 0.14825, 0.18535, 0.15958, 0.15271) for Maybridge HitFinder selected compounds.

Table 2. Orbital energy of the selected inhibitors of the Maybridge HitFinder database.

S. No.	Compound ID	HOMO energy	LUMO energy	HLG (eV)
1.	MH-12988	-0.29735	-0.17827	0.11908
2.	MH-11507	-0.22816	-0.08262	0.14554
3.	MH-12294	-0.18808	-0.04014	0.14794
4.	MH-9564	-0.29471	-0.15031	0.1444
5.	MH-3736	-0.22561	-0.05734	0.16827
6.	MH-10478	-0.23024	-0.07016	0.16008
7.	MH-6996	-0.22711	-0.06792	0.15919
8.	MH-7270	-0.22918	-0.06850	0.16068
9.	MH-12662	-0.21635	-0.06810	0.14825
10.	MH-14066	-0.22703	-0.04168	0.18535
11.	MH-13114	-0.22525	-0.06567	0.15958
12.	MH-13757	-0.21423	-0.06152	0.15271



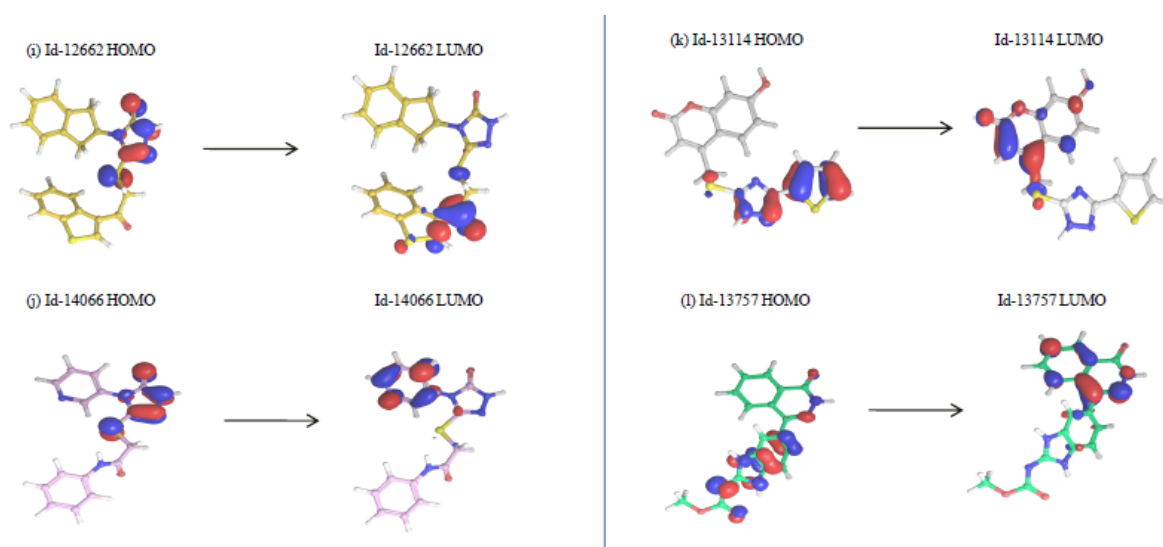


Figure 5. Plot shown highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) of Maybridge HitFinder screened selected compounds. The red color indicates positive electron density while the blue color indicates negative electron density respectively.

3.3. Superimpose Structure

The selected inhibitors from the Maybridge HitFinder database have been superimposed on crystal structure (PDB ID: 3BLR). The result itself showed that all the selected inhibitors

from the compound database occupied the ATP binding site fully and showed the similar binding patterns as the ATP binding shows. The superimposed diagram has been shown in Fig. 6.

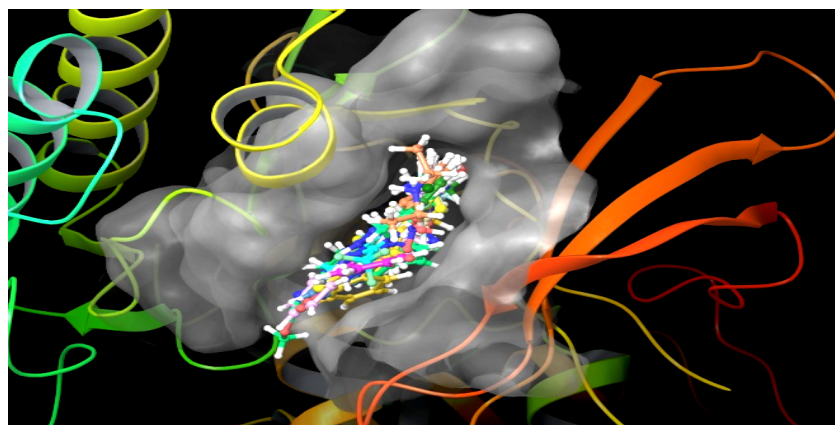


Figure 6. Selected Inhibitors after screened Maybridge HitFinder database has been superimposed in the ATP binding site of the target protein.

3.4. Drug-likeness property analysis

The compounds which has been shown a good binding affinity with the target has been selected for further evaluation. The Drug-Likeness properties was analysis of it. These compounds follows the Lipinski Rules of five. We have selected various properties such as Molecular Weight (MW), Hydrogen Bond Donor (HBD), Hydrogen Bond Acceptor (HBA), Human Oral

Absorption and Predicted Aqueous Solubility. The chemical properties and the characteristics has been shown in Table 3.

3.5. Binding energy assessment analysis

Protein-ligand complex binding energy was analysis using a Prime MMGBSA module of the Schrodinger software. It combines various terms like OPLS Molecular Mechanics Energies (E_{MM}),

Surface Generalized Born Solvation Model for Polar Solvation (G_{SGB}) and a Non Polar Solvation term. (G_{NP}). The total free energy of binding calculation as:

$$\Delta G_{\text{bind}} = G_{\text{complex}} - (G_{\text{protein}} + G_{\text{ligand}}) \quad (1)$$

ΔG_{bind} : total binding free energy of complex

G_{complex} : total energy of the complex

G_{protein} : energy of the receptor without ligand

G_{ligand} : energy of the unbound ligand

$$\text{Where } G = E_{\text{MM}} + G_{\text{SGB}} + G_{\text{NP}} \quad (2)$$

The binding energy of selected compounds was illustrated in Table 4.

Table 3. Drug-like properties of the selected inhibitors from Maybridge HitFinder Database.

S. No.	Compound Id ^a	Mol. Wt. ^b	SASA ^c	Human Oral Absorption ^d	HB donor ^e	HB acceptor ^f	QP log S ^g
1	MH-12988	319.406	681.750	100	0	4	-6.065
2	MH-11507	294.337	543.785	84.44	2	6	-4.932
3	MH-12294	235.289	477.367	96.599	3	2	-3.654
4	MH-9564	379.384	694.406	95.870	3	6	-4.453
5	MH-3736	285.322	501.083	91.557	1	4	-3.412
6	MH-10478	338.431	566.977	87.581	1	5	-4.380
7	MH-6996	367.384	617.201	71.277	2	6	-4.681
8	MH-7270	357.311	566.771	85.073	1	5	-4.279
9	MH-12662	407.516	667.552	100.000	1	5	-6.242
10	MH-14066	327.367	599.570	77.106	2	7	-3.899
11	MH-13114	357.413	596.996	81.906	2	6	-4.769
12	MH-13757	335.322	605.527	77.792	1	3	-5.726

^aMaybridge HitFinder Database Inhibitors Id

^bMolecular weight (acceptable range is: ≤ 500)

^cTotal solvent accessible surface area (acceptable range is: 300-1000)

^dHuman oral absorption (acceptable range is: $<25\%$ less & $>80\%$ high)

^eHydrogen bond donor (acceptable range is: ≤ 5)

^fHydrogen bond acceptor (acceptable range is: ≤ 10)

^gPredicted aqueous solubility (acceptable range is: -6.5-0.5)

Table 4. Prime MM-GBSA energy calculation result of the selected inhibitors from Maybridge Hit Finder database with the target.

S.No.	Compound Id ^a	ΔG_{bind} ^b	G_{vdw} ^c	G_{coul} ^d	G_{covalent} ^e	G_{solGB} ^f	G_{solLipo} ^g
1	MH-12988	-71.44	-42.66	-10.81	4.44	13.54	-34.58
2	MH-11507	-54.31	-38.77	-17.27	0.17	26.34	-24.10
3	MH-12294	-59.88	-31.88	-22.56	0.50	23.00	-26.98
4	MH-9564	-72.36	-43.37	-28.16	8.08	27.90	-33.43
5	MH-3736	-68.09	-40.45	-14.88	2.08	20.18	-32.32
6	MH-10478	-78.80	-41.67	-16.00	1.29	15.80	-37.28
7	MH-6996	-66.91	-49.42	-11.75	7.29	23.49	-34.55
8	MH-7270	-66.30	-45.87	-12.90	5.49	19.25	-31.14
9	MH-12662	-61.44	-35.87	-16.90	10.57	27.91	-40.83
10	MH-14066	-63.72	-42.54	-22.71	1.48	24.34	-23.28
11	MH-13114	-70.88	-48.20	-17.95	8.92	25.41	-37.96
12	MH-13757	-63.38	-47.55	-20.26	4.82	28.30	-26.83

^aInhibitors Id from Maybridge HitFinder database

^bFree binding energy

^cVan der Waal energy

^dCoulomb energy

^eCovalent energy(internal energy)

^fGeneralized born electro-static solvation energy

^glipophilic energy (nonpolar contribution estimated by solvent accessible surface area).

[V] CONCLUSION

In this research work Structure based virtual screening and Docking techniques were used for finding out the selective and most potent drug candidates from the very large number of drug library against CDK9/ Cyclin T1 complex. Top Twelve compounds have been found against 14,400 drug compounds (Maybridge HitFinder database). Lipinski filtration and MMGBSA analysis were also done on selected drug Compounds. Compound 1 {2[4diethylamino)phenyl]hydrazono}-1,2dihydronaphthalen-2-one (MH-12988) was found as most potent and selective with docking score (-11.963) and showing H-bonding with CYS 106, ASP 109 active site residues present in the target protein. It is clear that these compounds could be used as anticancer agents against the Cyclin Dependent Kinase/ Cyclin T1 complex for reducing the death leading disease such as Cancer and Cardiac Hypertrophy.

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