Insilico Analysis of Protein-Ligand Docking of DHFR (Dihydro Folate Reductase) and Quassinoids

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ABSTRACT

Quassinoids are the naturally available plant extracts which exhibit a wide range of biological activities , that include anti malarial, anti amoebic, anti tumor properties etc. The present experiment aims to exploit the antitumor properties of the quassinoids. 68 quassinoid analogues were designed and were docked with hDHFR (human dihydrofolate reductase) a potential cancer target. The docking results showed compound 33 to be the best ligand for DHFR**.**

KEYWORDS

Quassinoids, quassinoid ligands, quassinoids analogues, anti tumor, DHFR.

1. INTRODUCTION:

Quassinoids are the naturally available plant agents which exhibit a host of biological activities [1,2], seen mostly in Simaroubaceae species [3]. These biologically active phytochemical agents belong to the triterpene chemical family. The main active groups of the Quassinoids are Ailanthionone, Glaucorubinone and Holacanthone besides Benzoquinone, Canthin, Dehydroglaucarubinone and also Glaucarubine, Simarolide, Sitosole and Melianone [4].

The picrasane skeleton, a pentacyclic derivative of the Quassinoids have shown a remarkable antitumor activity [5]. Quassinoids also are known for their potent antimalarial [6], antimicrobial and antiprotozoal activities [7,8].

The present experiment aims to create the analogues for Quassinoids exploiting their antitumor property and study the binding affinity of these analogues for hDHFR, a target for Cancer [9].

Fluoropyrimidine – Antifolate based chemotherapeutic are extensively used to decrease the number of tumors.Human DHFR (hDHFR) Dihydrofolate reductase EC 1.5.1.3 is an enzyme with 186 amino acids and bears the molecular weight of 21.3 k Da. The gene coding the human DHFR is found in q11-q22 region of the chromosome 5 [10].

DHFR catalyses the reduction of 7,8 dihydro folate to folate 5,6,7,8-tetrahydrofolate (THF) which is an NADPH dependent reduction. THF are needed for a majority of one-carbon transfer reaction, in the biosynthesis of serine, glycine, thymidylate, purine, pyrimidine synthesis etc.

DHFR is useful in maintaining the intracellular folate pool and its depletion results in depletion of folate pool and hence effects the one-carbon transfer reactions.[11]

2. MATERIALS AND METHODS:

2.1 Ligands Preparation:

The quassinoid acts as a ligand for the present study and were drawn using chemsketch (ACDLABS 12.0). Removal of duplicates were done and bonds were then added to it. The CHARM m force field was used to minimize the energy and thereafter using catalyst the 3D structures were generate

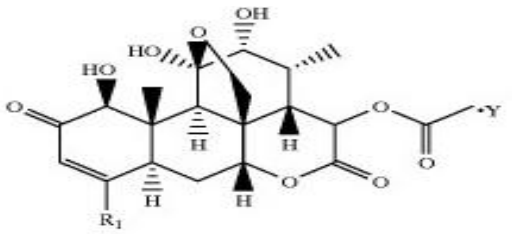


Figure 1: Structure of Quassinoid.

Further, 68 ligands were designed by substituting the groups at R_1 position and Y position [12]. This was followed by the ligand optimization.

Table 1. Substitution at R_1 and Y positions.

Compound	R_1	Y
1	H	CH_3
2	H	C_6H_5
3	H	COOH
4	H	CYCLOPROPYL
5	CH_3	CH_3
6	CH_3	C_6H_5
7	CH_3	COOH
8	CH_3	CYCLOPROPYL
9	F	CH_3
10	F	C_6H_5
11	F	COOH
12	F	CYCLOPROPYL
13	ARYL	CH_3
14	ARYL	C_6H_5
15	ARYL	COOH
16	ARYL	CYCLOPROPYL
17	SULFO	CH_3
18	SULFO	Ph
19	SULFO	COOH
20	SULFO	CYCLOPROPYL
21	NITRO	CH_3
22	NITRO	Ph
23	NITRO	COOH
24	NITRO	CYCLOPROPYL
25	CARBOXYL	CH_3
26	CARBOXYL	Ph
27	CARBOXYL	COOH
28	CARBOXYL	CYCLOPROPYL
29	OH	CH_3
30	OH	Ph
31	OH	COOH
32	OH	CYCLOPROPYL
33	H	H
34	OMe	CH_3
35	OMe	Ph
36	OMe	COOH
37	OMe	CYCLOPROPYL
38	OMe	H
39	Nitro	H
40	Sulfo	H
41	Aryl	H
42	F	H
43	CH_3	H

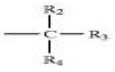
If Y side chain is referred by the formula [11]  ,then the substituents for the ligand would be

Table 2. Substitution at Y positions for R_2, R_3 , R_4.

COMPOUND	R1	R2	R3	R4
44	H	CH_3	CH_3	CH_3
45	OCH_3	CH_3	CH_3	CH_3
46	ARYL	CH_3	CH_3	CH_3
47	SULFO	CH_3	CH_3	CH_3
48	NITRO	CH_3	CH_3	CH_3
49	F	CH_3	CH_3	CH_3
50	CH_3	CH_3	CH_3	CH_3
51	CARBOXYL	CH_3	CH_3	CH_3
52	H	CH_3	CH_3	OH
53	OCH_3	CH_3	CH_3	OH
54	ARYL	CH_3	CH_3	OH
55	SULFO	CH_3	CH_3	OH
56	NITRO	CH_3	CH_3	OH
57	F	CH_3	CH_3	OH
58	CH_3	CH_3	CH_3	OH
59	CARBOXYL	CH_3	CH_3	OH
60	H	C_2H_5	OH	C_2H_5
61	OMe	C_2H_5	OH	C_2H_5
62	ARYL	C_2H_5	OH	C_2H_5
63	SULFO	C_2H_5	OH	C_2H_5
64	SULFO	C_2H_5	OH	C_2H_5
65	NITRO	C_2H_5	OH	C_2H_5
66	F	C_2H_5	OH	C_2H_5
67	CH_3	C_2H_5	OH	C_2H_5
68	COOH	C_2H_5	OH	C_2H_5

2.2 Protein Preparation:

The protein for the present study is imported from PDB [Protein Data Bank]. The X-Ray crystal structure with high resolution of human DHFR (Dihydrofolate reductase) PDB ID: 1KMS was imported into the discovery studio (Accelrys 2.1).

The protein chemistry of the missing hydrogen was corrected after which the hetero atoms and the crystallographic water molecules were removed from the protein. Valence monitor options and alternate conformations were used to connect the crystallographic disorder and the unfilled valence atoms. The protein was then subjected to energy minimization steps by applying the steepest descent method which was then followed then followed by the conjugate gradient method until the convergence gradient was satisfied. The active site pockets of the protein hDHFR were identified using eraser algorithm and a sphere was created around the active site.

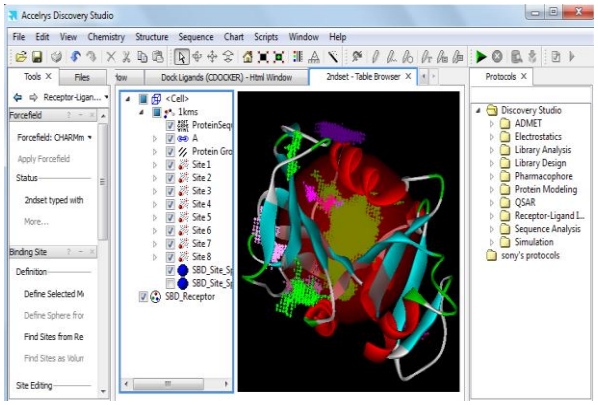


Figure 2. Active site pockets by Eraser algorithm

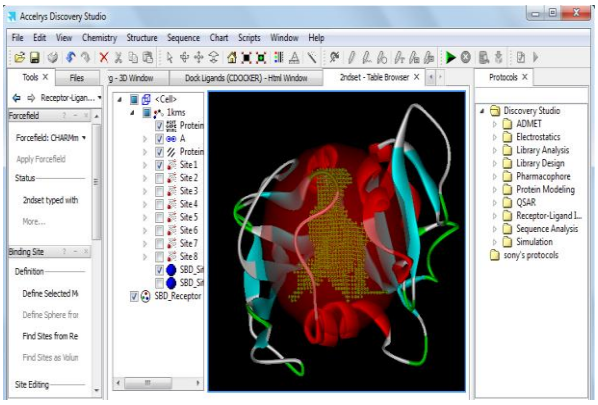


Figure 3. Sphere around the active site pocket

2.3 Ligand – Protein Docking:

Protein – Ligand docking is a molecular modeling technique aims at predicting the position and orientation of the ligand when it binds to the proteins. This method is mostly employed in designing the new drugs. A CHARMm-based docking program DOCK algorithm which offers a full ligand flexibility (including bonds, angles, dihedrals) was employed to find the potential binding mode between both protein and the ligand. In the present experiment the Quassiniod analogues were docked with the hDHFR and the binding affinity was studied.

3. Results And Discussions:

The Protein – Ligand interaction of hDHFR and Quassinoids was studied. Compound-33 shows Highest binding affinity-78.591K.cal/mol and good hydrogen bonding interactions with active site residues of Human DHFR , which shows Hydrogen bonding interactions with Tyr121, Ala9, Phe34, Ile16, and Val 115.

Table 3. Dock results

Compound	**Lig Score 1**	**Lig Score 2**	**Plp1**	**Plp2**	**Jain**	**Pmf**	**Dock score**
Comp 1							
Comp 2	2.2	2.77	87.21	85.42	4.52	124.95	36.73
Comp 3	1.72	2.01	85.73	82.21	4.32	113.8	33.054
Comp 4	1.75	2.42	85.67	81.96	4.66	114.65	33.19
Comp10	1.6	2.5	71.39	75.1	4.12	121.83	34.321
Comp 11	1.73	2.44	55.09	58.31	4.73	83.28	29.543
Comp 12	1.83	2.82	63.43	66.48	3.98	106.15	31.539
Comp 13	1.51	2.53	69.84	77.22	5.73	81.37	34.417
Comp 14	1.79	2.96	83.63	88.86	5.86	111.11	42.248
Comp 15	1.41	2.23	70.24	77.17	5.28	88.98	35.831
Comp 16	1.77	2.86	78.2	83.29	6.16	99.67	39.013
Comp 17	2.36	2.78	59.93	63.28	4.3	80.48	34.668
Comp 18	2.9	3.29	86.32	87.15	4.82	108.74	35.66
Comp 19	2.52	2.6	75.68	79.48	4.3	94.51	36.636
Comp 20	2.23	2.66	71.01	74.97	5.41	98.58	3.649
Comp 21	1.35	2.47	59.12	57.36	4.27	91.56	24.52
Comp 22	1.34	2.84	74.78	70.95	4.68	109.32	32.143
Comp 23	1.21	2.37	69.35	64.92	4.41	94.85	27.259
Comp 24	1.37	2.81	75.11	70.84	4.73	109.41	31.651
Comp 30	2.33	2.45	93.49	92.6	4.3	124.97	36.971
Comp 31	3..1	2.38	80.71	79.53	4.52	90.44	34.581
Comp 32	1.76	2.94	75.59	76.14	3.6	117.79	34.363

Comp 33	2.53	3.82	56.7	58.68	4.13	116.05	45.855
Comp 34	1.63	2.86	64.08	63.36	5.15	88.26	33.754
Comp 35	1.84	3.39	82.97	81.35	5.29	101.15	42.721
Comp 36	1.83	2.81	70.55	69.33	4.62	87.29	36.224
Comp 37	1.86	3.15	79.85	73.79	3.67	115.49	39.101
Comp 38	1.61	3.44	56.87	56.43	3.74	101.42	40.981
Comp 39	2.46	3.16	69.16	68.09	4.16	114.71	37.346
Comp 40	2.41	3.09	85.16	80.27	3.55	99.75	36.392
Comp 41	1.51	2.97	62.39	64.75	5.18	95.34	39.423
Comp 42	2.36	3.42	54.43	60.39	4.75	109.06	42.487
Comp 43	2.2	3.81	81.8	79.47	4.32	127.25	42.182
Comp 44	2.06	2.94	82.61	83.56	4.23	121.74	36.481
Comp 53	1.21	2.01	70.92	80.63	6.45	97.75	26.188

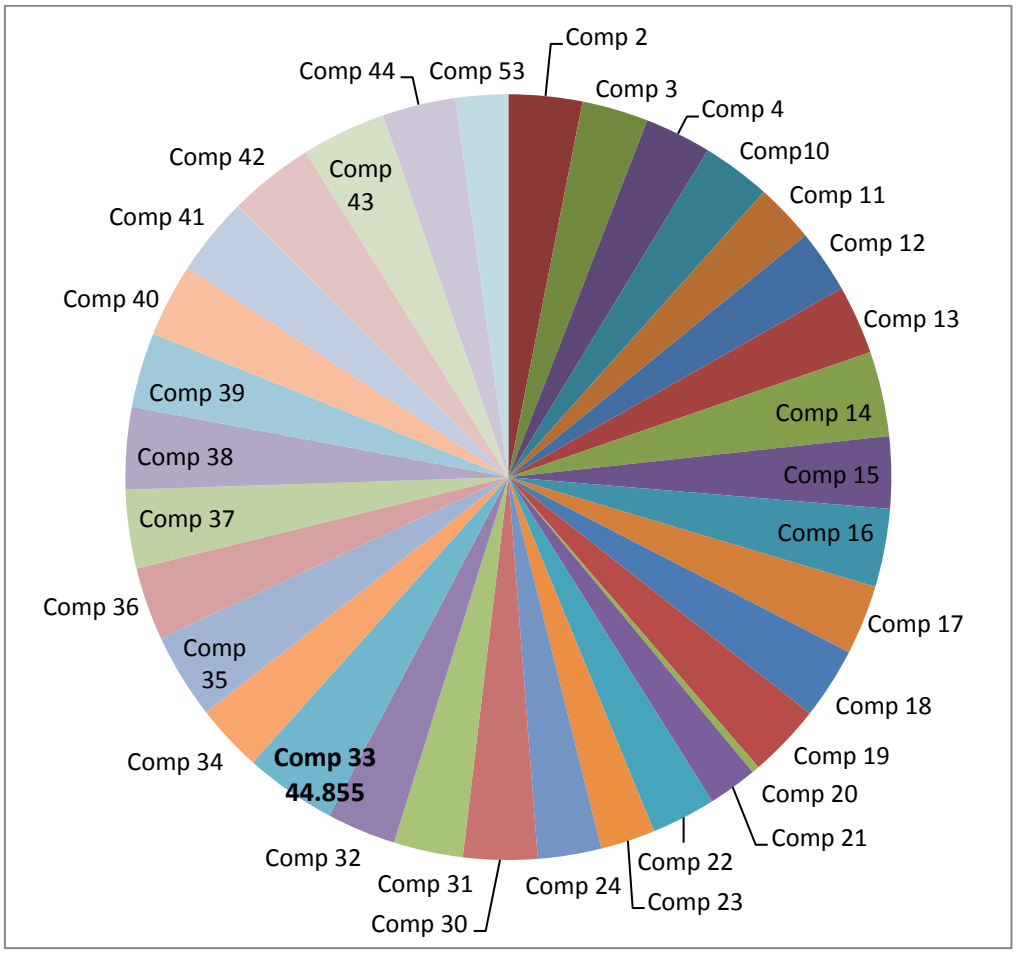


Figure 4. Graphical representation of the Dock results.

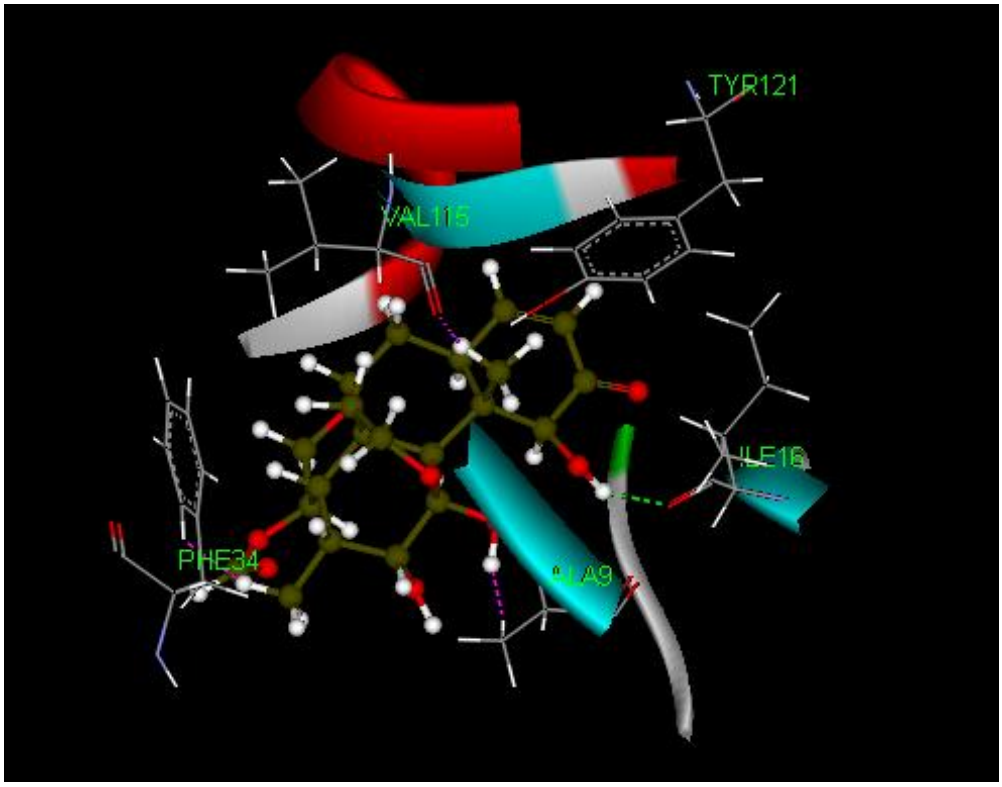


Figure 4. Docking of compound 33 with DFHR

4. CONCLUSION:

In the present experiment, the protein-ligand docking studies were performed to explore the binding affinity of hDHFR and the Quassinoids.The study also explores Quassinoids and its analogues to act as an alternative drugs for cancer which acts effectively on hDHFR, a cancer target.

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