Computer Aided Molecular Docking Studies on Diarylsulfonylureas as Potential Anticancer Agents

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ABSTRACT
Molecular docking study was performed on a series of 28 Diarylsulfonylureas **LD1-LD28** as potential cyclin-dependent kinase 2 (CDK2) inhibitors. The docking technique was applied to dock a set of representative compounds within the active site region of **3PY1** using Molegro Virtual Docker v 5.0. For these compounds, the binding free energy (kcal/mol) was determined. The docking simulation clearly predicted the binding mode that is nearly similar to the crystallographic binding mode with 1.02A^o RMSD. Based on the validations and hydrogen bond interactions made by R substituents were considered for evaluation. The results avail to understand the type of interactions that occur between diarylsulfonylureas with **3PY1** binding site region and explain the importance of R substitution on diarylsulfonylurea basic nucleus.

Keywords
Molecular Docking, Diarylsulfonylureas, Cyclin-dependent kinase 2 (CDK2), Molegro Virtual Docker (MVD).

1. INTRODUCTION
Computer aided drug design (CADD) can be done in two ways: ligand-based or structure-based. With the availability of the 3D structure of a biological target, it is feasible to use a structure-based approach to evaluate and predict the binding mode of a ligand within the active site of the receptor with docking methods [1-8]. Now it is a popular technique used for increasing the speed of drug designing process. This was made possible by the availability of many protein structures which helped in developing tools to understand the structure function relationships, automated docking and virtual screening.

Cancer is characterized by alterations in the expression of multiple genes, leading to dysregulation of the normal cellular program for cell division and cell differentiation. This results in an imbalance of cell replication and cell death that favours growth of a tumor cell population. The characteristics that delineate a malignant cancer from a benign tumor are the abilities to invade locally, to spread to regional lymph nodes, and to metastasize to distant organs in the body. At the molecular level, all cancers have several things in common, which suggests that the ultimate biochemical lesions leading to malignant transformation and progression can be produced in an unidentical pattern which is due to alterations in gene expression. In general, malignant cancers cause significant morbidity and will be lethal to the host if not treated. Exceptions to this appear to be latent, indolent cancers that may remain clinically undetectable (or *in situ*), allowing the host to have a standard life expectancy. Clinically, cancer appears to posses different phenotypic characteristics. As cancerous growth progresses, genetic drift in the cell population produces cell heterogeneity such as cell antigenicity, invasiveness, and as well metastatic potentials [9-12].

Cyclin-dependent kinases are the key regulators of cell-cycle transitions. In mammalian cells, Cdk2, Cdk4, Cdk6 and associated cyclins control the G1 to S phase transition. Because proper regulation of this transition is critical for an organism's survival, these protein kinases are exquisitely regulated at different mechanistic levels and in response to a large variety of intrinsic and extrinsic signals. Cyclin-dependent kinase 2 (CDK2) in complex with cyclins E and/or A is a key cell cycle regulator and continues to be an attractive target for the discovery of new anti-tumor agents. In particular, inhibitors of CDK-2/cyclin A/E have already progressed into clinical trials with encouraging early results [13, 14]. The X-ray crystal structure was obtained from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb) (PDB.ID: **3PY1**).

In India cancer is one of the ten leading causes of death today and advancing in rank year by year. According to the Indian Cancer Society, about 1.5 million people suffer from cancer at any point of time in India and India has the world's highest incidences of cancer of the gall bladder, mouth and lower pharynx. In view of present scenario, development of drugs with target specific predefined anticancer potential is more essential to fight against various types of cancers. Recently, the CDK2 inhibitory activity has been hypothesized to possess therapeutic potential for treatment of cancer. Thus there is a need for rapid and efficient computational methods capable of differentiating compounds with acceptable biopharmaceutical properties, e.g. solubility, lipophilicity, ionization constant etc at an early stage in the drug discovery process. In the present study, Ligand Protein Inverse Docking (LPID) stratagies were employed on set of 28 diarylsulfonylureas which earlier reported as potential cytotoxic agents. Through *In Silico* docking procedures different modes of interactions exhibited by these ligands will be recognized and further examined for their predicted binding energies.

2. MATERIALS AND METHODS

2.1 Software Methodology
In the present molecular docking study, software Molegro Virtual Docker (MVD) v 5.0 (www.molegro.com) along with Graphical User Interface (GUI), MVD tools was utilized to generate grid, calculate dock score and evaluate conformers. Molecular docking was performed using MolDock docking engine of software. The scoring function used by MolDock is derived from the Piecewise Linear Potential (PLP) scoring functions. The active binding site region was defined as a spherical region which encompasses all protein within 15.0 A^o

of bound crystallographic ligand atom with selected co-ordinates of X, Y and Z axes, respectively. Default settings were used for all the calculations. Docking was performed using a grid resolution of 0.30 A^o and for each of the 10 independent runs; a maximum number of 1500 iterations were executed on a single population of 50 individuals. The active binding site was considered as a rigid molecule, whereas the ligands were treated as being flexible, i.e. all non-ring torsions were allowed [15].

2.2 Molecular Modeling

A set of 28 new diarylsulfonylureas **LD1-LD28** listed in Table 1, were synthesized, characterized and which earlier reported as potential cytotoxic agents by one of the authors Dr. Vasudeva Rao Avupati *et al* [16]. In the present study, a set of 28 new diarylsulfonylureas **LD1-LD28** were modeled by using ISIS DRAW 2.2 software.

2.3 Ligand Preparation

The structures of diarylsulfonylureas **LD1-LD28** were converted into suitable chemical information using Chemdraw ultra v 10.0 (Cambridge software), copied to Chem3D ultra v 10.0 to create a 3D model and, finally subjected to energy minimization using molecular mechanics (MM_2). The minimization was executed until the root mean square gradient value reached a value smaller than 0.001kcal/mol. Such energy minimized structures are considered for docking and corresponding pdb files were prepared using Chem3D ultra v 10.0 integral option (save as /Protein Data Bank (pdb)) (Table 1) [17].

2.4 Protein Selection

The selection of protein for docking studies is based upon several factors i.e. structure should be determined by X-ray diffraction, and resolution should be between 2.0-2.5A^o, it should contain a co-crystallized ligand; the selected protein should not have any protein breaks in their 3D structure. However, we considered ramachandran plot statistics as the important filter for protein selection that none of the residues present in disallowed regions [18].

2.5 Protein Preparation

All CDK2 X-ray crystal structures were obtained from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb). Subsequent to screening for the above specific standards the resultant protein target (**PDB Code: 3PY1**) was selected and prepared for molecular docking simulation in such a way that all heteroatoms (i.e., nonreceptor atoms such as water, ions, etc.) were removed and Kollmann charges were assigned [19].

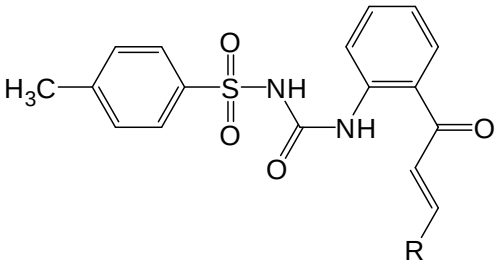
2.6 Software Method Validation

Software method validation was performed in MVD using Protein Data Bank (PDB) protein 3PY1. The x-ray crystal structure of 3PY1 complex with co-crystallized ligand was recovered from PDB. The bio active co-crystallized bound ligand was docked with in the active site region of 3PY1. The RMSD of all atoms between the two conformations is 1.02 A^o indicating that the parameters for docking simulation are good in reproducing X-ray crystal structure.

2.7 Molecular Docking

In the present investigation, we make use of a docking algorithm called MolDock. MolDock is based on a new hybrid search algorithm, called guided differential evolution. The guided differential evolution algorithm combines the differential evolution optimization technique with a cavity prediction algorithm. We used MVD because it showed higher docking accuracy than other stages of the docking products (MVD: 87%, Glide: 82%, Surflex: 75%, FlexX: 58%) in the market [20, 21].

Table 1. Diarylsulfonylureas LD1-LD28 with their Moldock Scores (kcal/mol) and H-bonds interactions against Cyclin-dependent kinase 2 (CDK2)



Ligand Code	'R' Group Substituent	Moldock Score (kcal/mol)	No. of H-Bonds / H-bond Interacting Residues
LD1	C_6H_5	-142.793	1/Leu 66
LD2	4-MeC_6H_4	-148.509	1/Lys 134
LD3	4-$NMe_2C_6H_4$	-147.976	1/Ser 283
LD4	3-$OMeC_6H_4$	-154.771	1/Arg 509
LD5	4-$OMeC_6H_4$	-146.259	1/Tyr 308
LD6	3,4-di$OMeC_6H_3$	-154.008	Nil
LD7	2,4-di$OMeC_6H_3$	-154.88	Nil
LD8	3,4,5-tri $OMeC_6H_2$	-152.219	1/Cys 29
LD9	2-OHC_6H_4	-144.888	2/Gly 104, His 151
LD10	3-OHC_6H_4	-151.099	2/Glu 211, Asp 274
LD11	4-OHC_6H_4	-147.838	2/Arg 506, Trp 450
LD12	3-OEt,4-OHC_6H_3	-147.356	2/Glu 208, Phe 210
LD13	3-OMe,4-OHC_6H_3	-141.17	1/Met 74
LD14	2-$NO_2C_6H_4$	-131.464	Nil
LD15	3-$NO_2C_6H_4$	-148.493	Nil
LD16	5-OH,2-$NO_2C_6H_3$	-148.088	1/Asp 274
LD17	3-FC_6H_4	-154.175	Nil
LD18	4-FC_6H_4	-151.071	2/Glu 211, Asp 274
LD19	**2-ClC_6H_4**	**-214.426**	**3/Phe 146, Val 64**
LD20	4-ClC_6H_4	-151.658	1/His 183
LD21	2,4-diClC_6H_3	-141.447	1/Tyr 67
LD22	3-BrC_6H_4	-146.84	1/Glu 122
LD23	**4-Allyl-OC_6H_4**	**-160.316**	**2/Lys 721, Asp 831**
LD24	**Phenylethene-yl**	**-184.534**	**1/Arg 132**
LD25	**Pyrrol-2-yl**	**-156.15**	**2/Phe 146, Val 64**
LD26	Pyridin-3-yl	-139.734	2/Ser 148, Lys 147
LD27	Pyridin-4-yl	-141.422	1/Glu 73
LD28	**Anthracen-9-yl**	**-165.854**	**1/Tyr 67**

Molecular docking technique was employed to dock the designed diarylsulfonylureas **LD1-LD28** listed in (Table 1) against CDK2 receptor 3PY1 using MVD to locate the interaction between various compounds and CDK2. MVD

requires the receptor and ligand coordinates in either Mol2 or PDB format. Non polar hydrogen atoms were removed from the receptor file and their partial charges were added to the corresponding carbon atoms. Molecular docking was performed using MolDock docking engine of Molegro software. The binding site was defined as a spherical region which encompasses all protein atoms within 15.0 A^o of bound crystallographic ligand atom (dimensions X (22.34 A°), Y (-83.32 A°), Z (-22.11 A°) axes, respectively). Default settings were used for all the calculations. Docking was performed using a grid resolution of 0.3 A^o and for each of the 10 independent runs; a maximum number of 1500 iterations were executed on a single population of 50 individuals.

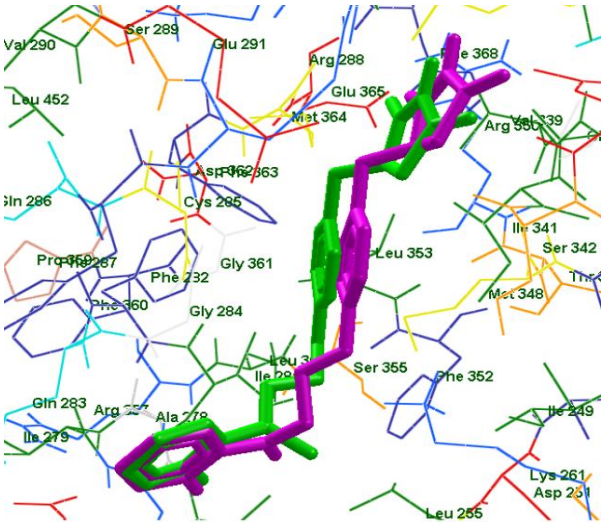


Fig 1: Superimposed binding orientation of docked conformer (pink) and most stable ligand (green) within the active binding site region of 3PY1.

3. RESULTS AND DISCUSSION

Ligand-Protein Inverse Docking (LPID) approach has been used as a useful tool in facilitating drug design. In this approach, docking single or multiple small molecules in single or multiple conformations to a receptor site is attempted to find putative ligands. A number of flexible docking algorithms have been introduced. These include multiple-conformer shape matching, genetic algorithm, evolutionary programming, simulated annealing, fragment-based docking, and other novel algorithms. Testing results have shown that these algorithms are capable of finding ligands and binding conformations at a receptor site close to experimentally determined structures. Because of their capability in identifying potential ligands and binding conformations, these algorithms are expected to be equally applicable to an inverse-docking process for finding multiple putative protein targets to which a small molecule can bind or weakly bind. This may be applied to the identification of unknown and secondary therapeutic targets of drugs, drug leads, natural products and other ligands. LPID approach is now applied to the database of 28 compounds in the present study for finding possible binding orientation, binding mode and binding interaction within the active site region of CDK2. The compound with least binding energy against target protein is considered as 'hit compound'. By this means, it is possible to understand how the compounds with observed cytotoxicity interact with the target protein. The results emerging out of this study can be used to establish the possible inherent mechanism of action of diarylsulfonylureas as potential cytotoxic agents.

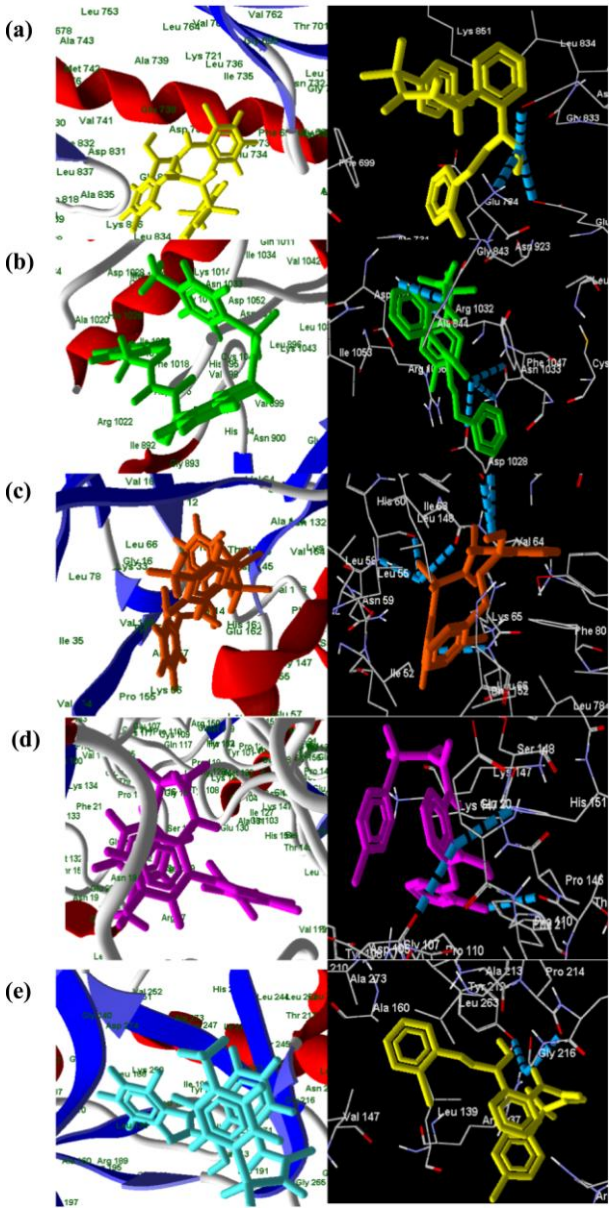


Fig 2. a) Active binding mode and H-bond interactions of LD19 against CDK-2 b) Active binding mode and H-bond interactions of LD23 against CDK-2 c) Active binding mode and H-bond interactions of LD24 against CDK-2 d) Active binding mode and H-bond interactions of LD25 against CDK-2 e) Active binding mode and H-bond interactions of LD28 against CDK-2

The ligand-protein inverse docking simulation technique was performed using MVD program with 28 synthetic ligands diarylsulfonylureas **LD1-LD28** with basic α,β-unsaturated ketone and sulfonylurea moieties reported to be having Cyclin-dependent kinase 2 (CDK2) inhibitory activity. Docking simulations with 3PY1 bound ligand resulted in a Moldock score of **-128.38 kcal/mol** and a RMSD value of **1.72 A^o** showed no hydrogen bond interactions with in the active binding site region. Docking studies on experimental compounds (Table 1) showed that most stable binding ligand **LD19** involved in **3** hydrogen bonds with amino acid residues **Phe 146** and **Val 64** within the binding site region of 3PY1. Although, other H-bond interactions exist, these hydrogen bonds are relevant for inducing intrinsic activity towards highly selective and CDK2 specific inhibitory property.

Moreover, from the data given in (Table 1), it appears that the compound **LD19** represent most significant among the diverse range of compounds. The amino acids **Phe 146** and **Val 64** were appeared to be the most important binding site residues that participate in H-bond interactions with in the active binding site region of 3PY1. The noteworthy hypothesis recognized by our studies on experimental compounds is useful in predicting the key interacting ligand **LD19** and its binding properties to exhibit CDK2 specific inhibitory property. Among all the compounds with stable binding conformations as seen in case of compounds such as **LD19, LD23, LD24, LD25** and **LD28** and **LD17** with Moldock Score i.e. least binding energies with corresponding H-bonds and interacting residues -214.426 kcal/mol, 3/Phe 146, Val 64, -160.316 kcal/mol, 2/Lys 721, Asp 831, -184.534 kcal/mol, 1/Arg 132, -156.15 kcal/mol, 2/Phe 146, Val 64 and -165.854 kcal/mol, 1/Tyr 67 respectively (Fig 2)

4. CONCLUSIONS

In this study the ligand-protein molecular docking simulation was used to preliminarily investigate and to confirm the potential molecular target for the diarylsulfonylureas **LD1-LD28** with observed cytotoxicity. The analysis of the best docked ligands against selected anticancer drug target revealed the binding mode of compounds involved in this study and confirm the role as CDK2 inhibitors. Binding energies of the drug–enzyme (receptor) interactions are important to describe how fit the drug binds to the target macromolecule. The residues participated in the hydrogen bond formation within the active binding site region revealed the importance of these residues towards the observed binding energy with respect to the hit identified against CDK2 target protein. The obtained hypothesis could be the remarkable starting point to develop some new leads as potential CDK2 inhibitors with enhance the affinity as well as intrinsic activity. The results of this work indicate efficient computational tools are capable of identify potential ligands such as LD19, LD23, LD24, LD25 and LD28 which earlier reported in our work as potential cytotoxic agents.

5. ACKNOWLEDGMENTS

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