Research Article

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3-PHENYLQUINOLINYLCHALCONE DERIVATIVES: PHARMACOPHORE MODELLING, 3D-QSAR ANALYSIS AND DOCKING STUDIES AS ANTI-CANCER AGENTS

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Abstract: Certain 3-Phenylquinolinylchalcone derivatives were evaluated for their anti-proliferative activities and found to exhibit anti-cancer and anti-inflammatory activities. 3D-QSAR and molecular docking approaches were performed on 3-Phenylquinolinylchalcone derivatives to understand their structural requisites and binding mode of the best fitted ligand for cancer inhibitory activity. Among them, (E)-3-(3-(4-methoxyphenyl)quinolin-2-yl)-1-phenylprop-2-en-1-one (6a) was the most active compound against the growth of H460, MCF-7, MDA-MB-231 and SKBR-3 cancer cell line respectively. Four featured hypothesis AHRR.521 of H460 was considered to be the best hypothesis which yielded a statistically significant 3D-QSAR model built with PLS values 3, Regression coefficient (R^2) = 0.8986, Cross validation coefficient (Q^2) = 0.9542, Root Mean Square Deviation (RMSD) = 0.0067, Pearson-R = 1. Interestingly, the result of docking was found to correlate with the pharmacophore study where this compound was active against all six oncoproteins p53, Raf Kinase, Aurora-A-Kinase, CDK-2, Resveratrol and HSP90. The results provide detailed insights of 6a compound which can afford guidance for rational drug design of novel potent anti-cancer agents.

Keywords: 3-Phenylquinolinylchalcone derivatives, Cancer, Pharmacophore, 3D-QSAR, Docking

INTRODUCTION

Medically Cancer is well known as a malignant neoplasm, is a broad group of diseases involving unregulated cell growth. In Cancer, cells divide and grow uncontrollably, forming malignant tumors and pervade nearby parts of the body. The Cancer may also spread to more distant parts of the body or throughout the body. There are over 200 different known cancers that affect humans (1).

Among various types of cancer such as lung, colon, breast, skin, bones, or nerve tissues; breast and lung cancer are the most commonly diagnosed as well as the leading cause of cancer death. Cancer is the major cause of death in developed countries and the second major cause of death in developing countries (2).

Lung cancer was considered to be rare in the beginning of the century (3) but has now reached almost epidemic proportions. It is the major cause of cancer deaths in developed countries and is also arising at alarming rates in developing countries. Deaths due to lung cancer are more than those due to breast, colorectal and prostate cancers put together (4).

Breast cancer is a malignant tumor that starts in the cells of the breast. A malignant tumor is a mass of cancer cells that can grow into (invade) surrounding tissues or spread (metastasize) to distant areas of the body. Almost, this disease occurs irrespective of age in mammals, entirely in women, but men can get it too (5).

The Chalcones are natural products which have been reported to possess many useful biological properties including anti-inflammatory, anti-tumor, antimicrobial, antioxidant, anticancer, antiproliferative, antimalarial, antiprotozoal, antiviral, antimiotic and cytotoxic activities. On the other hand, quinoline skeleton is one of the key building elements for a large number of natural and synthetic heterocycles which possess a wide variety of biological effects such as antimalarials, antitumor, bactericidal, antiproliferative, anti-inflammatory, and antiviral activities (6). So, certain 3-Phenylquinolinylchalcones derivatives were evaluated for their anti-proliferative activities.

These 3-Phenylquinolinylchalcones derivatives were evaluated against three non-small cell lung cancer cells (H1299, H460 and A549) and three breast cancer cells (MCF-7, MDA-MB-231 and SKBR-3). These cancers are the common malignancies in the world, and especially are the leading cause of cancer death in Asian Countries (7-11). Hence with the view of exploring single target drug mechanism an attempt was made to generate selective pharmacophore for cancer which can be prospectively employed to treat breast and lung

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cancer diseases. Feature-based pharmacophore models have been extensively used in the field of drug design and discovery for hit and lead identification and also during the subsequent lead-to-candidate optimization. 3-Dimensional Quantitative Structure Activity Relationship (3D-QSAR) and docking studies incorporate 3D data for ligands and provide more detailed analysis of ligand-receptor interactions (12).

In this research article common pharmacophore model for their anti-proliferative activities is generated from previously published 3-phenylquinolinylchalcone compounds. Atom-based 3D-QSAR was performed in order to analyze the structure activity relationship of these cancer inhibitors. Further structure based drug design approaches like docking study was performed to evaluate the common pharmacophore and 3D-QSAR model.

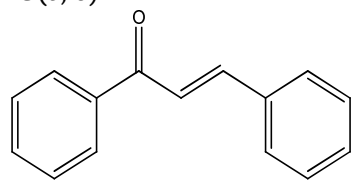
Among all these 3-phenylquinolinylchalcone derivatives, (E)-3-(3-(4-methoxyphenyl)quinolin-2-yl)-1-phenylprop-2-en-1-one (6a) was highly active compound and active against the growth of H460, MCF-7, MDA-MB-231 and SKBR-3 cell line, respectively.

MATERIALS AND METHODS

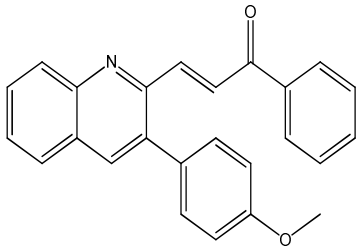
Dataset Collection

A dataset of 21 previously synthesized and evaluated 3-Phenylquinolinylchalcone derivatives with available IC_{50} (half maximal inhibitory concentration) data were taken from the literature (C-H Tseng *et al*., 2012) to generate common pharmacophore hypothesis. The computational work was run on a 2.40 GHz Intel core i3 system. The ligand preparation, protein preparation, grid preparation, ligand docking and 3D QSAR were run from Schrödinger 2011 software. The 21 3-Phenylquinolinylchalcone derivatives were designed in ChemBioOffice 2010 software and saved in mol format. The basic structure of Chalcone and (E)-3-(3-(4-methoxyphenyl) quinolin-2-yl)-1-phenylprop-2-en-1-one (**6a**) derivative are shown in figure 1 (a, b).

Figure 1: Basic structures of Chalcone and (E)-3-(3-(4-methoxyphenyl) quinolin-2-yl)-1-phenylprop-2-en-1-one (6a) derivative (a, b)



a. Chalcone



b. **(E)-3-(3-(4-methoxyphenyl) quinolin-2-yl)-1-phenylprop-2-en-1-one (6a)**

Selection of Target protein

Following six cancer proteins (listed in Table 3), with their resolution and ligand interaction diagram is retrieved from the Protein Data Bank (PDB), were targeted in this study. These target proteins were selected based on their best appropriate ligand interactions. Structural and active site studies of the proteins were done by using CASTp (Computed Atlas of Surface Topography of Protein).

Table 3: Docking Result showing XP G Scores of 6a drug molecule with Six Oncoproteins

S.No.	PROTEIN NAMES	PDB ID	CELL LINE	G SCORE	NO. OF HYDROGEN BONDS	RESIDUES	H-BOND DISTANCE(A°)
1.	p53	4AGO	H1299	-5.1	1	Val147	1.872
2.	Raf Kinase	3OG7	H460	-7.5	2	Cys532 Asp594	2.254 2.299
3.	Aurora-A-Kinase	1MQ4	A549	-7.2	1	Lys162	2.065
4.	CDK-2	4BGH	MCF-7	-7.8	1	Leu83	1.833
5.	Resveratrol	1JWH	MDA-MB-231	-9.8	1	Val116	2.095
6.	HSP90	1UYE	SKBR-3	-10	1	Phe138	2.000

Phase-Methodology

3-Dimensional Quantitative Structure Activity Relationship (3D-QSAR) study was carried out using PHASE (13). It is an ideal tool for structure arrangement, pharmacophore perception, activity prediction, and 3D database searching. It provides support for structure-activity relationship development (SAR), lead optimization, lead expansion and lead discovery. The common pharmacophore hypotheses display characteristics of 3D chemical structures that were meant to be difficult for binding. It is also well suited to drug discovery projects for which no receptor structure is available. It executes fine conformational sampling and a range of scoring techniques to identify common pharmacophore hypotheses. A given hypothesis along with known activity data will create 3D-QSAR models that identify overall aspects of molecular structure for potent activity. These generated models may be used in conjunction with our hypothesis, for a database of 3D molecules that were most likely to exhibit strong activity towards the target.

Preparing Ligands

For structure optimization and energy minimization the molecules were processed with Ligprep2.5 program. Conformers were generated by using force field OPLS-2005 (14) and root mean square deviation (RMSD) with 1.0A°. The conformer with least potential energy was subjected for the further study. Each ligand was then selected based on low energy conformation and subjected to Impact minimization by Impact 5.7 to minimize the energy of ligands further and then the prepared structures were imported to PHASE along with their activity values to develop pharmacophore model. All the compounds used in study have known IC_{50} values of different range therefore the values (in micro-Molar) were converted into negative logarithm of IC_{50} (pIC_{50}). The pIC_{50} ranged values varies for all 21 compounds and on the basis of average value, they were divided into active and inactive compounds. The dataset of every 21 compounds was divided randomly into training and test set according to their pIC_{50} values.

Pharmacophore Hypothesis Generation

PHASE (Pharmacophore alignment and scoring engine) can identify the spatial arrangements of functional groups that are common and essential for the biological activity of the ligands used in the study (15). Next step to develop pharmacophore model after preparing ligands is to create sites. The pharmacophore sites were created from a set of four pharmacophore features, including hydrogen bond acceptor (A), hydrophobic group (H), and aromatic ring (R) by setting the pharmacophore matching tolerance to 1.2A°. Hypothesis were generated by the number of matching active compounds, variation of number of active sites and common pharmacophore hypothesis were considered, which indicates at least four sites common to all molecules. The common pharmacophore hypotheses with significant statistical values were selected for molecular alignments, as the quality of alignment is measured in terms of Survival score. The Best common pharmacophore hypothesis was selected based on highest survival score. In the hypothesis scoring step default parameters for site, number of matches, vector, volume, energy and selectivity terms were employed to align the actives to the hypothesis and calculate the score for the actives. Each pharmacophore and its related ligand were treated temporarily as reference and assigned a score according to the alignment score, volume score and a vector score.

All the pharmacophoric features were then used to build 3D QSAR models. The QSAR model was validated by looking into different regression analysis derived parameters. The model was selected based upon the following criterion. R^2 value should be around 0.8-1.0, Q^2 value should be close to R^2 value, Pearson-R values should be greater than Q^2 value and around 0.9, survival score for generated hypothesis should be more than 2, the presence of compound having a fitness score of 3 for which the residual value should not be high, fitness range of 0.5-3 should be revolved around in some dataset compounds and the SD values should be below 0.3.

Validation of Pharmacophore hypothesis

The external validation is considered to be a conclusive proof to determine the predictability of a model. The data set has to be divided into training set and test set for this model. The training set was used to generate pharmacophore model. Validation is an important feature of pharmacophore design when the model is built for the predicting activities of molecules in external test set (16-17). In the present work, the developed pharmacophore model was externally validated by predicting the activity of test set molecules. The correlation between the predicted and experimental activities of the molecules of training and test sets were shown in tables 1a and 1b. The graphical representations were shown in figures 2a and 2b.

Table 1a: Experimental and predicted IC_{50} values of training set molecules based on hypothesis AHRR.521

Serial No.	Ligand Name	Experimental Activity (IC_{50})	Predicted Activity (IC_{50})	Pharm Set	Fitness
1.	5	5	5.01	Inactive	2.43
2.	6a	5.17	5.16	Active	3
3.	6b	5.15	5.16	Active	2.98
4.	6d	5	5.05	Inactive	2.96
5.	6e	5.15	5.16	Active	2.89
6.	6f	5.17	5.22	Active	2.84
7.	6g	5.12	5.12	Active	2.92
8.	6h	5.12	5.10	Active	2.88
9.	6i	5.13	5.09	Active	2.98
10.	7	5.18	5.19	Active	2.92
11.	8	5.19	5.17	Active	2.95
12.	9	5.26	5.28	Active	2.96
13.	10	5.20	5.13	Inactive	2.97
14.	11	5.28	5.29	Inactive	2.95
15.	12	5.15	5.15	Inactive	2.97
16.	13	5	5.05	Inactive	2.97
17.	14	5	5.01	Inactive	2.94
18.	15	5	4.99	Inactive	2.95
19.	Topotecan	5.13	5.13	Active	1.44

Table 1b: Experimental and predicted IC_{50} values of test set molecules based on hypothesis AHRR.521

Serial No.	Ligand Name	Experimental Activity (IC_{50})	Predicted Activity (IC_{50})	Pharm set	Fitness
1.	6c	5.15	5.17	Active	2.98
2.	16	5.09	5.10	Inactive	2.9

Docking methodology

Docking techniques are computational techniques for exploration of possible binding mode of a substrate to a given receptor, enzyme or other binding site. 6a compound was subjected for docking studies with the 6 different Oncoproteins and it is performed by using the GLIDE 5.7 (Grid-based Ligand Docking with Energetics) module. All the 6 Oncoproteins show good fitness in this study. The crystal structures of these Oncoproteins were downloaded from protein data bank. Protein pre-processing, optimization and minimization were carried out in the protein preparation wizard using OPLS-2005 force field and root mean square deviation (RMSD) of 0.30A°. Grid was generated by using centroid of selected residue in the receptor grid generation panel by specifying the active site range. Finally impact minimized ligands were docked into the grid generated active site residues using the extra precision docking mode in GLIDE 5.7.

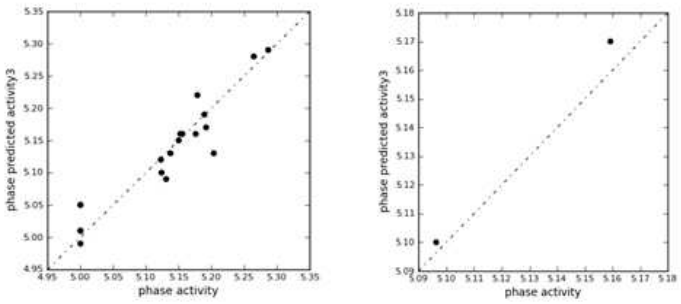


Figure 2 (a and b): Plots of experimental and predicted activity for (a) training and (b) test set molecules using model AHRR.521

Overall, the van der Waals' energy contributed most to the interaction energy, but the electrostatic energy disclosed the greatest variation and was therefore the major factor for the ranking of the molecules. Docking result revealed that all the molecules were docked efficiently as it is evident from the extra precision (XP) Glide scores.

RESULTS AND DISCUSSION

All the previously synthesized 3-Phenylquinolinylchalcone derivatives were evaluated against a panel of six cancer cell lines including 3 non-small cell lung cancer cells (H1299, H460 and A549), 3 breast cancer cells (MCF-7, MDA-MB-231 and SKBR-3), respectively.

3D-QSAR Analysis

In this ligand based pharmacophore model development, we developed a model that screened important pharmacophoric features necessary for a set of 3-Phenylquinolinylchalcone derivatives to function as anti-proliferative agents. Among the 21 compounds tested for anti-proliferative activity, (E)-3-(3-(4-methoxyphenyl)quinolin-2-yl)-1-phenylprop-2-en-1-one (6a) was the most active compound against the growth of H460, MCF-7, MDA-MB-231 and SKBR-3 cell lines, shown in table 2 and among these 6a was highly active against the growth of H460 cell line, respectively.

Table 2: Result of PLS statistics of the Selected QSAR Models of the Six Cancer Cell Line

ID	CELL LINE	SD	R^2	F	P	STABILITY	RMSE	Q^2	PEARSON-R
AARR.338	H1299	0.205	0.8564	27.8	3.7e-006	0.0904	0.3223	0.5704	0.9953
AHRR.521	H460	0.031	0.8986	44.3	1.088e-007	0.0451	0.0067	0.9542	1
ARRR.15	A549	0.123	0.7638	16.2	5.754e-005	0.0751	0.0174	0.7575	1
AHRR.509	MCF-7	0.169	0.849	42.2	6.959e-007	0.0299	0.0596	0.6349	0.8053
AHRR.18	MDA-MB-231	0.273	0.8646	31.9	9.334e-007	0.017	0.3468	0.5882	1
AARR.103	SKBR-3	0.193	0.8988	44.4	1.071e-007	0.0029	0.1930	0.7793	1

SD= standard deviation of the regression, R^2= correlation coefficient, F= variance ratio, P= significance level of variance ratio, RMSE= root-mean-square-error, Q^2= for the predicted activities, Pearson-R= correlation between the predicted and observed activity for the test set.

So, to find common pharmacophore hypothesis, the data sets were divided into active and inactive sets (18) depending upon the observed activity. Among the 19 compounds in training set, 14 were active and 5 were inactive and test set comprised of 2 compounds. The hypothesis (AHRR.521) aligned with the best fit ligand was shown in figure 3, the bond distances between different sites and all ligands alignment to AHRR.521 were shown in figure 4 & 5. The hypothesis depicted a decent survival score (3.0), best regression coefficient (R^2=0.8986), variance (F=44.3), and Standard deviation (SD=0.0314). The squared predictive correlation coefficient (Q^2) for this model is 0.9542. Studies show that for a reliable model, the Q^2 should exceed 0.60 (19-20). For each ligand, one aligned conformer based on the lowest RMSE of feature atom coordinates from those of the corresponding reference feature was superimposed on AHRR.521 and the fitness scores for all ligands were observed. Fitness score evaluate the activity of a compound, the greater the fitness score, the activity prediction of the compound is also greater. The fit function examines if the feature is mapped or

not and also contains a distance term which measures the distance that differentiates the features on the molecule from the centroid of the hypothesis feature.

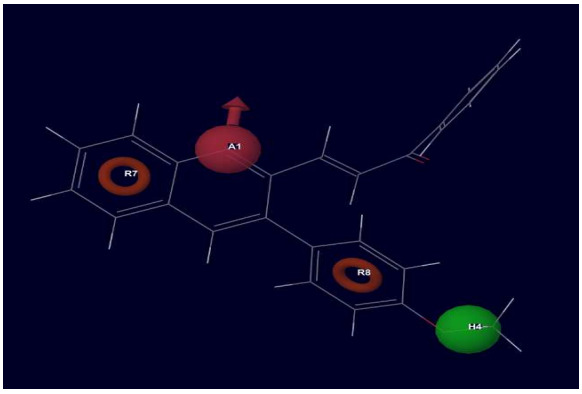


Figure 3: Best pharmacophore model AHRR.521 aligned with compound 6a. Pharmacophore features are color coded: 1 hydrogen bond acceptor (A1; pink), 1 hydrophobic group (H4; green) and 2 aromatic rings (R7, R8; orange)

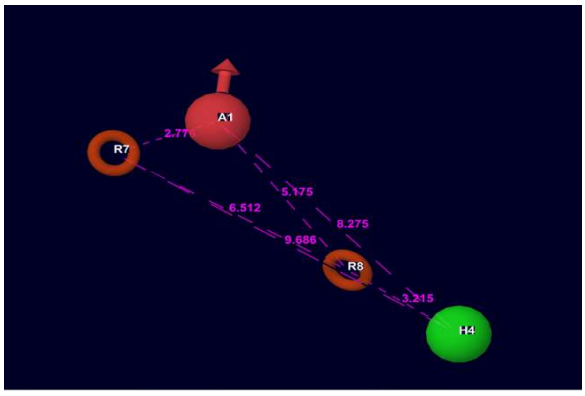


Figure 4: PHASE-generated pharmacophore model AHRR.521 illustrating hydrogen bond acceptor (A1; pink), aromatic ring (R7, R8; orange) and hydrophobic group (H4; green) features showing distances (in A°).

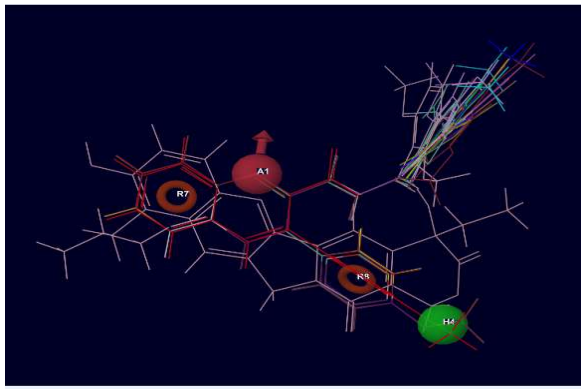


Figure 5: PHASE-generated pharmacophore model AHRR.521 illustrating all ligands alignment

According to the results of this study, model AHRR.521 can best fit for the prediction of 3-Phenylquinolinylchalcone anti-proliferative activity.

Hydrophobicity Field Prediction

The 3D-QSAR model shown in figure 6 depicts the hydrophobicity field prediction. Blue regions show that the substitutions at these positions by groups having more hydrophobic characteristics favor 3-Phenylquinolinylchalcone anti-proliferative activity. Red regions show that groups having more hydrophobic property do not favor 3-Phenylquinolinylchalcone anti-proliferative activity.

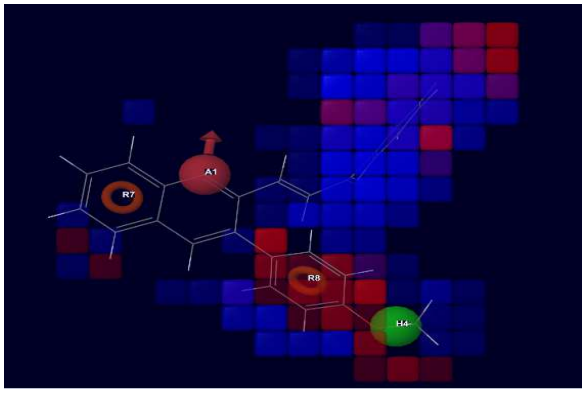


Figure 6: 3D-QSAR visualization model based on compound 6a of training set illustrating electron withdrawing characteristics and hydrophobicity features

Docking Analysis

Further to validate common pharmacophoric features and 3D-QSAR model, docking studies were performed. The result in the form of XP G Scores of 6a compound with six different Oncoproteins p53 (4AGO), Raf Kinase (3OG7), Aurora-A Kinase (1MQ4), CDK-2 (4BGH), Resveratrol (1JWH), HSP90 (1UYE) were tabulated in the table 3 and represented in figure 7. The stability of docking between ligand and the target protein depends on the binding interaction and thus the G Score describes how well the drug has interacted with the protein. In this study, results were found to be complementary with 3D QSAR studies. The hydrogen bonding interaction which is a vital parameter for the stability of *drug-protein complex* is found in all the best scoring molecules.

CONCLUSION

Studies have shown that 3-Phenylquinolinylchalcone derivatives exhibited clear evidence for their anti-proliferative activities and have provided insights into the structural requirement of novel series of these derivatives as inhibitors of lung cancers and breast cancers in human. In this study, a highly predictive atom-based 3D-QSAR model is generated using training and test set of 19 and 2 molecules, respectively, which consist of four featured pharmacophore hypothesis (AHRR.521):- one hydrogen bond acceptor (A), one hydrophobic group and two aromatic ring (RR). Atom-based 3D-QSAR visualization of model in the context of the structure of molecules under study provides details of the relationship

between structure and function, and thus gives information regarding structural modification with which to design analogs with better activity prior to synthesis. Moreover, docking evidences also correlate with the 3D-QSAR results. Thus, the obtained results provides hypothetical image to rationally design new 3-Phenylquinolinylchalcone derivative molecules as cancer inhibitors.

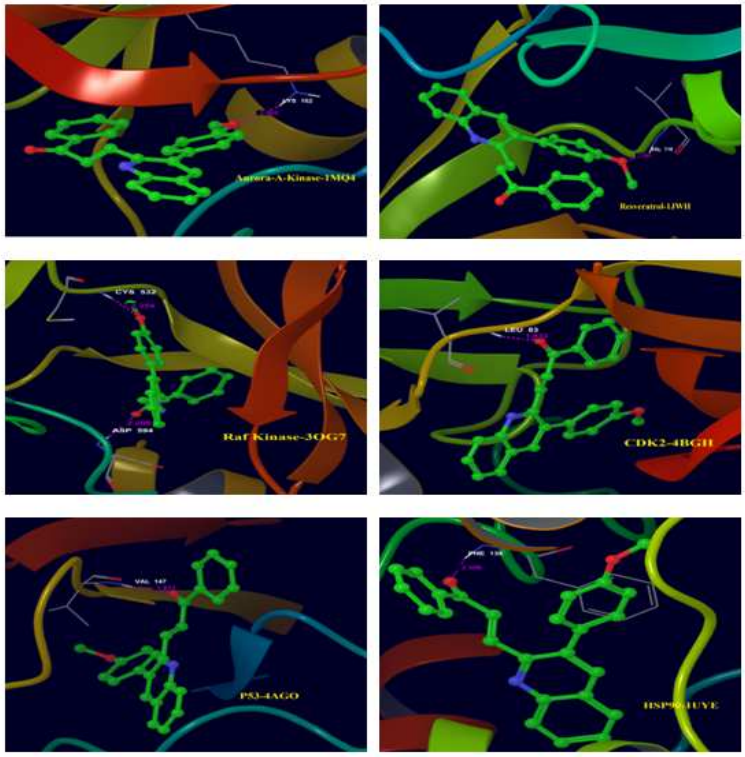


Figure 7: Docking maps of 6a Compound with Six Oncoproteins

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