ANTICANCER EVALUATION, AND MOLECULAR DOCKING STUDIES OF SOME NOVEL SUBSTITUTED PYRIDO[4,3-D]PYRIMIDINES AS CYCLIN-DEPENDENTKINASE 2 (CDK2) INHIBITORS

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ABSTRACT:

The wide occurrence of the heterocyclic molecules in bioactive natural products and pharmaceuticals has made them as important synthetic targets. The chemistry of pyrimidines has become increasingly important as a result of recent developments in medicinal chemistry. Two moles of substituted aldehyde and substituted ketones were stirring 12 hrs. Then the mixture was cooled and filtered. Recrystallised with ethanol producing substituted chalcone **(2a-k).** The chalcones are treated with urea refluxed at 80ºC in a heating mantle for 6 hrs in the presence of 10% sodium hydroxide in ethanol produced trisubstituted pyrimidine derivatives **(3a-k).** Cyclin-dependent kinases (CDK) control the cell division cycle (CDC). The deregulation of CDK2 is closely related to many cancers. CDK2 is utilized as one of the most studied kinase targets in oncology. It plays an important role in regulating various events of eukaryotic cell division cycle. Accumulated evidences indicated that over expression of CDK2 should cause the abnormal regulation of cell-cycle, which would be directly associated with hyper proliferation in cancer cells. Therefore, CDK2 was regarded as a potentially therapeutic target for cancer therapy. It is possible to develop pharmacologically relevant cytotoxic agents by specifically inhibiting CDK2 activity with lesser toxicity than traditional chemotherapeutic agents. The *insilico* docking studies was performed Schrodinger Maestro 11.9. From the docking results, The heterocycle of 4-(4-Chlorostyryl)-6-(4-Chlorophenyl)pyrimidin-2-(1H)-one, (AS010) shown interaction with THR-14,LYS-33,LEU-83,ASN-132,LYS-129. However, it was noted that there is a hydrogen bond between the derivatives of Pyrido(4,3-d)Pyrimidine and AS010 was potent inhibitor for CDK2.

Keywords: Pyrido[4,3-d]pyrimidine, Cyclin dependent kinase ,Anti-cancer activity, AS010, Schrodinger Maestro 11.9**,** Molecular docking.

INTRODUCTION:

Pyrazolopyrimidines are versatile scaffolds, which have been exploited for developing potential anticancer agents. Cancer is one of the most serious diseases in the world.The cyclin-dependent kinases (CDKs) play a crucial role in cell cycle progression and are validated targets of cancer therapy[1].Cancer is one of the most serious diseases in the world[2]. Being a basic nucleus in DNA and RNA, the pyrimidine fragment played an important role in pharmaceutical chemistry. Pyrido[1,2-a]pyrimidine also possesses diverse biological activities[3]. Fluorinated organic molecules are known to have a wide range of biological functions and fluorinated anticancer agents have become more and more popular as new therapies for cancer[4].Protein kinases are important molecular drug targets for developing novel anticancer agents and several kinase inhibitors are in clinical trials[5]. These enzymes play a crucial role in cell division, transcription and posttranscriptional modification[6]. It is becoming noteworthy to investigate new druggable molecular targets, identify and develop their modulators as novel drugs for the treatment of cancer. Amongst others, protein kinases have become an important group of drug targets and number of kinase inhibitors in clinical development is rapidly increasing[7]. Fluorinated organic molecules are known to have a wide range of biological functions and fluorinated anticancer agents have become more and more popular as new therapies for cancer[8]. Cyclin-dependent kinase (CDK) is a type of serine/threonine family protein kinase that regulates mammalian cell cycles[5]. It governs the transition from quiescence or cytokinesis to cell proliferation, and through its checkpoints, ensures genome stability[9]. The G1-S phase cell cycle transition is governed by two cyclin-cdk complexes, cyclin D-cdk4/6 and cyclin E-cdk2. Cyclin D-cdk4 (hereafter D-K4) phosphorylates the G1 gatekeeper Rb, causing the release of S-phase specific transcription factors, such as E2F. E2F causes the transcriptional induction of Cyclin E, which in turn partners with cdk2 to further phosphorylate Rb and irreversibly cause the transition into S-phase[10]. Targeting of CDK2/cyclin E activity by RNA interference or by small molecules is effective against human breast cancer cell lines exhibiting *CCNE1* amplification[11]. The binding of CDK to a cyclin protein drive cells from preparation phase (G1 phase) to DNA and cellular division forming two daughter cells (M phase) [12].Different from CDK2, the transcriptional CDK9 appears to have a minimal effect on cell cycle transition[13]. A number of structural diverse CDK inhibitors are currently under active clinical investigations for the treatment of cancer[14,15]. Some of the CDK2 inhibitors were investigated clinically for their potential as anti-cancer agents. In this review, we present the structure, functions and activation of CDK2 by cyclin binding with special focus on recent advances in the development of different classes of CDK2 inhibitors[16]. Even though the targeting of CDK2 does not have to be an optimal strategy for cancer treatment because of the redundancy of CDKs in cell cycle regulation[17]. Maintenance of the pyrimidine nucleus since it forms hydrophobic and hydrogen bond interactions with the hinge region residues at ATP binding site of CDK2[18]. The CDK2 enzyme, which should be useful to aid the designing of new inhibitors with CDK2 improved biological response[19].

MATERIALS AND METHODS:

In continuation of our efforts, herein we report the synthesis and biological evaluationof some novel substituted pyrido[4,3-d]pyrimidines derivatives.All the ten pyrido[4,3-d] pyrimidinesare prepared from standard procedure. Chalcones are converted in to pyrido[4,3-d] pyrimidinesby Sodium hydroxide and urea condensation with standard solvents the compounds are purified using column chromatography the melting points are checked.All the compounds possesanti bacterial and anti fungal activity. Present study deals with the usage of the above compounds for anticancer activity. Chemical structures were sketched by Chemdraw software in Structure Data Format (SDF). The docking studies were performed with standard precision (SP) Glide, and extra precision (XP) Glide and MGBSA Prime in Schrodinger software.

Preparation of Ligands:

Structures of ligands sketched and saved in SDF format were imported via selecting file. The imported ligands (AS001-AS011) were set to minimize under force field OPLS3e. Minimization calculations can be performed on all structures of pyrido[4,3-d]pyrimidinesderivatives.

Preparation of Protein:

X-ray crystalline Structure of protein 2A4L was imported from Protein Data Bank (PDB) to workspace, which further set to preprocess followed by review and modify to remove unwanted chains and residues, further refined under forcefield of OPLS3e. The results were monitored in job monitor.

Molecular Docking:

The compoundsselected for molecular docking have some collective structural features. All the lead compoundsshowed good binding energy and exhibited interactions and better lower free energyvalues, indicating more thermodynamically favored interaction. As for Glide docking, crystal structures of 2A4L have been prepared by the protein preparation wizard in Schrodinger ¨ suite. Afterwards, receptor grids were generated before docking with the active site determined by the position of co crystal ligand. Crystal structures of 2A4L were imported into Glide, defined as the receptor structure and the location of active site with a box. The OPLS3e force field was used for grid generation. The standard precision (SP) and the extra precision (XP) protocols were set for docking studies with crucial residues, in constrained binding to get accurate results. Binding affinity was retrieved running Prime MM-GBSA. All other parameters were maintained as default.

RESULT AND DISCUSSION:

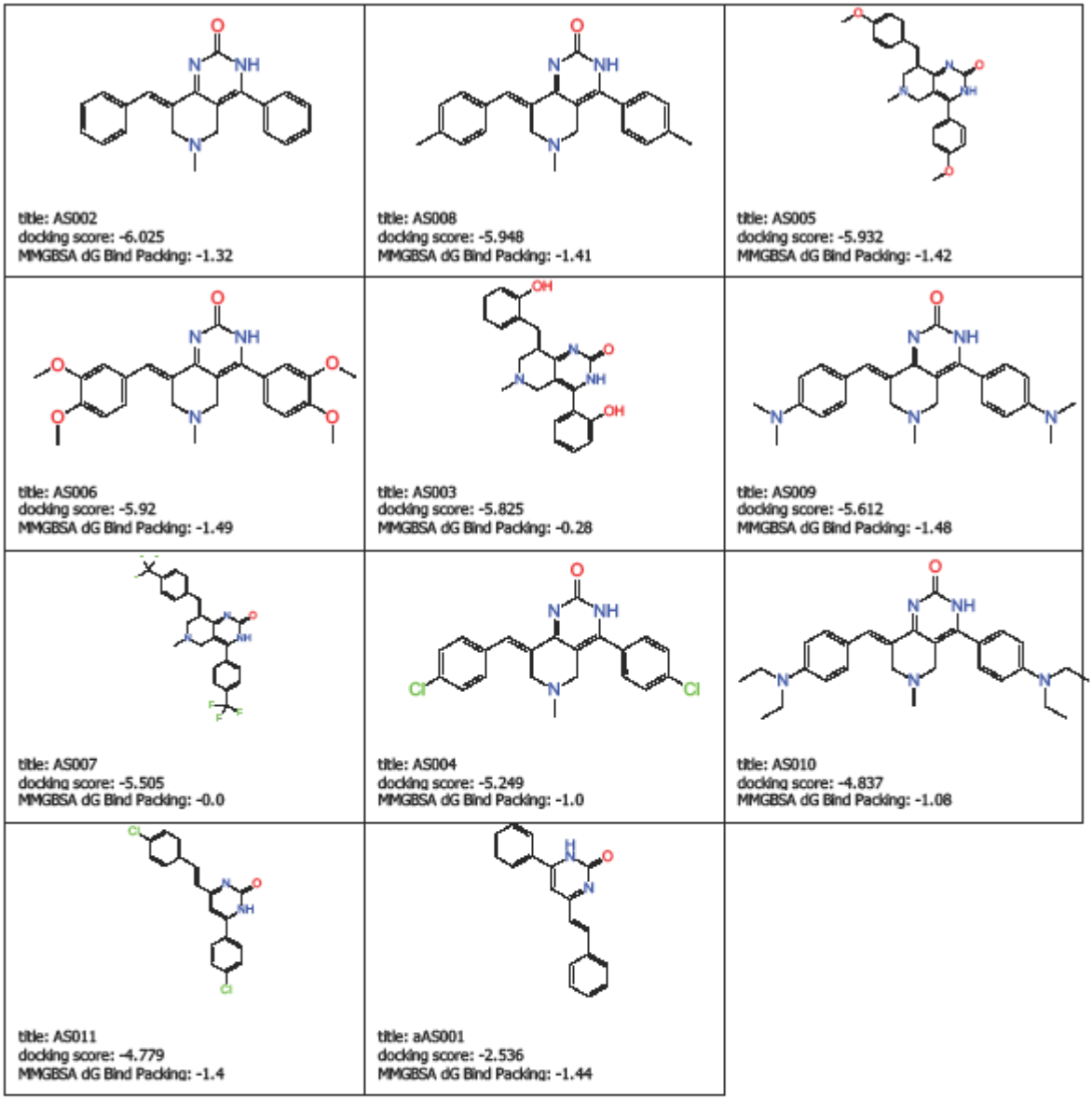
Molecular Docking:

To dock, 11 structures of ligands have been determined. Meanwhile, these ligands were used to conduct native docking to measure the docking conformations. Three different docking programsSP Glide, and XP Glide, Prime MM-GBSAwere used for improving the accuracy of prediction. Then, Xscore followed by molecular docking was reliable and accurate for forecasting protein-ligand binding free energies (Table 1).The docking results were evaluated by comparing values of score energy, SP Glide, XP Glide, and Binding energy. Through analysis of these results of docking simulations, most binding energy scores could accurately forecast the ligand activities. The lowest binding energy and the highest docking score demonstrated that these compounds (ligands) presented well favorable interactions. The docked ligands AS002,AS008,AS006,AS005, pyrido[4,3-d] pyrimidines derivatives showed the best range of Docking score, XP Gscore and Binding energy.(Table 1).

Table 1: Glide Docking and binding energy scores

Title	docking score	glide gscore	glide emodel	XP GScore	Prime Energy	MMGBSA dG Bind
AS002	-6.025	-6.025	-57.87	-6.025	-10947.6	-39.24
AS008	-5.948	-5.948	-57.846	-5.948	-10973.1	-47.06
AS005	-5.932	-5.932	-63.794	-5.932	-10977.4	-49.84
AS006	-5.92	-5.92	-72.705	-5.92	-10946.9	-57.21
AS003	-5.825	-5.825	-59.841	-5.825	-10976.9	-40.16
AS009	-5.612	-5.612	-72.687	-5.612	-10950.2	-48.3
AS007	-5.505	-5.505	-53.779	-5.505	-10924.5	-33.6
AS004	-5.249	-5.249	-61.227	-5.249	-10965.7	-46.94
AS010	-4.837	-4.837	-57.719	-4.837	-10999.1	-40.97

AS011	-4.779	-4.779	-56.971	-4.779	-10972.4	-45.83
aAS001	-2.536	-2.536	-55.077	-2.536	-10958.1	-42.89



Inhibitor Binding Analysis:

The least binding energy and the most rational binding pattern between the inhibitors and 2A4L were Selected by the three docking protocols. As expected, Pyrido(4,3-d)Pyrimidine derivatives (compounds AS001-ASO11) bound in the active site validating the prediction by molecular docking with 2A4L. Among the set, three compounds were selected, which represented good interactions with the target protein, including compounds ASO10, AS009. From the docking results, The heterocycle of 4-(4-Chlorostyryl)-6-(4-Chlorophenyl)pyrimidin-2-(1H)-one, (AS010) shown interaction with THR-14,LYS-33,LEU-83,ASN-132,LYS-129.However,it was noted that there is a hydrogen bond between the derivatives of Pyrido(4,3-d)Pyrimidineand AS010was potent inhibitor.

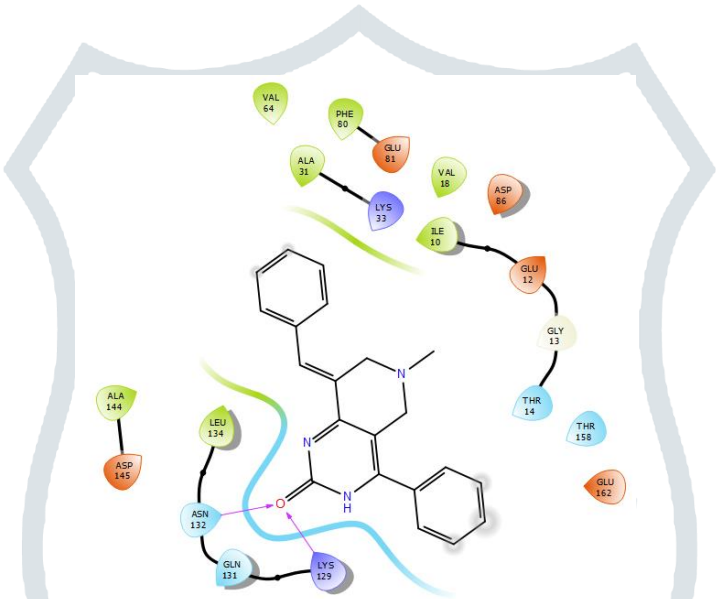


Fig 1. Schematic 2D representation of AS001 in the binding pocket of 2A4L

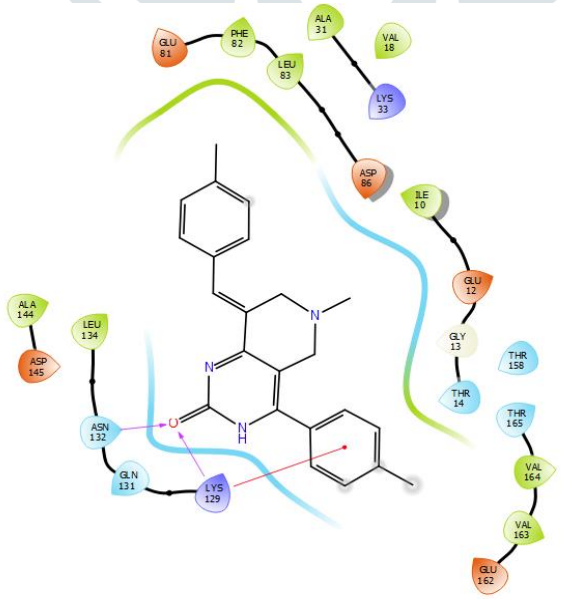


Fig 2. Schematic 2D representation of AS002 in the binding pocket of 2A4L

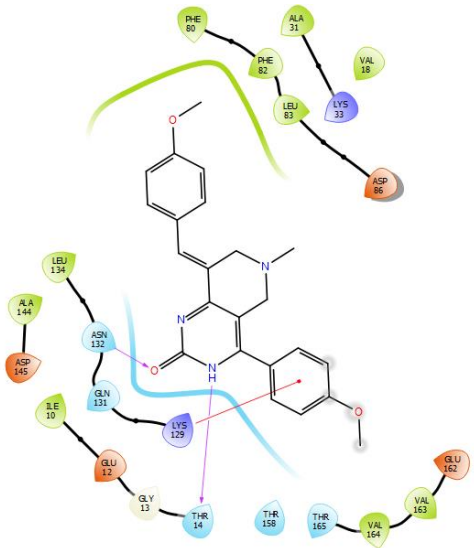


Fig 3. Schematic 2D representation of AS003 in the binding pocket of 2A4L

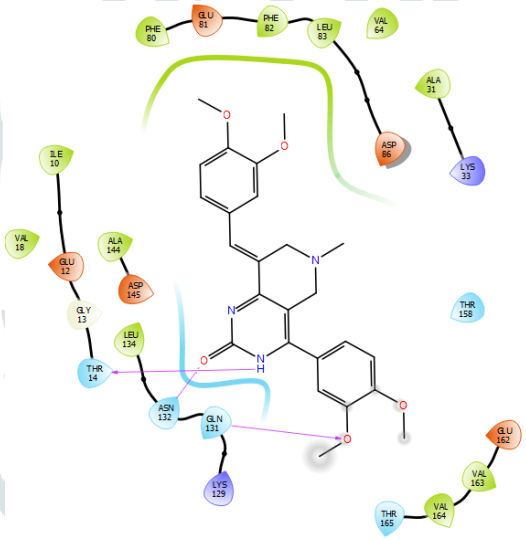


Fig 4. Schematic 2D representation of AS004 in the binding pocket of 2A4L

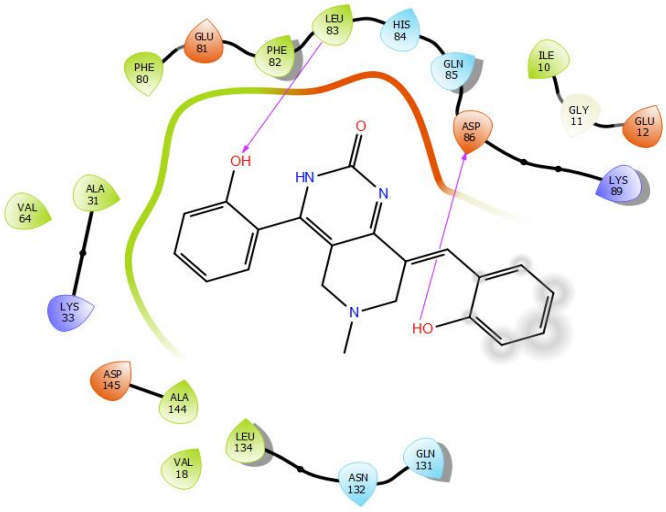


Fig 5. Schematic 2D representation of AS005 in the binding pocket of 2A4L

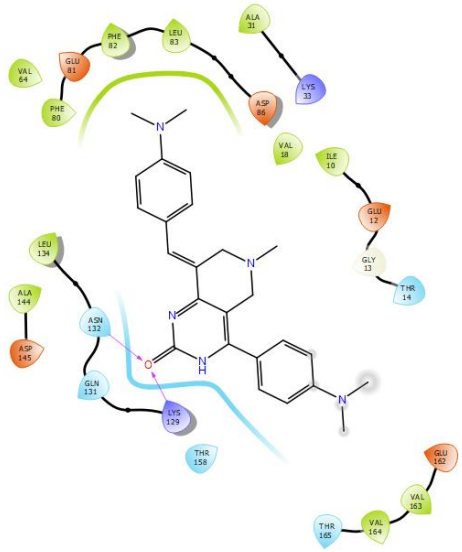


Fig 6. Schematic 2D representation of AS006 in the binding pocket of 2A4L

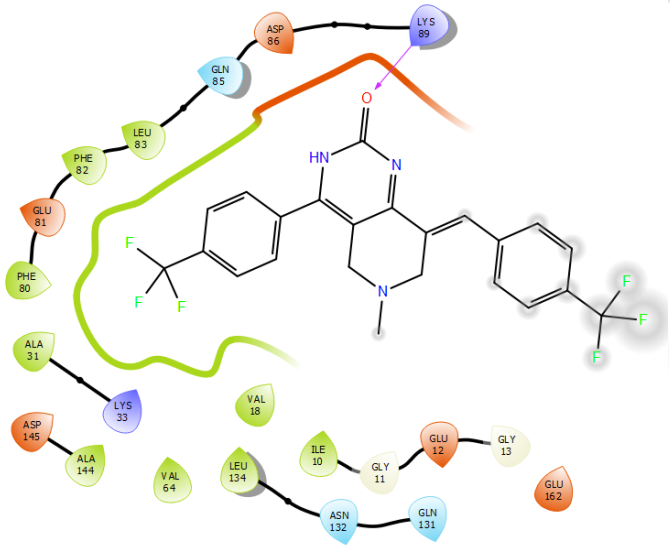


Fig 7. Schematic 2D representation of AS007 in the binding pocket of 2A4L

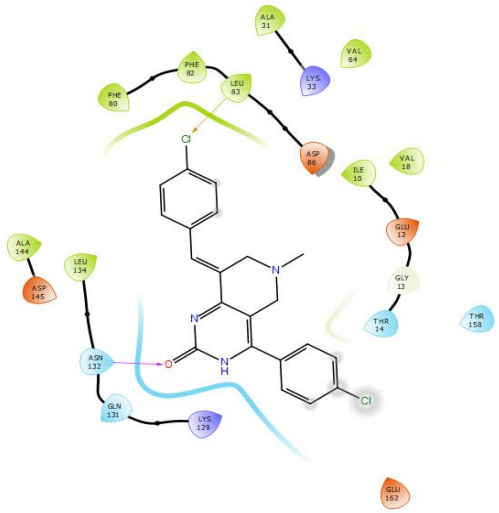


Fig 8. Schematic 2D representation of AS008 in the binding pocket of 2A4L

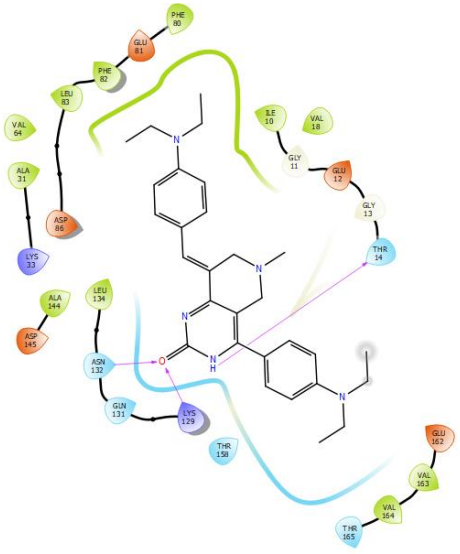


Fig 9. Schematic 2D representation of AS009 in the binding pocket of 2A4L

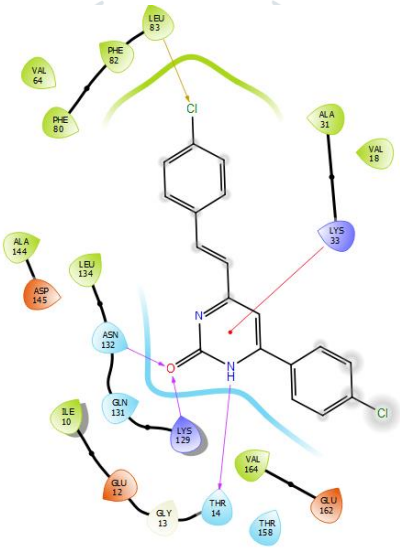


Fig 10. Schematic 2D representation of AS0010 in the binding pocket of 2A4L

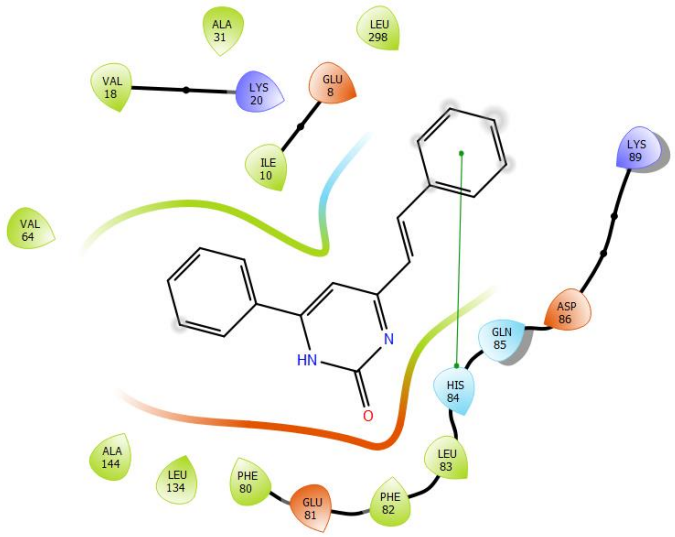


Fig 11. Schematic 2D representation of AS0011 in the binding pocket of 2A4L

CONCLUSION:

In order to understand binding modes between 2A4Land 11 competitive ligands (AS001-ASO11), the molecular docking was developed to reproduce experimental binding affinities for 11inhibitors. To identify the docking accuracy about this target, docking studies were evaluated by different docking programs. Interestingly, these docking results showed that a sole reference could not represent binding modes of all inhibitors.Interactions between compounds and the 2A4L active site. Docking results were merged which allowed us to weigh different binding patterns in the active sites. In a word, we identified that two hydrogen bond acceptors and an aromatic ring were essential anchoring points in4-(4-Chlorostyryl)-6-(4-Chlorophenyl)pyrimidin-2-(1H)-one (AS010) played a pivotal role in binding affinity. This provides lowest energy ligands, docked into the target pocket with best possible pose. The compounds4-(4-Chlorostyryl)-6-(4-Chlorophenyl)pyrimidin-2-(1H)-one (AS010) are quantified using the docking score to act against anti cancer activity. The CDK2 enzyme, which should be useful to aid the designing of new inhibitors with CDK2 improved biological response.

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