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DRUG DESIGNING AND DOCKING STUDIES OF BREAST CANCER TYPE1 SUSCEPTIBILITY PROTEIN (BRCA1) INVOLVED IN BREAST CANCER

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ABSTRACT: Cancer is a disease that begins in the cells of the body which is characterized by uncontrolled, uncoordinated and undesirable cell division. If a cell accumulates critical mutations in five or six of the proto-oncogenes, tumour suppressor genes and DNA repair genes are likely to result in a fully malignant cell, capable of forming a tumour. In this work we identified the inhibitors using three dimensional structures for Breast cancer susceptibility protein1 (BRCA1) crystal structure using GOLD software. After collecting 3D structure, structure validation studies was performed, and the generated model was reliable. Active site of BRCA1 was identified using CASTp server and these active residues are used to find better inhibitor. New drug derivatives of Podophyllotoxin and Fosbretabulin were designed using Chemsketch software and these were docked to the BRCA1 active residues in order to find better inhibitor. From the docking results the best inhibitors were identified and it can be used for further studies.

Key words: Cancer, BRCA1, Drug Designing, Docking

INTRODUCTION

Breast cancer, the most common type of cancer among women in India where nearly a million women are affected with breast cancer annually. Breast cancer is responsible for 23 % of all cancer cases [1]. Breast cancer Type 1 suceptibility protein early onset (*BRCA1*) is a human estrogen sensitive tumor suppressor gene and is an established DNA repair gene. The gene is a specific, caretaker gene found in all humans [2-4]. The *BRCA1*gene produces either a full-length breast cancer type 1 susceptibility protein (BRCA1) or through alternatively splicing two documented variants, BRCA1Δ11 or BRCA1Δ11b, both of which lack a nuclear localization signal [5]. The full-length *BRCA1* transcript includes 23 exons (22 coding exons) that encode 1863 amino acid protein (nuclear phosphoprotein) involved in multiple DNA repair pathways and cell-cycle checkpoint-14 regulation[8]. The protein is involved in protein modification and protein ubiquitination pathways. In translational modifications phosphorylation of BRCA1 protein at Ser-308 by Aurora Kinase A is required for normal cell cycle progression from G_2 to mitosis. Phosphorylated to IR, UV, and various stimuli causes checkpoint activation by ATM or ATR. Phosphorylation at Ser-988 by CHEK2 regulates mitotic spindle assembly and Autoubiquitinated undergoes 'Lys-6'-linked polyubiquitination. 'Lys-6'-linked polyubiquitination does not promote degradation[17]. *BRCA1* and *BRCA2* are normally expressed in the cells of breast and other tissues, where they help repair damaged DNA or destroys cells if DNA cannot be repaired. If *BRCA1* and *BRCA2* itself is damaged by a BRCA mutation, damaged DNA is not repaired properly, and this increase the risk for breast cancer [6]. The *BRCA1* gene mutation was discovered in 1994[7], exposing women with these mutations to six to eight fold risk of developing breast cancer compared to the average female population. BRCA mutation carriers have a life time estimate of breast cancer that ranges from 36-90% [8-9]. Females who carry a germline mutation in either the *BRCA1* or *BRCA2* gene have a lifetime risk of breast cancer of 60-70%[9], and once diagnosed as having breast cancer, have a high risk of a second primary breast cancer and or tumorigenesis in reproductive tissues [11,12].

Loss of function germ-line mutations in BRCA1 confer markedly increased risk of breast and ovarian cancer [13]. BRCA1 is a regulator of lipid metabolism in human breast cancer cells (MCF7) and this will directly interact with the phosphorylated form of acetyl CoA carboxylase (ACC-p) at the BRCA1 C-terminal BRCT domains and the interaction between these two leads to the maintenance of the phosphorylated state of ACC thereby altering lipid metabolism in the cancer cell line [13]. ACC1 or ACC2 are the isoforms of ACC and ACC2 containing an extra 146 amino acids in the NH2-terminus region. ACC activity is negatively regulated by phosphorylation of residue Ser79 on ACC1 and Ser221 on ACC2. In the active form, ACC catalyzes the carboxylation of acetyl CoA into malonyl CoA (MaCoA). Changes in cellular MaCoA content alter intracellular lipid dynamics in two specific manners. MaCoA directly contributes to de novo synthesis of palmitate via fatty acid synthase (FAS) and MaCoA also allosterically inhibits carnitine palmitoyltransferase-1 (CPT-1) a mitochondrial long chain fatty acid transporter. Thus, in mammary tissue the ability of BRCA1 to affect ACC activity alters cellular lipid concentrations by indirectly regulating rates of fatty acid synthesis and/or the flux of fatty acids into the mitochondria [14-20].

METHODOLOGY

The sequence of BRCA1 was obtained from UNIPROT. The structure was collected from Protein Dara Bank. After that the unnecessary chains and hetero atoms were removed using SPDBV software. Later hydrogens were added to the protein and used for active site identification.

Finally, the structure having the least energy with low RMSD (Root Mean Square Deviation) was used for further studies. In this step, the quality of the initial model was improved. The final structure obtained was analyzed by Ramachandran's map using PROCHECK (Programs to check the Stereo chemical Quality of Protein Structures) and environment profile using ERRAT graph (Structure Evaluation server). This model was used for the identification of active site and for docking of the substrate with the enzyme.

Active site Identification

Active site of BRCA1 was identified using CASTp server. A new program, CASTp, for automatically locating and measuring protein pockets and cavities, is based on precise computational geometry methods, including alpha shape and discrete flow theory. CASTp identifies and measures pockets and pocket mouth openings, as well as cavities. The program specifies the atoms lining pockets, pocket openings, and buried cavities; the volume and area of pockets and cavities; and the area and circumference of mouth openings.

Docking method

Docking was carried out using GOLD (Genetic Optimization of Ligand Docking) software which is based on genetic algorithm (GA). This method allows as partial flexibility of protein and full flexibility of ligand. The compounds are docked to the active site of the BRCA1. The interaction of these compounds with the active site residues are thoroughly studied using molecular mechanics calculations. The parameters used for GA were population size (100), selection pressure (1.1), number of operations (10,000), number of island (1) and niche size (2). Operator parameters for crossover, mutation and migration were set to 100, 100 and 10 respectively. Default cutoff values of 3.0 A° (dH-X) for hydrogen bonds and 6.0 A° for vanderwaals were employed. During docking, the default algorithm speed was selected and the ligand binding site in the BRCA1 was defined within a 10 A° radius with the centroid as CE atom of ALA61. The number of poses for each inhibitor was set 100, and early termination was allowed if the top three bound conformations of a ligand were within 1.5A° RMSD. After docking, the individual binding poses of each ligand were observed and their interactions with the protein were studied. The best and most energetically favorable conformation of each ligand was selected.

Gold Score fitness function

Gold Score performs a force field based scoring function and is made up of four components: 1. Protein-ligand hydrogen bond energy (external H-bond); 2. Protein-ligand vander Waals energy (external vdw); 3. Ligand internal vander Waals energy (internal vdw); 4. Ligand intramolecular hydrogen bond energy (internal- H- bond). The external vdw score is multiplied by a factor of 1.375 when the total fitness score is computed. This is an empirical correction to encourage protein-ligand hydrophobic contact. The fitness function has been optimized for the prediction of ligand binding positions.

GoldScore = S (hb_ext) + S (vdw_ext) + S (hb_int) + S (vdw_int)

Where S (hb_ext) is the protein-ligand hydrogen bond score, S (vdw_ext) is the protein-ligand van der Waals score, S (hb_int) is the score from intramolecular hydrogen bond in the ligand and S (vdw_int) is the score from intramolecular strain in the ligand.

RESULTS AND DISCUSSION

From the PDB databank, the PDB file was collected and the PDB code was IJM7. The final stable structure of the BRCA1 protein obtained is shown in Figure 1.

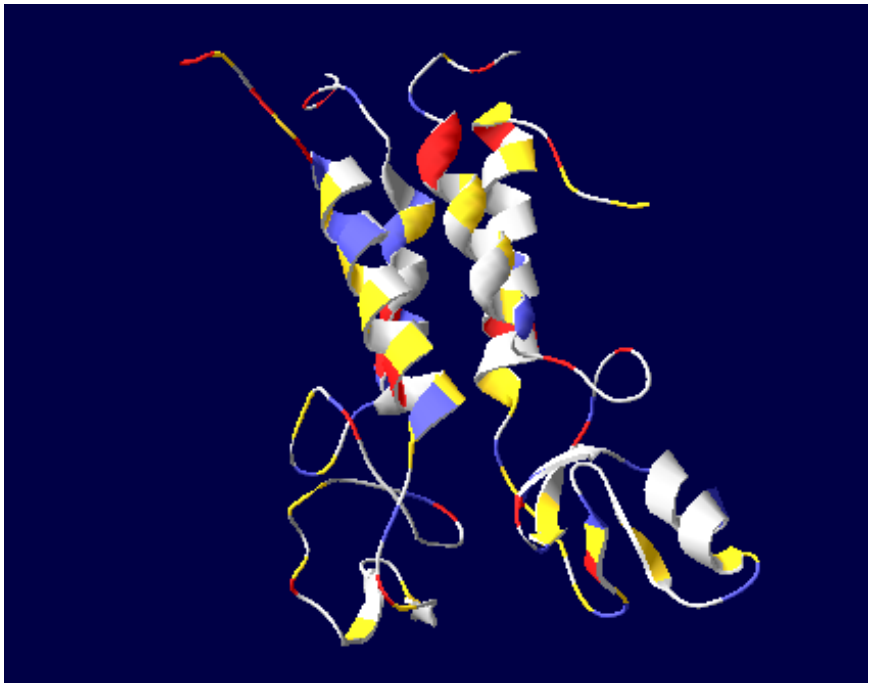


Fig 1: structure of BRCA1

Active site Identification of BRCA1

After the final model was built, the possible binding sites of BRCA1 was searched based on the structural comparison of template and the model build and also with CASTP server and was shown in Figure 2. Infact from the final refined model of BRCA1 domain using SPDBV program. It was found that secondary structures are highly conserved and the residues, ASP-65, CYS-66, CYS-88, TYR-112, LEU-113, LEU-115, ASP-117, ILE-118, GLN-136.

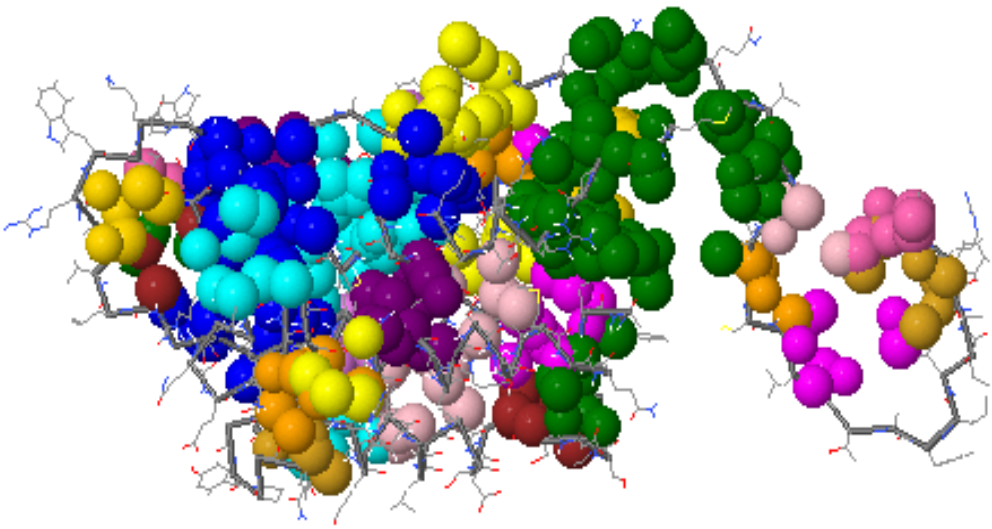
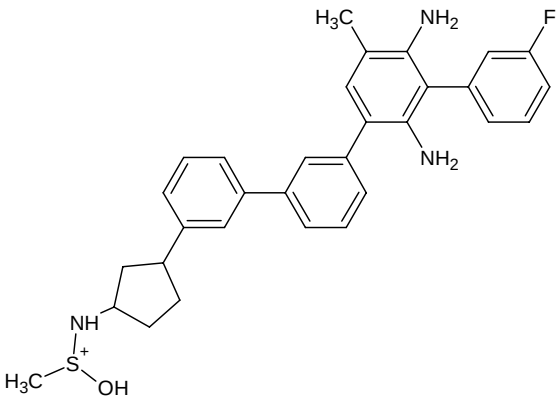


Fig 2: active site of BRCA1



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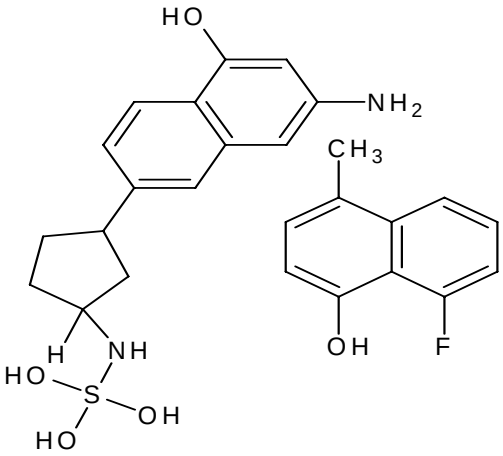

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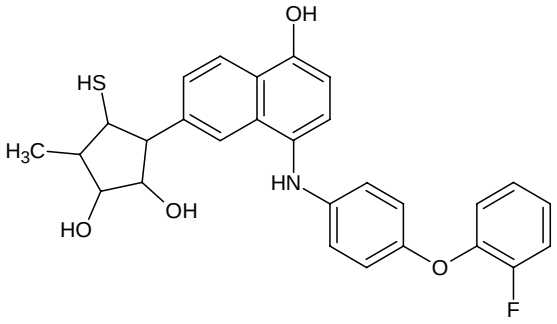

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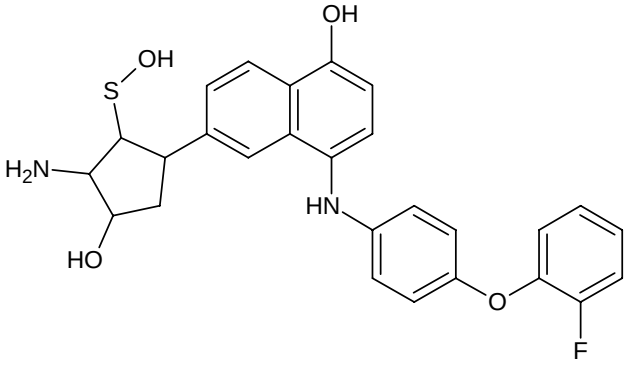

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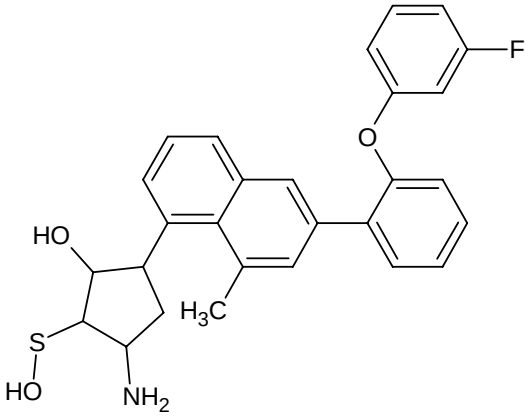

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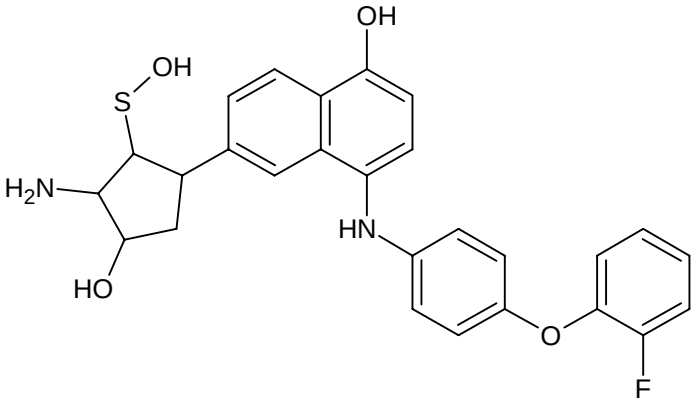

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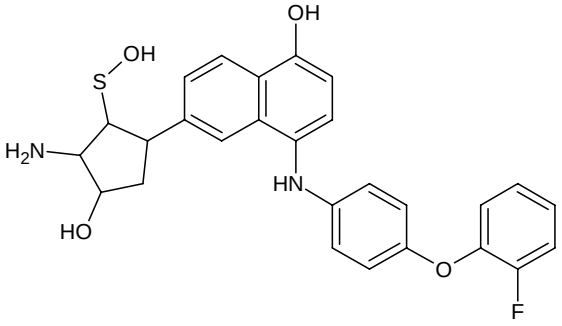

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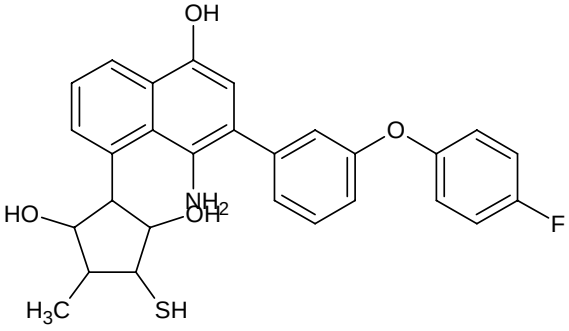

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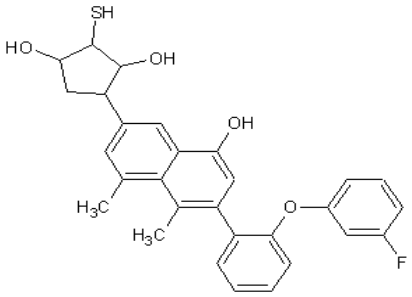

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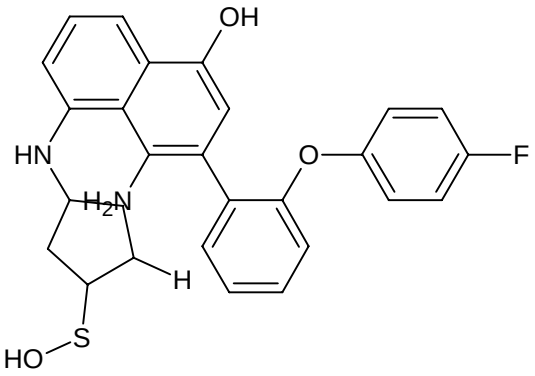

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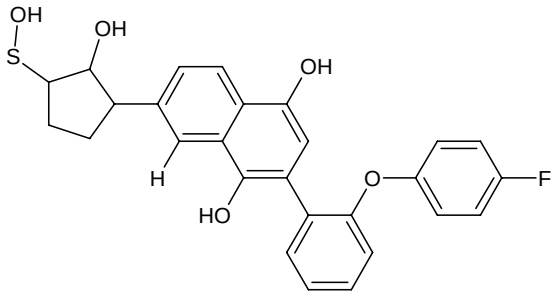

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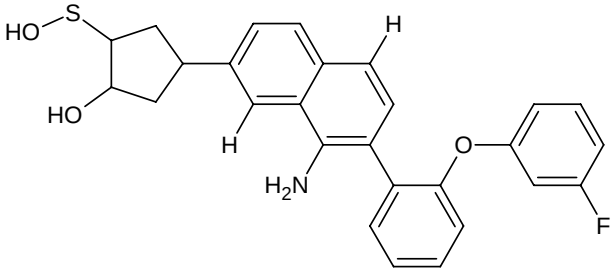

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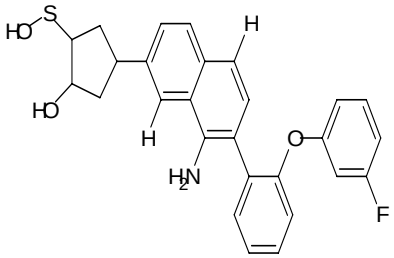

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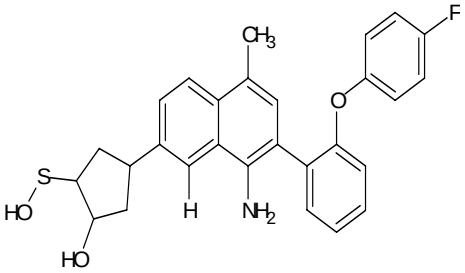

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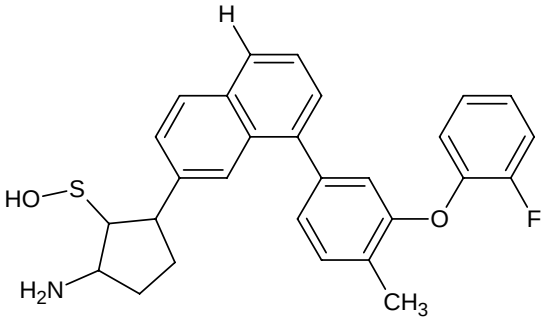

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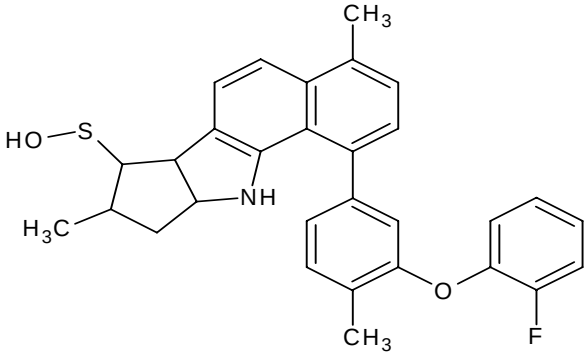

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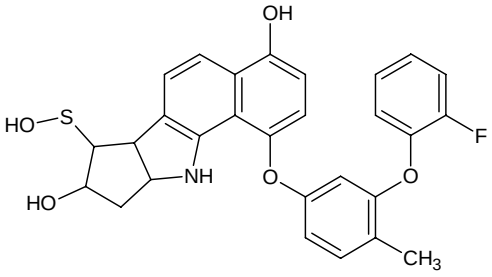

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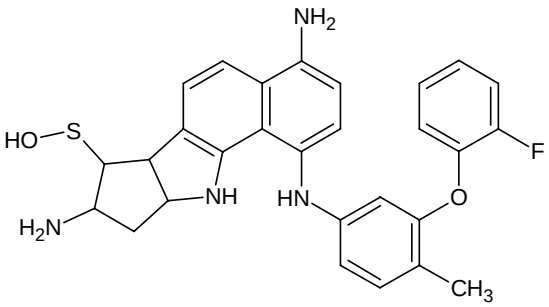

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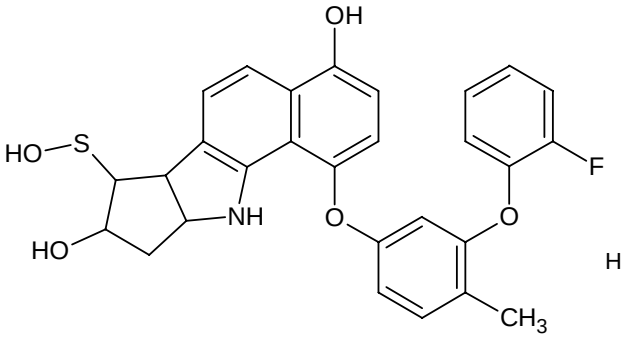

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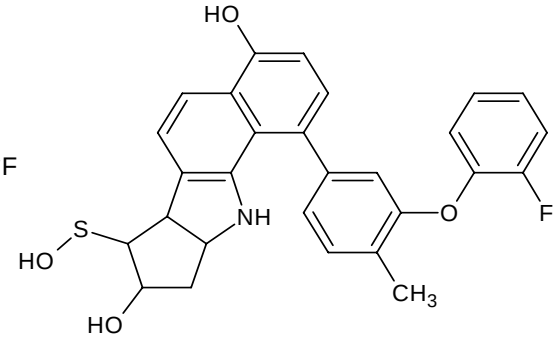

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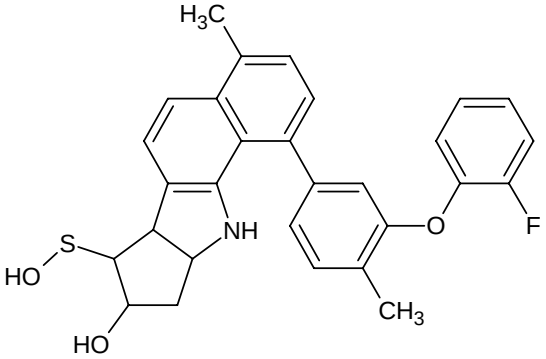

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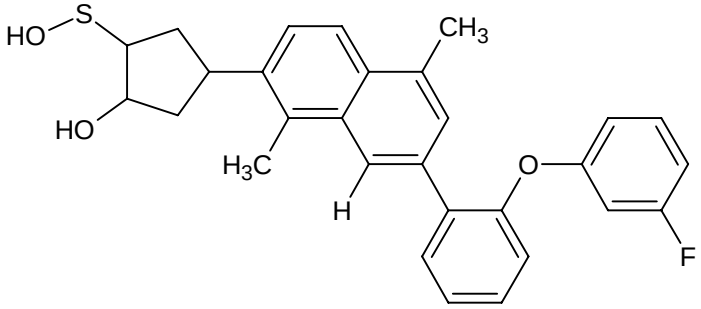

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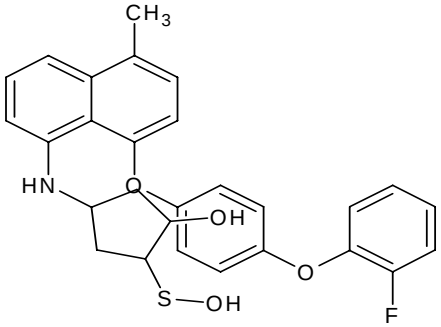

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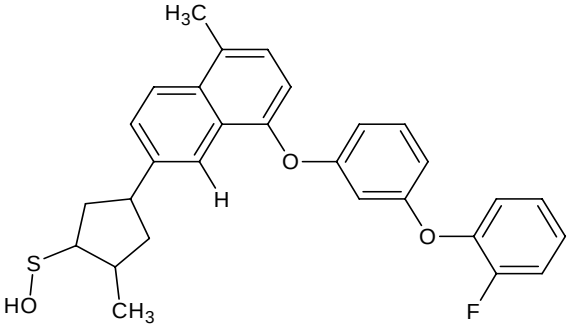

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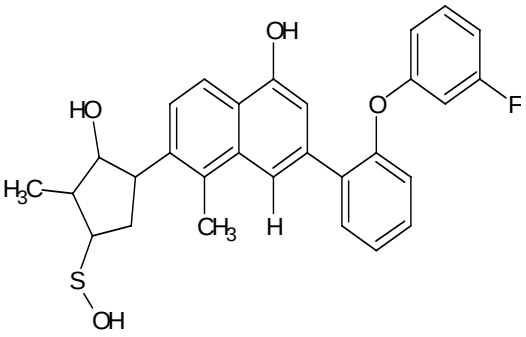

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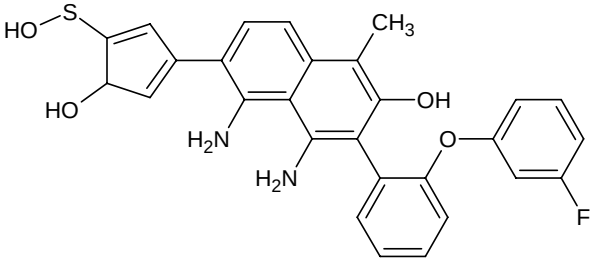

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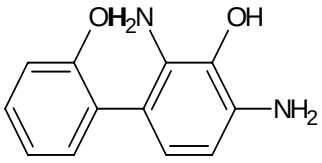

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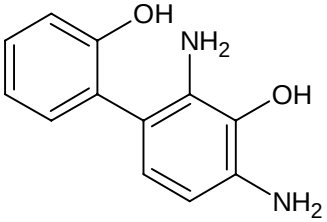

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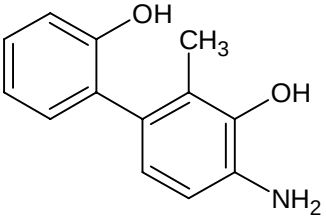

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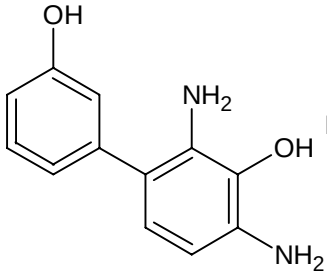

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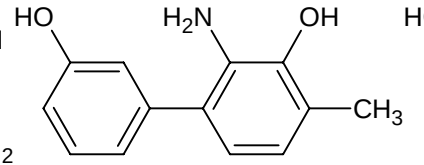

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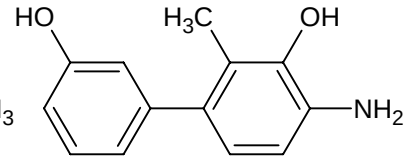

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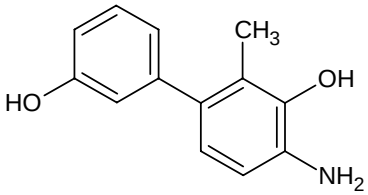

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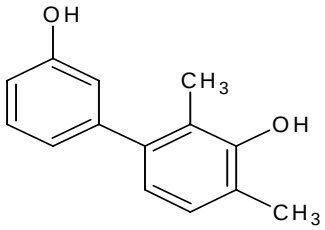

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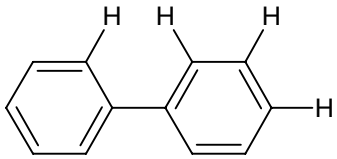

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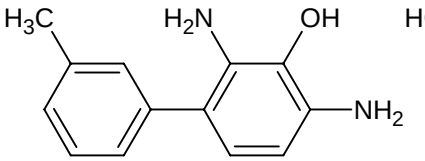

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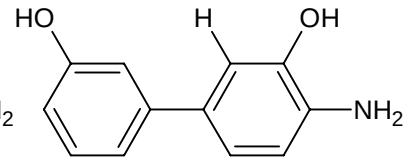

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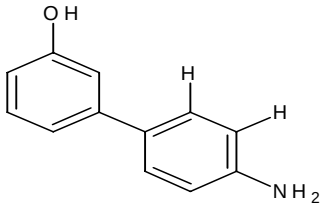

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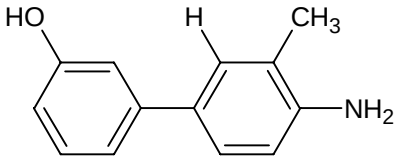

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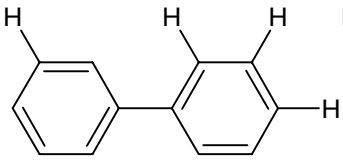
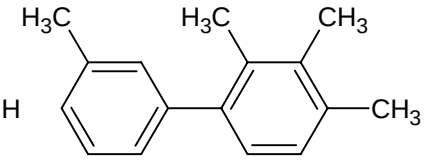


Derivative 40



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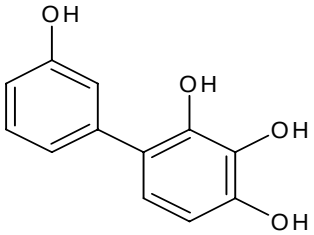

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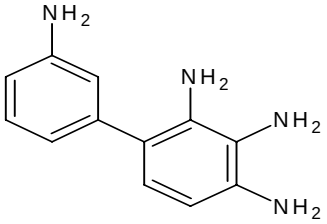

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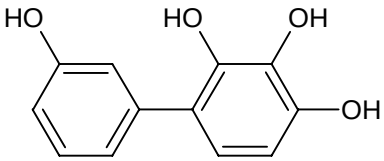
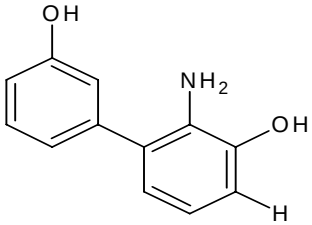
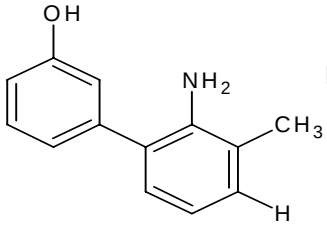


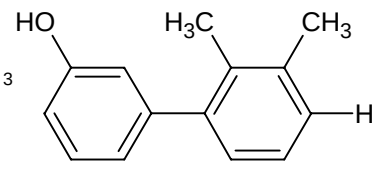
Derivative 44



Derivative 45



Derivative 46



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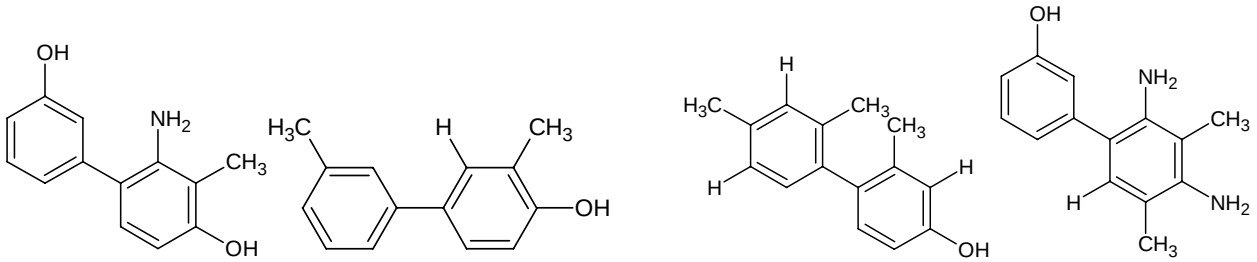


Derivative 48 Derivative 49 Derivative 50 Derivative 51

Fig-3: The inhibitors used for docking

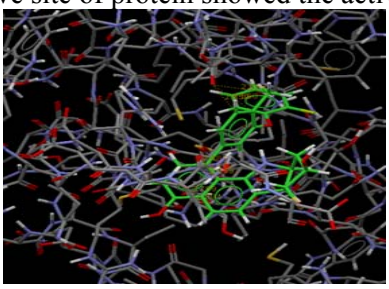
Table 1: properties of designed derivatives

Molecule	Molecular Formula	Formula Weight	Molar Refractivity cm^3	Index of Refraction	Density g/cm^3	Polarizability cm^3
1	$C_{31}H_{33}F_3N_3$	366.3392296	96.27 ± 0.5	1.632 ± 0.05	1.35 ± 0.1	38.16 ± 0.5 10^{-24}
2	$C_{26}H_{29}F_9N_2$	500.5822288	96.93 ± 0.5	-	-	-
3	$C_{28}H_{25}F_3NO4$	491.577986	139.63 ± 0.5	1.720± 0.05	1.41 ± 0.1	54.43± 0.5 10^{-24}
4	$C_{27}H_{25}FO4N_2$	492.561897	96.60 ± 0.5	1.752± 0.05	1.47 ± 0.1	54.29 ± 0.5 10^{-24}
5	$C_{28}H_{25}FNO3$	428.408609	116.95 ± 0.5	1.645 ± 0.05	1.32 ± 0.1	46.36 ± 0.5 10^{-24}
6	$C_{28}H_{26}F_4O4S$	491.573772	137.45 ± 0.5	1.736 ± 0.05	1.43 ± 0.1	54.9 ± 0.5 10^{-24}
7	$C_{30}H_{31}FO4$	366.339229	96.27 ± 0.5	1.632 ± 0.05	1.35 ± 0.1	38.16 ± 0.5 10^{-24}
8	$C_{30}H31F_4N_4$	506.628147	-	-	-	-
9	$C_{27}H_{25}F_3N_4$	476.562409	134.98 ± 0.5	1.735± 0.05	1.42 ± 0.1	53.57± 0.5 10^{-24}
10	$C_{20}H_{10}F_6N_4$	420.3106192	96.60 ± 0.5	1.594 ± 0.05	1.47 ± 0.1	38.29 ± 0.5 10^{-24}
11	$C_{27}H_{23}F_4$	478.53192	130.75 ± 0.5	1.645 ± 0.05	1.32 ± 0.1	51.86 ± 0.5 10^{-24}
12	$C_{21}H_{13}F_3N_4O_3$	426.3481296	104.16 ± 0.5	1.644 ± 0.05	1.48 ± 0.1	41.29 ± 0.5 10^{-24}
13	$C_{30}H_{26}FNO_3$	475.5748856	135± 0.4	1.719 ± 0.04	1.48 ± 0.1	53.89± 0.5 10^{-24}
14	$C_{21}H_{13}FN_4O_3$	426.3481296	104.16 ± 0.4	1.644 ± 0.03	1.48 ± 0.1	41.29 ± 0.5 10^{-24}
15	$C_{19}H_9FN_6$	378.3101696	95.86 ± 0.4	1.696 ± 0.03	1.52 ± 0.1	38.00 ± 0.5 10^{-24}
16	$C_{21}H_{14}FNO_2$	425.3633696	105.22 ± 0.4	1.654 ± 0.03	1.48 ± 0.1	41.71 ± 0.5 10^{-24}
17	$C_{16}H_{20}FN_6$	459.574955	134.41± 0.4	1.696 ± 0.02	1.32 ± 0.1	53.00 ± 0.5 10^{-24}
18	$C_{21}H_{14}FN_5O_2$	425.3633696	137.22 ± 0.4	1.654 ± 0.02	1.48 ± 0.1	41.71 ± 0.5 10^{-24}
19	$C_{19}H_{11}FN_4O$	489.557796	135. ± 0.4	1.766± 0.02	1.49 ± 0.1	53.23± 0.5 10^{-24}
20	$C_{26}H_{14}FN_4O_4$	487.58506	138.38 ± 0.4	1.733± 0.02	1.40 ± 0.1	54.391± 0.5 10^{-24}
21	$C_{23}H_{19}FN_4O_4$	472.4165696	114.38 ± 0.4	1.602 ± 0.02	1.41 ± 0.1	45.34 ± 0.5 10^{-24}
22	$C_{20}H_{11}FN_4O_3$	412.3215496	99.55 ± 0.4	1.654 ± 0.02	1.51 ± 0.1	39.46 ± 0.5 10^{-24}
23	$C_{20}H_{11}F_3N_4O_3$	474.567896	137.46± 0.5	1.672± 0.03	1.38 ± 0.1	`54.37± 0.5 10^{-24}
24	$C_{23}H_{28}FNO_4$	490.5856326	138 .46± 0.4	1.702 ± 0.03	1.38 ± 0.1	54.374 ± 0.5 10^{-24}
25	$C_{23}H_4FN_4O_4$	5025566324	140.49± 0.4	1.797 ± 0.03	1.52± 0.1	55.69 ± 0.5 10^{-24}
26	$C_{23}H_{11}FN_2O_4$	261.2358196	63.08 ± 0.3	1.74102± 0.02	1.384± 0.6	543.± 0.5 10^{-24}
27	$C_{23}H_{11}FNO_4$	472.4165696	114.38 ± 0.4	1.602 ± 0.03	1.41 ± 0.1	25.34 ± 0.5 10^{-24}

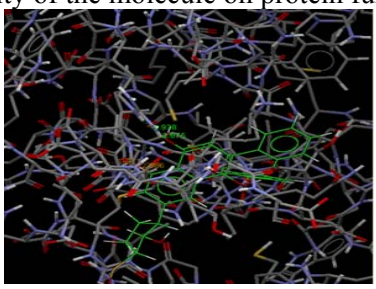
Docking of inhibitors with the active site of BCL2L10

Docking of the inhibitors with BCL2L10 was performed using GOLD 3.0.1, which is based on genetic algorithimn. This program generates an ensemble of different rigid body orientations (poses) for each compound conformer within the binding pocket and then passes each molecule against a negative image of the binding site. Poses clashing with this 'bump map' are eliminated. Poses surviving the bump test are then scored and ranked with a Gaussian shape function. We defined the binding pocket using the ligand-free protein structure and a box enclosing the binding site.

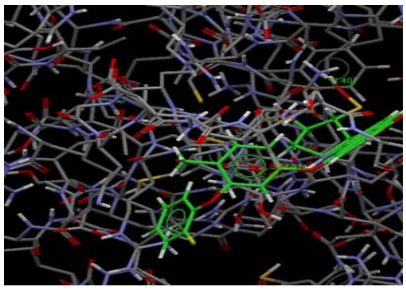
This box was defined by extending the size of a cocrystalized ligand by 4A. This dimension was considered here appropriate to allow, for instance, compounds larger than the cocrystallized ones to fit into the binding site. One unique pose for each of the best-scored compounds was saved for the subsequent steps. The compounds used for docking was converted in 3D with SILVER. To this set, the substrate corresponding to the modeled protein were added. Docking of best inhibitor with the active site of protein showed the activity of the molecule on protein function (Fig 4 and table 2).



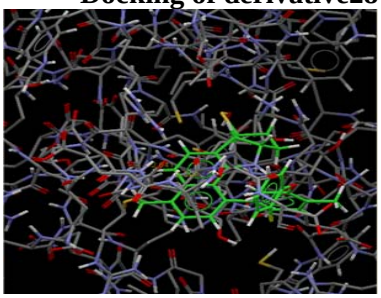
Docking of derivative16 with BRCA1



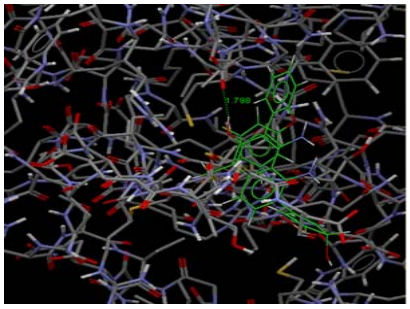
Docking of derivative28 with BRCA1



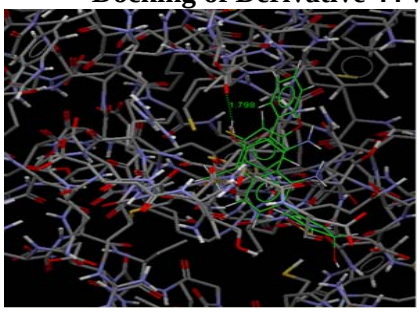
Docking of derivative 35 with BRCA1



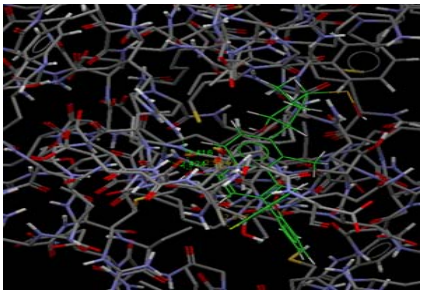
Docking of Derivative 44 with BRCA1



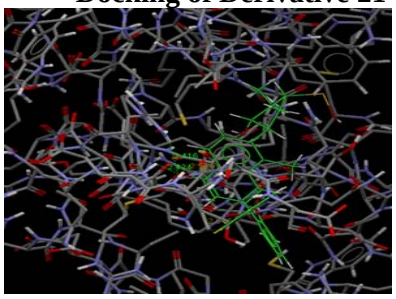
Docking of Derivative 11 with BRCA1



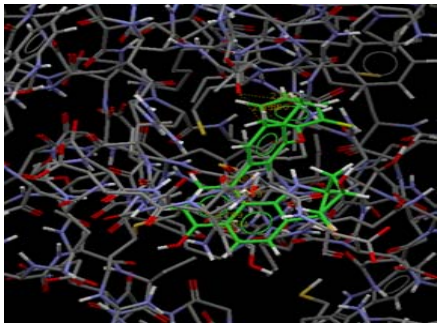
Docking of Derivative 21 with BRCA1



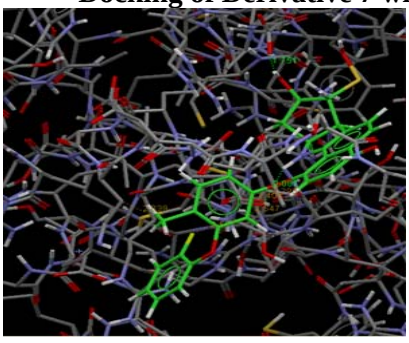
Docking of Derivative 5 with BRCA1



Docking of Derivative 7 with BRCA1



Docking of Derivative 19 with BRCA1



Docking of Derivative 28 with BRCA1

Figure 4: docking studies of best docked derivatives

Table 2: Docking studies of derivatives with BRCA1

Fitness	S(hb_ext)	S(vdw_ext)	S(hb int)	S(int)	Ligand name
29.59	5.55	20.74	0.00	-4.48	drug1
31.70	1.28	24.69	0.00	-3.54	drug10
34.66	8.96	20.35	0.00	-2.29	drug11
34.00	6.00	21.56	0.00	-1.64	drug12
29.32	2.00	22.80	0.00	-4.03	drug13
25.38	0.00	24.29	0.00	-8.01	drug15
35.70	8.07	23.10	0.00	-4.13	drug16
24.55	0.66	18.00	0.00	-0.86	drug17
32.52	6.43	21.90	0.00	-4.03	drug18
30.32	6.00	19.37	0.00	-2.32	drug19
24.64	3.56	17.57	0.00	-3.08	drug2
31.13	6.20	21.69	0.00	-4.89	drug20
31.48	6.23	22.44	0.00	-5.59	drug21
28.05	9.29	17.03	0.00	-4.65	drug22
29.31	0.92	22.90	0.00	-3.10	drug23
27.37	4.02	19.17	0.00	-3.01	drug24
2.79	3.47	20.94	0.00	-29.47	drug25
31.89	3.21	24.21	0.00	-4.61	drug3
27.57	6.12	21.76	0.00	-8.47	drug4
34.34	8.55	22.53	0.00	-5.18	drug5
27.57	6.12	18.86	0.00	-4.48	drug7
34.97	9.48	23.75	0.00	-7.16	drug8
26.28	0.00	19.60	0.00	-0.67	drug9
28.44	1.73	26.67	0.00	-9.95	molcule13
39.00	8.00	29.66	0.00	-9.78	molecule1
28.69	6.09	23.85	0.00	-10.20	molecule11
29.37	0.31	25.92	0.00	-6.59	molecule12
38.59	2.00	30.44	0.00	-5.27	molecule14
25.83	0.56	24.42	0.00	-8.31	molecule15
43.68	5.46	30.97	0.00	-4.36	molecule16
-103.50	1.89	21.28	0.00	-134.65	molecule17
42.07	6.05	28.86	0.00	-3.66	molecule18
34.89	0.00	29.19	0.00	-5.24	molecule19
25.94	6.76	26.97	0.00	-17.92	molecule2
28.92	0.00	26.08	0.00	-6.93	molecule20
10.72	5.00	30.50	0.00	-36.21	molecule21
-13.57	0.00	29.19	0.00	-53.71	molecule22
29.17	1.18	29.11	0.00	-12.03	molecule23
3.11	2.73	27.22	0.00	-37.05	molecule24
20.09	2.72	22.38	0.00	-13.40	molecule25
-80.66	0.00	31.46	0.00	-123.92	molecule3
-85.69	0.05	31.42	0.00	-128.94	molecule4
-44.46	0.00	21.08	0.00	-73.44	molecule6
-22.97	5.88	29.58	0.00	-69.53	molecule7
0.24	7.81	31.43	0.00	-50.78	molecule8
-6.29	0.34	30.33	0.00	-48.33	molecule9
-46.45	0.00	27.99	0.00	-84.93	molecule5

CONCLUSION

BRCA1 is one of the regulator protein, which specifically binds with the cell division proteins. In this work, we have collected 3D model of BRCA1 domain, from human using the SPDBV software and obtained a refined model after energy minimization.

The final refined model was further assessed by ERRAT & PROCHECK program, and the results show that this model is reliable. The stable structure is further used for docking of substrate with the derivatives of podophyllotoxin. Docking results indicate that conserved amino-acid residues in BRCA1 main play an important role in maintaining a functional conformation and are directly involved in donor substrate binding. The interaction between the domain and the inhibitors proposed in this study are useful for understanding the potential mechanism of domain and the inhibitor binding. As is well known, hydrogen bonds play important role for the structure and function of biological molecules. In this study it was found that ASP-61, CYS-66, CYS-88, TYR-112, LEU-113, LEU-115, ASP-117, ILE-118, GLN-136 of BRCA1 are important for strong hydrogen bonding interaction with the inhibitors. To the best of our knowledge ASP-65, CYS-66, CYS-88, TYR-112, LEU-113 are conserved in this domain and may be important for structural integrity or maintaining the hydrophobicity of the inhibitor-binding pocket. The molecules 35, 44, 5 showed best docking results with target protein.

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