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# CONCEPTION OF A POTENT DRUG THROUGH TOXICITY AND PHARMACOPHORE STUDY FOR INHIBITING CD 1 INVOLVED IN CANCER BY MOLECULAR DOCKING STUDIES

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**ABSTRACT:** Cancer is a class of disease, where the cells uncontrollably divide without any control over cell cycle and cell division. Various factors contribute to cause of cancer in many ways. Ultimately the cancer cell proliferates without control over cell cycle. Many factors involve in cell cycle amongst that one of the ideal target is Cyclin D1 which couples with cyclin dependent kinase 4, phosphorylates it and this complex promotes cell cycle to next phase. It has been proven that Cyclin D1 inhibition can prevent the progress of many cancers, particularly the breast cancer. So, in the present study Cyclin D1 is exclusively considered as a potent target and by using various commercial softwares and on line tools and databases a couple of drugs have been designed to bind to and inhibit Cyclin D1 and prevent the progress of cell cycle in cancerous cells, and all the designed molecules are evaluated for its pharmacokinetic properties, toxicities, potencies, pharmacophore and lastly its binding ability with the target has been studied and submitted. Considering all the necessity aspects of drug like pharmacokinetic, toxicity, and binding ability and binding energies amongst all the best molecules the ligand M718 is proven to be the best with all the acceptable properties to treat cancer.

**INTRODUCTION:** The term neoplasm denotes a mass of tissue formed as a result of abnormal, excessive, uncoordinated, autonomous and purposeless proliferation of cells [1]. The loss of check over and control in the Cell cycle is often found in many human cancers. Irregularity in the cell cycle regulator function and expression result not only in proliferative advantages, but also lead to tumor progression and invasiveness of the cancer.

In particular, cyclin D1 and p21 are often over-expressed in human cancers, correlating with high tumor grade, poor prognosis and increased metastasis [2]. Cyclin D1 is a key regulatory protein at G1/S checkpoint of the cell cycle.

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It forms complexes with CDK4 or CDK6 and it phosphorylates the retinoblastoma tumour suppressor protein, resulting in the release of E2F transcription factors that allow cell to enter into S phase[3]. Breast cancer is the most common female malignancy in the US, the second most common cause of cancer death in women, and the main cause of death in women ages 40-59. A part from this it affects younger women constitute a small proportion of breast cancer patients, but commonly have distinct concerns and issues compared with older women, including queries regarding fertility, contraception and pregnancy [4, 5, 6].

Loss of the retinoblastoma protein tumor suppressor gene (RB) coding for a nuclear phosphoprotein that regulates the cell cycle is found in many human cancers and probably leads to disruption of the p16-cyclin D1-CDK4/6-RB pathway. Cyclin D1 is known to activate CDK4, which then phosphorylates the RB protein, leading to cell cycle progression. p16 inhibits CDK4, keeping RB hypophosphorylated and preventing cell cycle progression. The significance of these three markers, cyclin D1, CDK4 and p16, for breast cancer

and carcinogenesis is nevertheless still controversial [7]. Cyclin D1 and Dicer expression significantly correlates in luminal A and basal- like subtypes of human breast cancer [8]. Cyclin D1 is also involved and is an ideal target for many cancers like, bladder cancancer (urinogenital cancer), gastric cancer [9, 10]. In the present study various new lead molecules have been designed by using various freeware bioinformatic and commercial software's. All the designed molecules have been evaluated for drug like properties and docking interactions with the target protein, to inhibit CD1 and CDK4 complex involved in various cancers, (particularly in breast cancer).

## MATERIALS AND METHODS:

**Disease selection:** In the present study cancer disease is extensively studied and considered exclusively.

**Target identification:** The method of target identification (novel target), extracts useful knowledge from the raw data and help to focus on the relevant items of data. The most sophisticated aspect is the generation of new insights through the combination of information from different sources. Knowledge on the three - dimensional structure (fold) of a protein provides clues on its function and aids in the search for inhibitors and other drugs. To retrieve and validate the Cyclin D1 protein sequence using computational tools such as NCBI, UniProtKB, Gene Cards, etc. the X ray structure of uniliganded human Cyclin D1 with Cyclindependent kinase 4 domain was used in the present study (pdb code: 2W99, chain A).For docking purpose the structure was minimized to a constant, stable energy structure using the conjugate gradient protocol and applying the CHARM M force field incorporated in Discovery studio software.

**Chemical library:** The chemical library is a collection of chemical compounds used for treating diseases. It consists in series of compounds. Each compound has associated information and its physiochemical properties such as chemical structure, molecular formula, molecular weight, logP value, hydrogen donor, hydrogen bond acceptor, e.t.c., for this library of screening Accelyrs Discovery Studio, ChemSpider, ChemSketch e.t.c., databases were used. There are millions of compounds available in these databases. Through the help of these tools we can find new chemical compounds against cyclin D1, to inhibit target protein. In the chemical compound screening the major part to test is that the chemical compound is having drug likeliness or must pass ADME properties. In the present study Accelry's Discovery Studio is used for these evaluations.

**Lead designing:** Lead library was designed based on Lipinski's rule of five. The functional group of all leads was kept changed on the course of our designing. Lead design was performed with ChemSketch Freeware. Care was taken not to include heavy atoms or carcinogenic atoms to the molecule.

**Lead optimization:** The prepared library of compounds (approximately 820 compounds) was then subjected to Toxicity Prediction (TOPKAT) in the "ADMET" protocol. NTP Carcinogenicity Call (Male Mouse) (v3.2), FDA Carcinogenicity Female Mouse Single vs. Multi (v3.1), Developmental Toxicity Potential (DTP) (v3.1), Rat Oral LD50 (v3.1), Skin Irritation (v6.1) and Aerobic Biodegradability (v6.1), were the six criteria selected for the toxicity prediction. Further analysis by ADMET Descriptors in the "ADMET" protocol was carried out to study the lead compounds pharmacokinetic properties.

**Receptor ligand interaction (Docking):** C DOCKER is used in the present study, C - DOCKER is a grid-based molecular docking method which employs CHARM M force field and assigns the partial charges of the atoms with those found in Merck Molecular Force Field (MMFF) [11, 12]. All the designed ligands were used to dock the target and ligands into the binding site. The resulting poses with higher C Dock score were investigated and the interacting ligand target complex was examined.

**Pharmacophore analysis:** The docked molecule with the best acceptable properties and docked energy is then subjected for the pharmacophore analysis. In this study the pharmacophore of the best chemical leads were determined by using and following the protocol of Ligandscout.

The present study is done by in silico method or by virtual screening method. The virtual screening method is the one of high troughput screening

method where it reduces the time, economy, and labour. Myriad number of drugs can be evaluated for number of targets involved in various diseases and the best drug like molecules are evaluated for drug like properties and docking interaction with the targets and the results can be interpreted. Accelry's Discovery studio (version 2.5) is commercial soft ware used in the present study to design lead molecules and to determine and estimate the docking interactions, complex of drug and protein binding, number of bonds formed by ligand with the target e.t.c., Ligand scout is the another commercial software used in the present study to estimate and determine the Pharmacophore nucleus of the evaluated lead molecules.

## RESULTS:

In the present study nearly about 820 ligand molecules have been designed and screened for the molecular properties test, and toxicity prediction amongst these 9 molecules were found to be the finest. The results of evaluated nine molecules for molecular property test are shown in **Table.1**. Topkat results for these molecules are shown in **Table.2** and the topkat interpreted values of the best two molecules viz, M718 and 507 are shown in **Figure 1** and **Figure 2.**

**TABLE.1: MOLECULAR PROPERTIES OF CHEMICAL COMPOUNDS USED FOR DOCKING.**

| Mol. Name | A logP | Molecular weight | Num of H acceptors | Num of H donors | Num of Rotatable bonds | Number of rings | Number of aromatic rings | Molecular fractional polar surface area |
|---|---|---|---|---|---|---|---|---|
| M718 | 1.752 | 182.067 | 9 | 3 | 4 | 0 | 0 | 0.792 |
| M507 | 0.115 | 152.084 | 7 | 4 | 2 | 0 | 0 | 0.792 |
| M500 | -0.187 | 134.094 | 7 | 5 | 2 | 0 | 0 | 0.895 |
| M691 | 0.068 | 163.11 | 7 | 4 | 3 | 0 | 0 | 0.717 |
| M620 | 1.073 | 165.149 | 5 | 2 | 3 | 1 | 1 | 0.525 |
| M692 | 1.017 | 166.086 | 6 | 3 | 3 | 0 | 0 | 0.595 |
| M808 | 2.293 | 142.22 | 5 | 2 | 2 | 0 | 0 | 0.977 |
| M627 | -0.462 | 184.171 | 5 | 3 | 2 | 1 | 1 | 0.533 |
| M628 | 0.487 | 187.147 | 4 | 2 | 2 | 1 | 1 | 0.417 |

**Inference**: the chemical compounds which obey the Lipinski's Rule of five were selected (chemical compounds listed in above table) for further screening and docking studies.

**TABLE.2: TOPKAT ANALYSIS OF CHEMICAL COMPOUNDS USED FOR DOCKING.**

| Mol.Name | NTP Carcinog-enicity Call (Male Mouse) (v3.2) | FDA Carcinog-enicity Female Mouse Single vs Multi(v3.1) | Developmental Toxicity Potential(DTP) (v3.1) | Rat OralLD50 (v3.1) | Skin Irritation (v6.1) | Aerobic Biodegradability (v6.1) |
|---|---|---|---|---|---|---|
| M718 | 0.656 | 0.000 | 0.000 | 1.6 g/kg | 0.668 | 0.000 |
| M507 | 0.075 | 0.129 | 0.076 | 6.4 g/kg | 0.824 | 0.000 |
| M500 | 0.203 | 0.075 | 1.000 | 3.1 g/kg | 0.607 | 0.000 |
| M691 | 0.000 | 0.000 | 0.000 | 5.3 g/kg | 0.797 | 0.000 |
| M620 | 0.000 | 0.009 | 0.000 | 4.4 g/kg | 1.000 | 0.01 |
| M692 | 0.000 | 0.000 | 0.000 | 837.6mg/kg | 0.993 | 0.000 |
| M808 | 0.001 | 0.994 | 0.983 | 1.3 g/kg | 0.001 | 0.000 |
| M627 | 0.001 | 0.000 | 1.000 | 273.3mg/kg | 0.248 | 0.000 |
| M628 | 0.025 | 0.000 | 1.000 | 783.8mg/kg | 0.999 | 0.000 |

**Inference**: 8 ligand molecules were identified to show positive results. Chemical compounds screened for TOPKAT screening were given flexible criterion on developmental toxicity, Skin irritation, and Rat oral LD50.

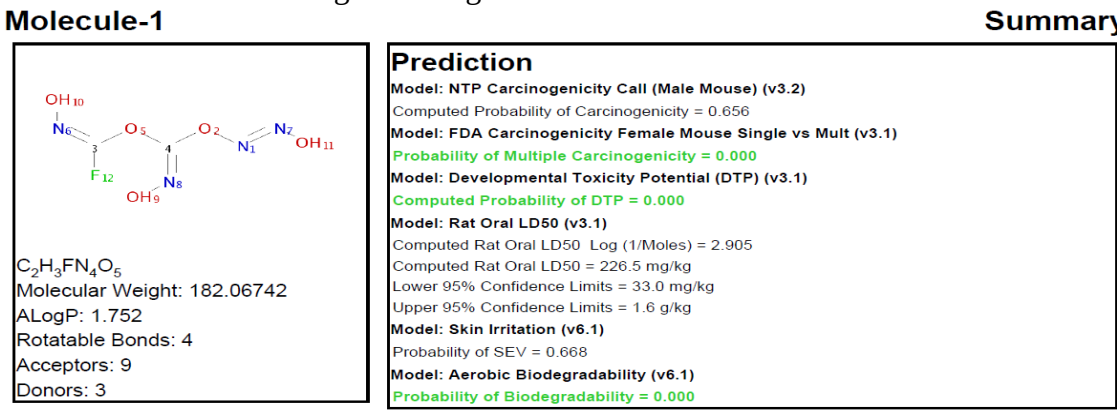


**FIG.1. TOPKAT SCREENING FOR LIGAND M718**

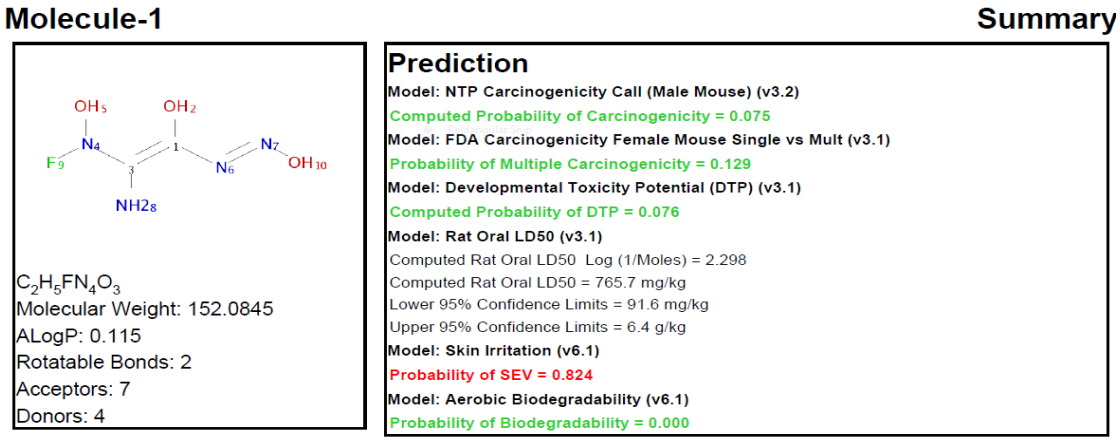


**FIG.2. TOPKAT SCREENING FOR LIGAND M507**

**TABLE.3. ADMET SCREENING FOR CHEMICAL COMPOUNDS USED FOR DOCKING STUDIES.**

| Mol. name | BBB | BBB LEVEL | Abso-rption level | Solu-bility | Solub-ility level | Hepato-toxicity | Hepato toxicit-y probability | CYP 2D6 | CYP 2D6 proba bility | PPB level | Alog P98 | Unknown Alog P98 | PSA_2D |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M718 | - | 4 | 0 | -2.386 | 3 | 1 | 0.635 | 0 | 0.019 | 0 | 1.624 | 0 | 125.599 |
| M507 | - | 4 | 0 | -0.752 | 4 | 0 | 0.496 | 0 | 0.019 | 0 | 0.17 | 1 | 14.985 |
| M500 | - | 4 | 1 | -0.138 | 4 | 0 | 0.496 | 0 | 0.019 | 0 | -0.132 | 1 | 124.443 |
| M691 | - | 4 | 0 | -1.26 | 4 | 1 | 0.569 | 0 | 0.019 | 0 | 0.29 | 1 | 117.257 |
| M620 | -0.87 | 3 | 0 | -2.961 | 3 | 1 | 0.622 | 0 | 0.039 | 0 | 2.148 | 0 | 87.181 |
| M692 | -1.20 | 3 | 0 | -1.794 | 4 | 1 | 0.569 | 0 | 0.019 | 0 | 1.239 | 1 | 90.717 |
| M808 | -0.20 | 2 | 0 | -1.35 | 4 | 0 | 0.278 | 0 | 0.019 | 0 | 1.959 | 0 | 41.631 |
| M627 | -1.87 | 3 | 0 | -0.724 | 4 | 1 | 0.549 | 0 | 0.009 | 0 | -0.46 | 0 | 99.811 |
| M628 | -1.16 | 3 | 0 | -1.828 | 4 | 1 | 0.543 | 0 | 0.009 | 0 | 0.487 | 0 | 73.271 |

**Inference**: ADMET screening of ligand molecules demonstrated their Blood- Brain Penetration and Hepatotoxicity. Flexible criterions based on probability (<0.6) were given to certain molecule having potential for further analysis.

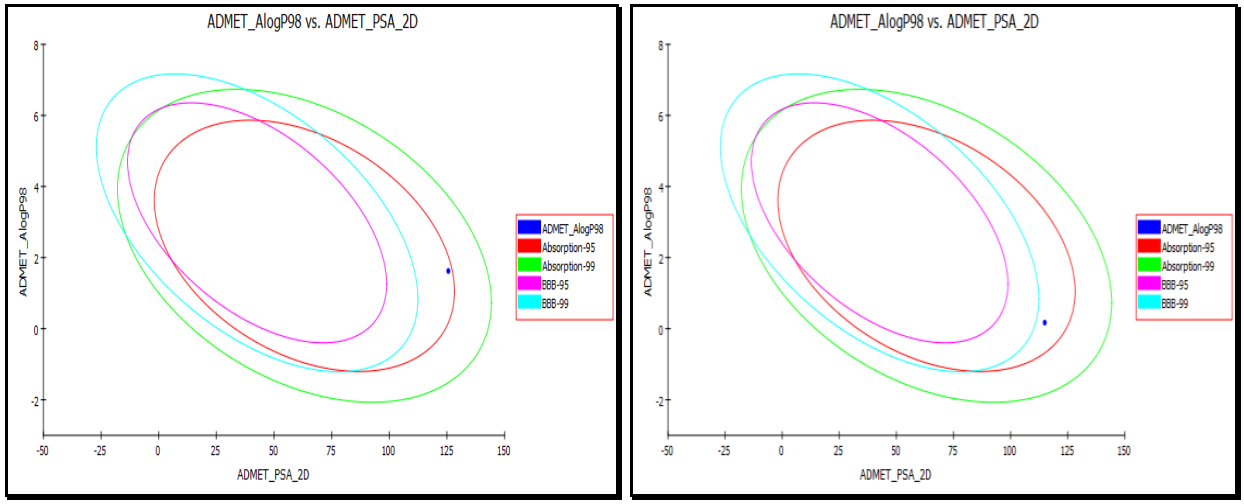


**FIG.3. ADMET GRAPHICAL DESCRIPTION PLOT FOR M718 (LEFT) AND M507 (RIGT) LIGAND MOLECULE.**

The docking interaction of evaluated nine lead molecules and the docking energies with the target protein CD1 are shown in **Table.4** and the binding orientation of ligand M718 and its bondings with the target protein is shown in **Figure 4.**

**TABLE.4.THE LIST OF DEVELOPED IDEAL TARGETS OF CYCLIN D1 WITH THEIR C-DOCKER INTERACTION ENERGY TO ACTIVE SITE OF TARGET RECEPTOR.**

| Molecule | Tagged | Visisble | Visibility locked | Calculate charges | Top hits | C docking energy | C docking interactions |
|---|---|---|---|---|---|---|---|
| M718 | No | No | No | No | 10 | 33.1631 | 32.1313 |
| M507 | No | No | No | No | 10 | 30.4793 | 28.8997 |
| M500 | No | No | No | No | 10 | 30.7455 | 29.748 |
| M691 | No | No | No | No | 10 | 23.0774 | 24.914 |
| M620 | No | No | No | No | 10 | 17.8696 | 22.4479 |
| M692 | No | No | No | No | 10 | 19.2228 | 30.5823 |
| M808 | No | No | No | No | 10 | 16.3461 | 16.9653 |
| M627 | No | No | No | No | 10 | 9.19593 | 20.5996 |
| M628 | No | No | No | No | 10 | 8.66088 | 25.1014 |

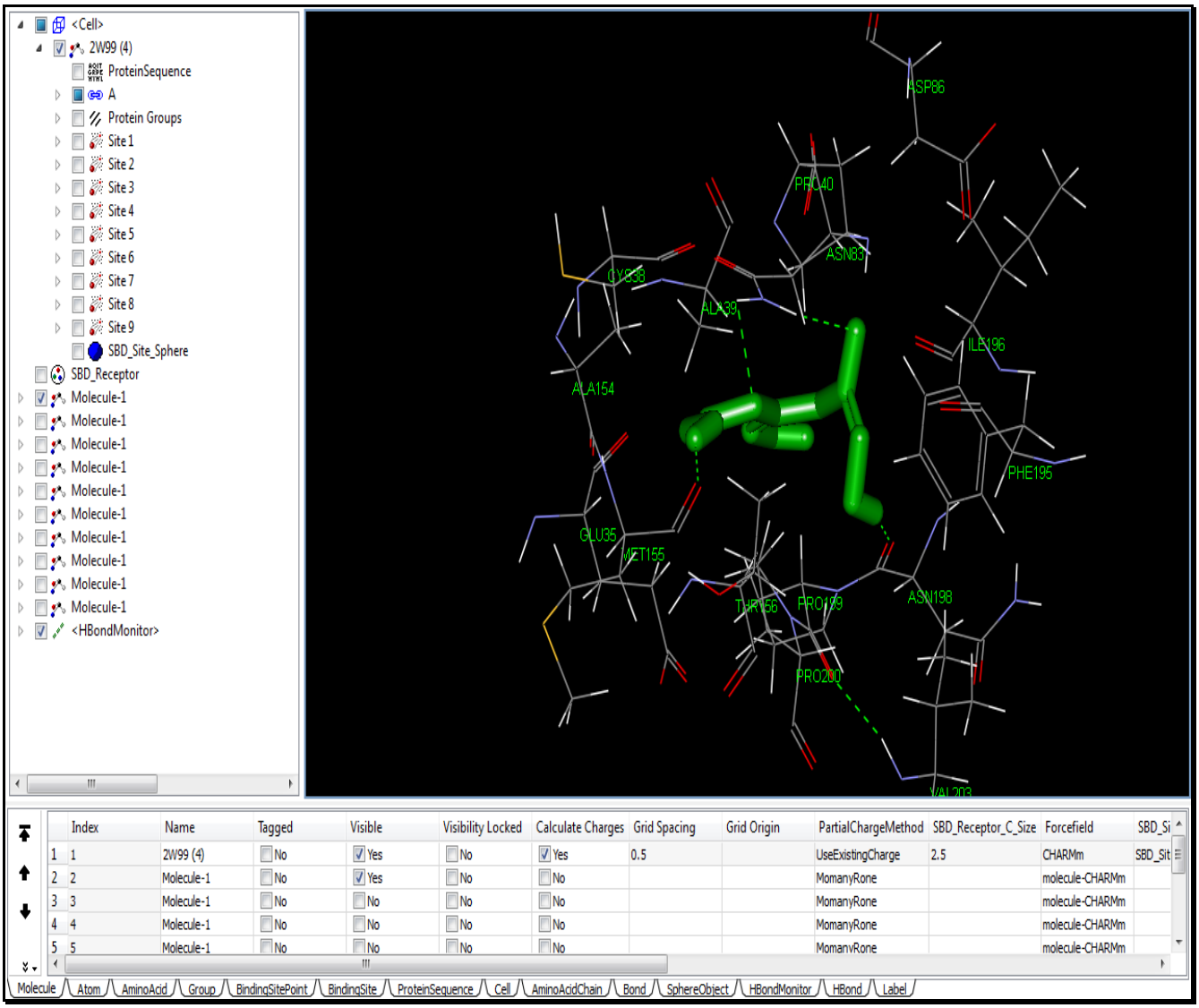


**FIGURE 4: BINDING ORIENTATION OF DESIGNED CHEMICAL COMPOUND M718 WITH TARGET AND SHOWING INTER MOLECULAR HYDROGEN BONDS WITH ALA39, ASN83, MET156, AND ASN198 OF THE LIGAND.**

**Pharmacophore:** The pharmacophore analysis or the pharmacophore of the designed molecule can be found using Accelrys Discovery studio but it can be shown and determined in a more sophisticated way by using the commercial software, "Ligandscout". The docked compound with binding site of receptor can be easily visualized on three feature of pharmacophore model. Aromatic ring features (yellow), hydrophobic region feature (blue), hydrogen bond acceptor feature (red). The pharmacophore nucleus of M718 is shown in **Figure 5.**

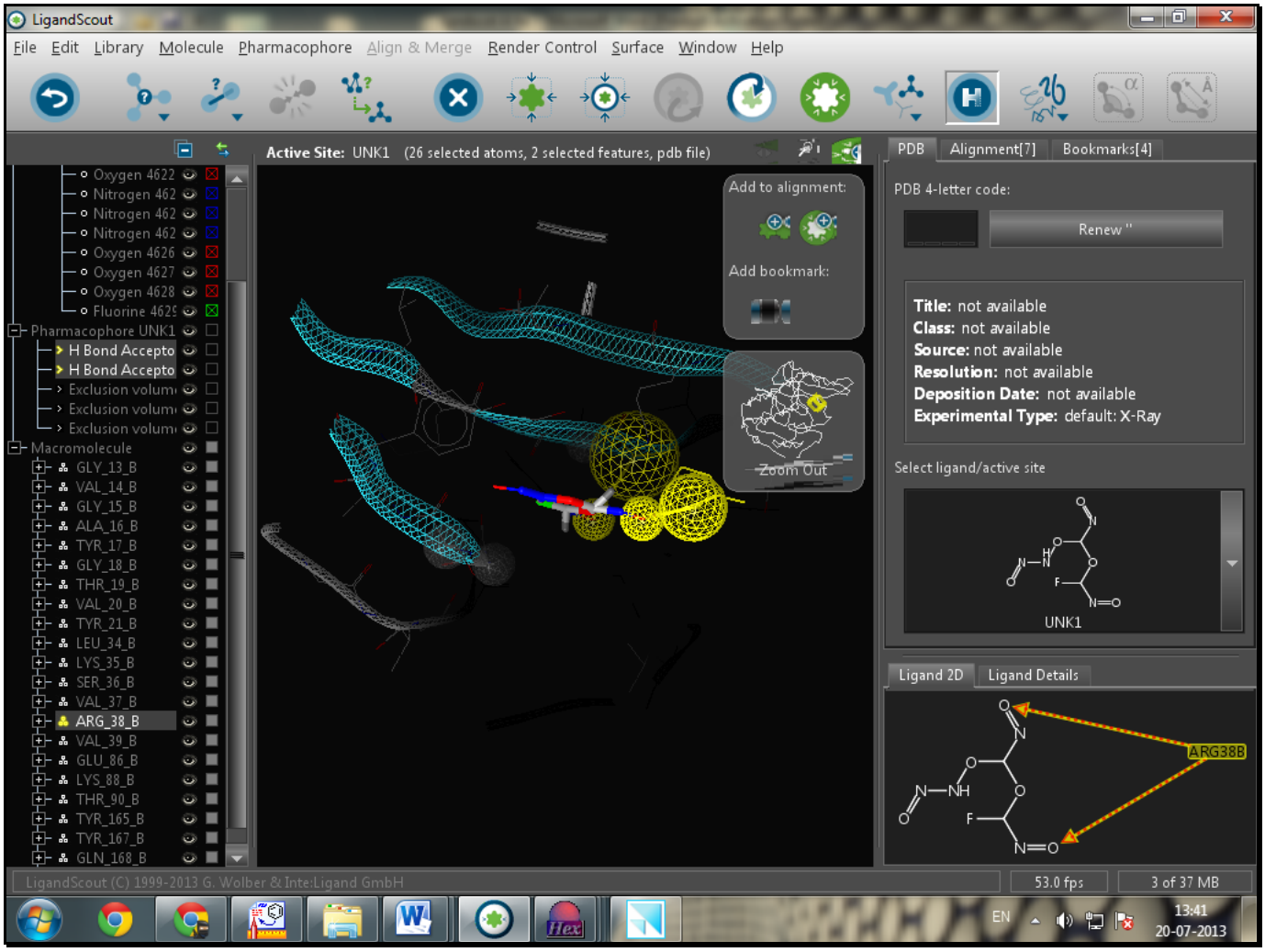


**FIGURE 5: EVALUATION OF PHARMACOPHORE OF CHEMICAL COMPOUND M718 USING LIGANDSCOUT SOFTWARE.**

**DISCUSSIONS:** The interaction and binding affinity between the potent chemical compounds and the target, Cyclin D1 were studied using various computational methods. Based on the pharmacokinetics properties (ADMET), and toxicity evaluation ( TOPKET), binding energy, hydrogen bonds formed and docking results were analyzed to find out the best ligand which can bind and inhibit the target Cyclin D1. Based on the observations from the results the molecule M718 has high values to bind and inhibit the target among the all ligands.

The virtual screening (In Silico) method adopted in the present study helped in identifying and developing the ligands using the commercial software and many online tools for the treatment of many types of cancers, particularly the breast cancer. This type of screening reduces the time and economy in designing a drug as well as in analyzing the drug toxicity, safety and potency before it is promoted for clinical trials. The further studies has to be carried out in both *in-vitro* and *in-vivo* pre-clinical studies.

**ONCLUSIONS:** The interaction between the Cyclin D1 and various ligand molecules were studied by using various commercial softwares, on line tools and data bases. Based upon the pharmacokinetic properties, toxicity data, docking interactions, docking energy, hydrogen bond formation in docking results. I conclude that it was the best molecule which inhibited the activity of targeted protein. And finally based on the observations the ligand M718 having high value to inhibit the action of the protein Cyclin D1 and it is having good pharmacokinetic properties and least toxicity profile. This molecule can be studied and evaluated for the treatment of various cancers and is believed to be a good chemical entity for treatment of breast cancer.

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