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SCREENING OF UNIQUE BINDING SITE SPECIFIC LIGAND FOR CARBONIC ANHYDRASE IX

Priyanka Padia, Kajal Kumari, Shikha Kumari and Vidya Niranjan*

Department of Biotechnology, R.V. College of Engineering, Mysore Road, Bengaluru- 560059, Karnataka, India.

Keywords:

Cancer, Carbonic Anhydrase IX, Inhibitor, cavities, docking

Correspondence to Author:
Vidya Niranjan

Department of Biotechnology, R.V. College of Engineering, Mysore Road, Bengaluru- 560059, Karnataka, India.

E-mail: priyanka_padia@hotmail.com

ABSTRACT: Cancer is a common and widespread cause of human death in the world. Treatments like chemotherapy and radiotherapy facilitate in prolonging the patient's life however in some cases cancer can be prevented. Cancers like breast, prostate, colon, renal cell carcinoma are caused by the overexpression of an enzyme termed Carbonic Anhydrase IX (CAIX). In 2015, 60,290 new cases of breast cancer alone are estimated. If this overexpression is controlled using an inhibitor, then the mortality rates due to cancer can be brought down by a significant margin. The primary objective of this research is to find an inhibitor that would reduce or down regulate the overexpression of CAIX in the human body. The inhibitor would specifically target CAIX. CAIX has a region which is unique and its cavities were identified. These were docked with the chosen inhibitor. Numerous acetazolamides and sulfanilamides were tested as inhibitors. Various bioinformatics tools such as Maestro and Hex were used for docking and grid generation. Docking scores were obtained and studied. The inhibitors with the highest negative docking score was selected. Two cavities were found to be the most efficient. Indane sulphonamide (PDB ID- 2QOA) was found to be the best fit for cavity 2 while Carbonic Anhydrase II Inhibitor (PDB ID- 2X7T) was found to be the best fit for cavity 6. The future in this direction of research is the possible production of anti-cancer drugs, immune therapy, cancer specific antibodies, usage of selective inhibitors and many such novel strategies to battle against cancer.

INTRODUCTION: Carbonic anhydrase is a metalloenzyme having zinc in its prosthetic group along with Histidine residues. It participates in the reversible hydration of carbon dioxide forming proton and bicarbonate, thereby maintaining the pH of the cellular environment. There are five distinct families of Carbonic Anhydrase namely α, β, γ, δ, ε. α being the mammalian CA constituting of 16 isozymes [1].

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These isozymes are similar in function in many aspect but also have differences based on their activity and localization. CAIX enzyme helps in the proliferation, differentiation in the body cells. It is generally found in the solid tumor cells and is induced by hypoxia. It is regulated by hypoxia inducible factor (HIF-1) [2].

CAIX is one of the mammalian trans membrane enzymes that participate in variety of biological reaction. Researchers have said that over expression of CAIX is linked to the proliferation of cancer. Therefore an inhibitor is desired that would down regulate the expression of CAIX. The inhibitor binds to the zinc present in the active site and blocks its reaction thus suppressing the action of CAIX. The well-known inhibitors for CAIX are sulphonamides, phenols, inorganic compounds, but

these inhibitors tend to bind with other residues present in and around the cell thus not being1. specific [3]. Hence a CAIX specific inhibitor is anticipated in this research that would bind only to a unique and specific site that would lower the expression of CAIX and prevent cancer.

The problem that has been encountered till now is that the inhibitor attacks all the isozymes instead of CAIX specifically and this is counterproductive. Hence the need of a specific inhibitor arises.

Research is being carried out to investigate the expression of CAIX in human fetus if it is regulated by hypoxia inducible factors. Many studies were done on mice to figure out the anti-convulsant potency by using electric shock, because of this property inhibition of CAIX is possible [4]. Many compounds like acetazolamide and sulphonamide are found to be the inhibitors for CAIX.

The Journal of medical chemistry and Journal of radiotherapy and oncology have published two articles about the proteins which help in the survival of cancer cells. The condition which is maintained by CAIX is in neutral pH that allows the cancer to survive [5]. Even in chemotherapy and radiotherapy, resistance to treatment develops. It was a major obstacle in cancer treatment. During the period of treatment it was observed that CAIX showed an up regulation in drug resistance in tongue cancer. Despite the new strategies and technologies in chemotherapy, the survival rate was only 5-10% [6].

Medical chemistry and bioinformatics have provided a good rostrum to conceal the chemical structure and topological indices. This encouraged in the development of large databases with rapid collection, retrieval, annotation, comparison and mining. This led to the seeking of relationship between the chemical structure and biological activity. A new area for drugs based on structural designs is rapidly growing with the emerging opportunities in the genomics, proteomics for hundreds of targets [7]. The analysis of the targets and its specificity are important to design a drug.

Objectives:

Prediction of unique and common binding sites in CAIX using MOLE 2.0 software. Screening and validation of isozyme specific inhibitor for CAIX.

MATERIALS AND METHODS:

As it research in the field of bioinformatics, no tangible material was used. All the data was collected online and the simulation was run using various software. The experimentation has been done in R.V. College of Engineering in 2015.

The complete procedure is described below:

1. The sequences are collected in PDB format from NCBI. The source is human beings.

2. Using the online tool, CLUSTAL W2, multiple sequence alignment is done and the guide tree and phylogram are obtained.

3. CAIX, CAXII, CAVI and CAXIV were closely related and they were studied further.

4. The variable and conserved regions are marked in the result of the multiple sequence alignment. These areas were studied and it was found that CAIX has a unique variable region.

5. Thus, CAIX was run in human genome blast and it was found that it had only 38% similarity compared to other organisms. This helped in determining that a specific inhibitor is be possible for CAIX.

6. Modeller was used to prepare the structures of Carbonic Anhydrase.

7. The Ramachandran Plots were created using RAMPAGE. They were studied and the ones with the most favorable regions were chosen.

8. Using the most favorable structures, **MOLE 2.0** was used to create cavities and the best ones were chosen. Cavities 2 and 6 were chosen from CAIX.

9. Inhibitors like acetazolamides and sulfanilamides were screened using Maestro and screening was done. It was shortlisted to 8 inhibitors.

10. He was used to do the docking procedure. Mutated enzyme was docked with inhibitors. Docked complexes were obtained.

11. The complexes were used for the process of validation. Maestro was used for this as well.

RESULTS AND DISCUSSION:

Modelling of the structure:

Found the variable region of CA IX. This region was used for human genome blast to find the uniqueness of the variable region.

CAIX, CAVI, CAXII, CAIV are the closely related ones.

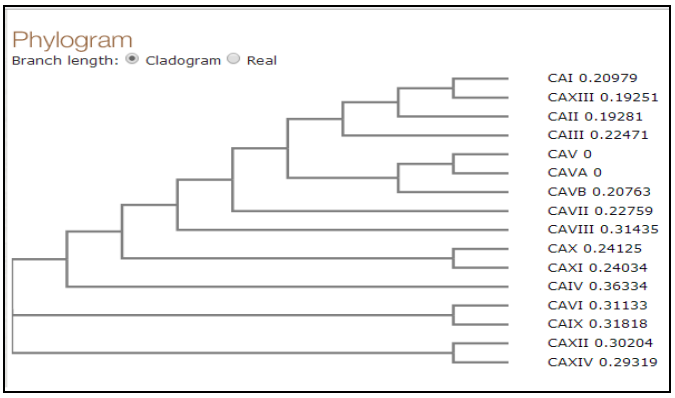

FIG. 1: PHYLOGRAM OF THE CLOSELY RELATED CA'S.

In **Fig.1** the phylogram analysis is done using cladogram branch. From the analysis it is clear that **Fig. 2** and **3** show the human genome blast and the variable region. Similarity was found to be 38%.

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenPept Graphics Distance tree of results Multiple alignment

Description	Max score	Total score	Query cover	E value	Ident	Accession
PREDICTED: carbonic anhydrase 9 isoform X1 [Homo sapiens]	194	194	100%	6e-62	100%	XP_006716932.1
PREDICTED: carbonic anhydrase 9 isoform X2 [Homo sapiens]	193	193	100%	7e-62	100%	XP_006716933.1
carbonic anhydrase 9 precursor [Homo sapiens]	198	198	100%	2e-61	100%	NP_001207.2
cerebellar degeneration-related antigen 1 [Homo sapiens]	30.4	87.0	57%	0.48	38%	NP_004056.2
PREDICTED: collagen alpha-1(XXIV) chain isoform X3 [Homo sapiens]	28.5	28.5	68%	2.6	28%	XP_011539494.1
collagen alpha-1(XXIV) chain precursor [Homo sapiens]	27.7	27.7	66%	5.8	29%	NP_690850.2
PREDICTED: collagen alpha-1(XXIV) chain isoform X2 [Homo sapiens]	27.3	27.3	66%	6.5	29%	XP_011539493.1

FIG. 2: HUMAN GENOME BLAST OF CARBONIC ANHYDRASE IX.

FIG. 3: VARIABLE REGION RUN AGAINST HUMAN GENOME BLAST.

Fig.4 and **5** show that model 5 has the least number of disallowed regions and the most number of favoured regions. Hence it is the best suited option. No. of residues in favoured region- 450 (98.5%), No. of residues in allowed regions: 4 (0.9%), No. of residues in outlier region: 2 (0.4%).

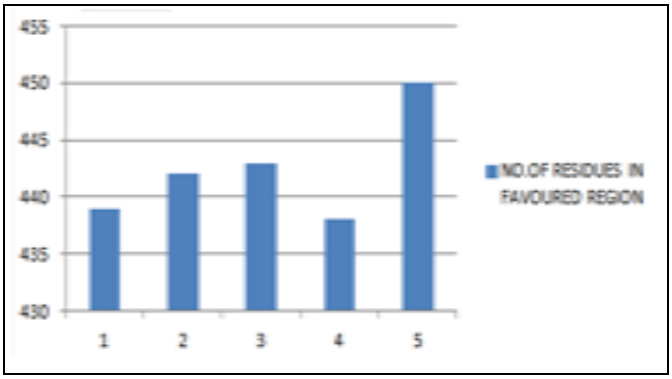

FIG. 4: FAVORED REGIONS SHOW THAT MODEL 5 IS THE BEST FIT.

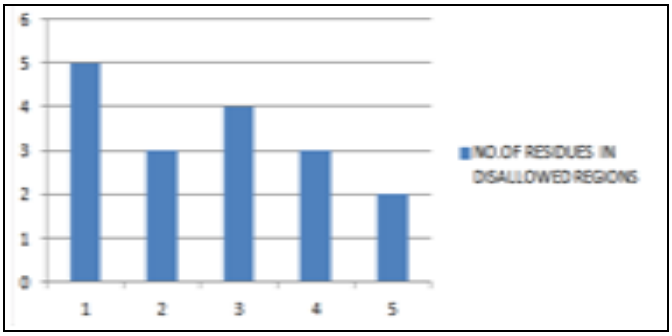

FIG.5: DISALLOWED REGIONS SHOWING THAT MODEL 5 IS THE BEST FIT.

Generation of cavities:

The MOLE2.0 software generated 4-6 cavities for both the CAs. On comparing the found variable region with the amino acid sequence of the cavities generated by the software, we were able to find one binding site sequence present in the variable region of the isozymes. This will help to generate inhibitors specific to that particular isozyme to produce an isozyme specific drug.

Fig. 6 and **7** show cavities 2 and 6 respectively. The residues were a close match to the unique region (1-160 and 350-459).

Screening of inhibitors:

Eight inhibitors were screened from 20 using Maestro and parameters like:

1. Toxicity
2. Crystallographic resolution < 2.5
3. Structure
4. Ligands present

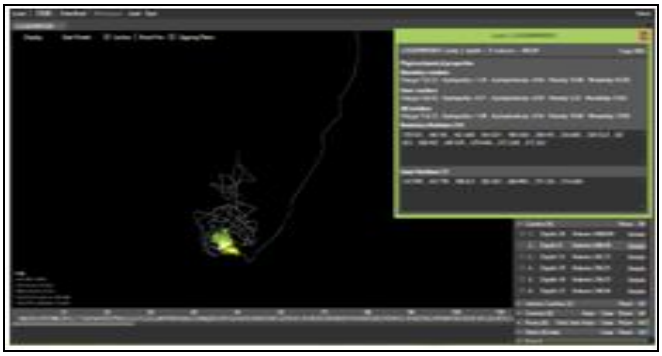

FIG. 6: CAVITY 2 OF CAIX

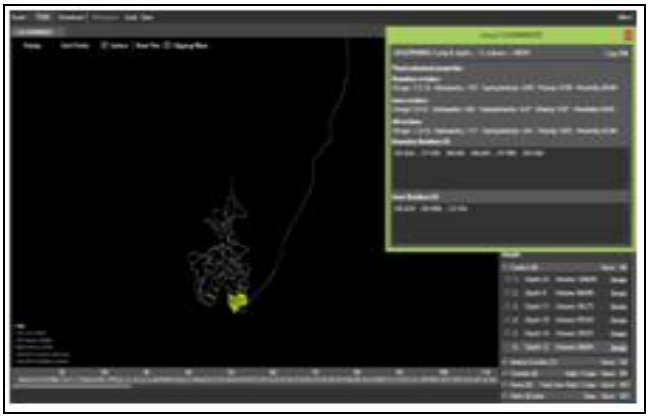

FIG. 7: CAVITY 6 OF CAIX

Fig.8 shows all the inhibitors along with the PDB IDs and whether they are valid or not after the first few screening processes.

Inhibitors	PDB ID	Valid/Not valid
G250	3IAI	Not valid
Bissulphonamide	2X7T	Valid
1,2,4 triazolethiol	4N1O	Not valid
Ureido Sulfonamide	4KUV	Not valid
Dimethyl sulfoxide	4QM0	Not valid
1,1 dioxide	3VQA	Valid
Indane Sulfonamide	2QOA	Valid
Nitroimidazolesufamate	4MO8	Valid
Methazolamide	4K0Z	Valid
1,3,5, triazine	4BRX	Valid
Ethoxzolamide	3CAJ	Valid
Ethoxzolamide	3DCW	Valid
Ethoxzolamide	3MDZ	Not valid

FIG. 8: LIST OF INHIBITORS AND THEIR VALIDITY.

Fig. 9 - 12 show the docking of cavity 2 and 6 with 2QOA and 2X7T respectively. These 2 were the best fits and were ergo chosen.

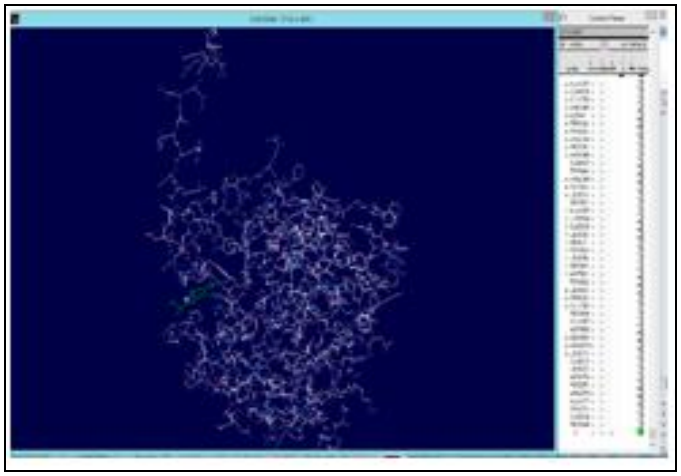

FIG. 9: DOCKED STRUCTURE OF CAVITY 2 WITH 2QOA IN SWISS PDB VIEWER. THE GREEN COLOUR IS THE INHIBITOR 2QOA.

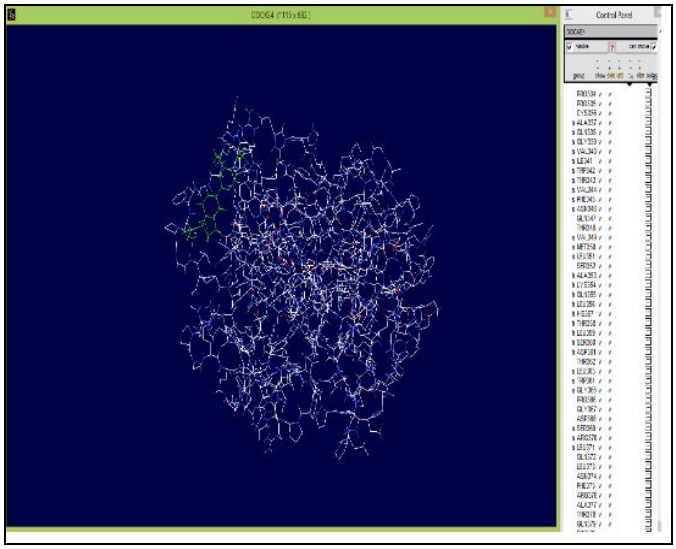

FIG. 10: DOCKED STRUCTURE OF CAVITY 2 WITH 2X7T IN SWISS PDB VIEWER. THE GREEN COLOUR IS THE INHIBITOR 2X7T.

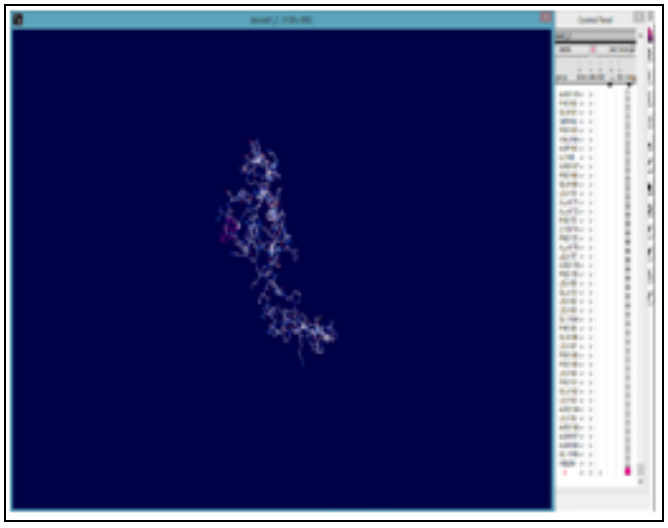

FIG. 11: DOCKED STRUCTURE OF CAVITY 6 WITH 2QOA IN SWISS PDB VIEWER. THE PINK COLOUR IS THE INHIBITOR 2QOA

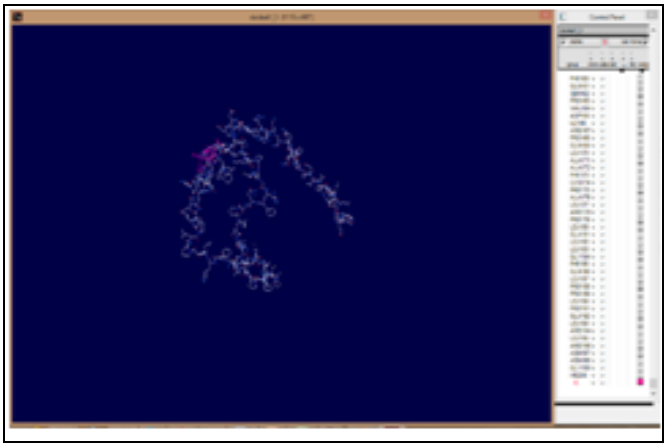

FIG. 12: DOCKED STRUCTURE OF CAVITY 6 WITH 2X7T IN SWISS PDB VIEWER. THE PINK COLOUR IS THE INHIBITOR 2X7T.

Validation using Maestro:

Maestro used the complexes generated by Hex 8.0 and grid generation was done. Docking scores were generated which aided in determining the inhibitor that would give optimum results when docked with cavities 2 and 6.

Fig.13-16, show the ligand interactions of indane sulfonamide and Carbonic Anhydrase II inhibitor in cavities 2 and 6.

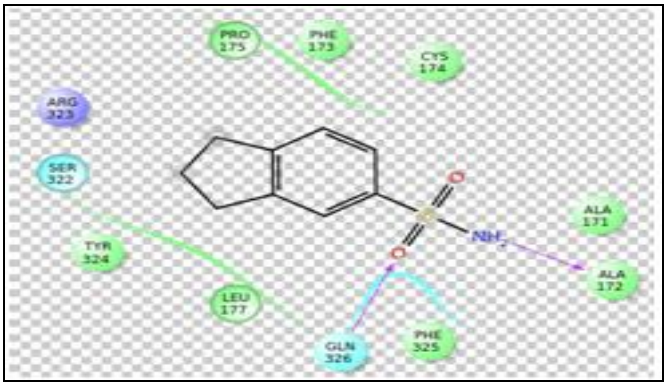

FIG. 13: LIGAND INTERACTION OF 2QOA (INDANESULPHONAMIDE) IN CAVITY 2.

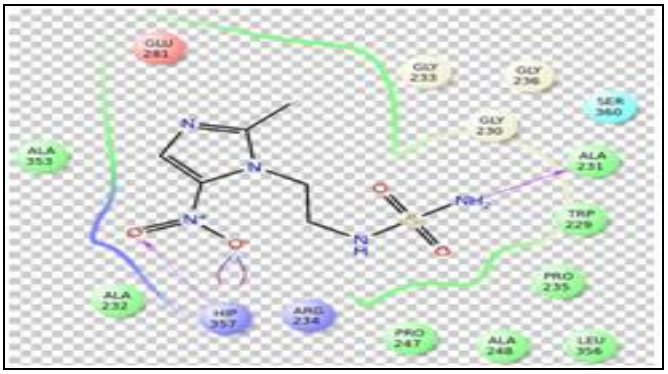

FIG. 14: LIGAND INTERACTION OF 2X7T (CARBONIC ANHYDRASE II INHIBITOR) IN CAVITY 2.

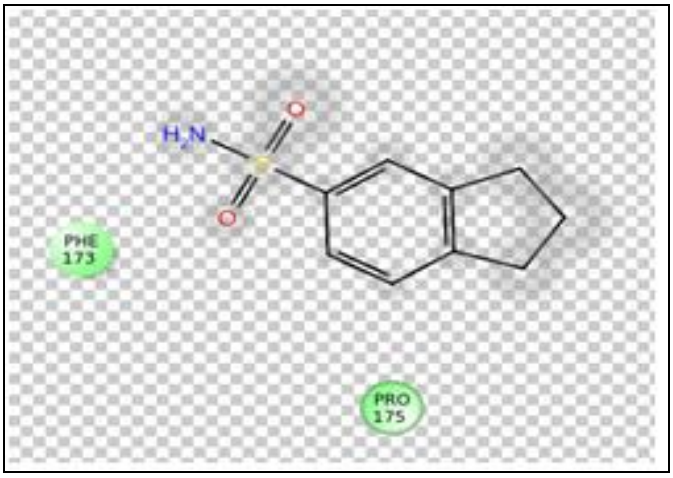

FIG. 15: LIGAND INTERACTION OF 2QOA (INDANESULPHONAMIDE) IN CAVITY 6

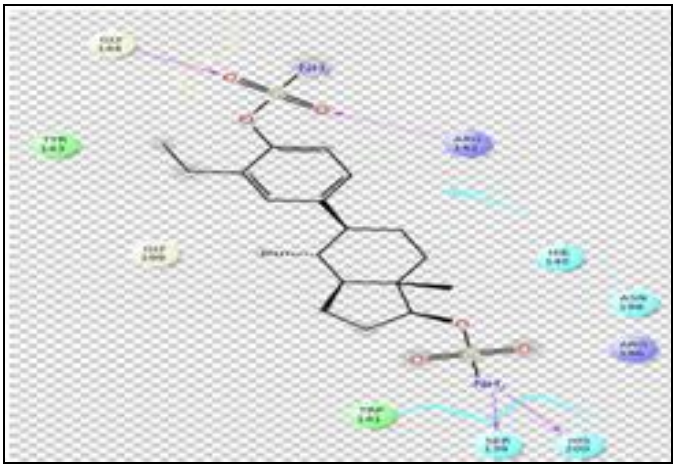

FIG. 16: LIGAND INTERACTION OF 2X7T (CARBONIC ANHYDRASE II INHIBITOR) IN CAVITY 6.

Fig.17 and 19 show the energy tables of cavities 2 and 6. **Fig. 18** and **20** show their respective graphs. The docking scores of the inhibitors were compared and the ones with the highest negative value were selected. Cavity 2- Carbonic Anhydrase II Inhibitor (2X7T) and Cavity 6- Indane Sulphonamide (2QOA).

Inhibitors PDB ID	Docking score	Glide gscore	E Model
2QOA	-6.079	-6.079	-37.827
2X7T	-5.336	-5.336	-46.428
4MO8	-5.788	-5.788	-41.187
3CAJ	-4.637	-4.367	-39.504
3DCW	-4.624	-4.624	-38.42
4BRX	-5.048	-5.048	-36.577
CAVITY 2			

FIG 17: DOCKING SCORES OF CAVITY 2

Inhibitors PDB ID	Docking score	Glide gscore	E Model
2QOA	-4.207	-4.207	-22.441
2X7T	-4.521	-4.521	-46.282
3CAJ	-3.909	-3.909	-28.766
3DCW	-4.113	-4.113	-27.938
4MO8	-3.876	-3.876	-31.444
4BRX	-3.518	-3.518	-25.627
CAVITY 6			

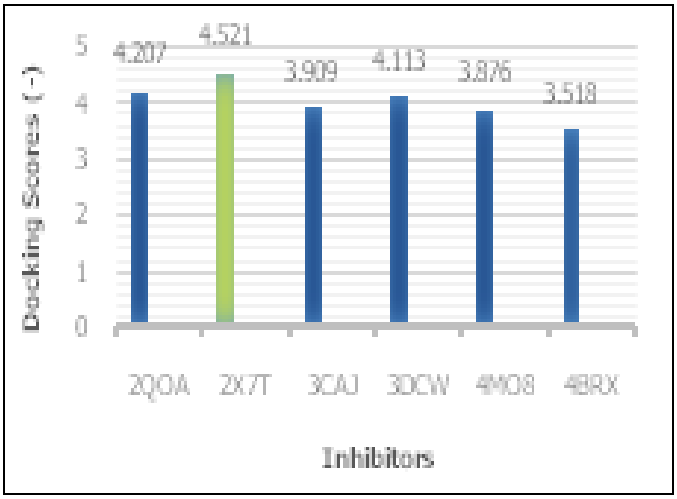

FIG. 18: GRAPH ILLUSTRATING THAT 2X7T HAS THE LEAST ENERGY VALUE IN CAVITY 2.

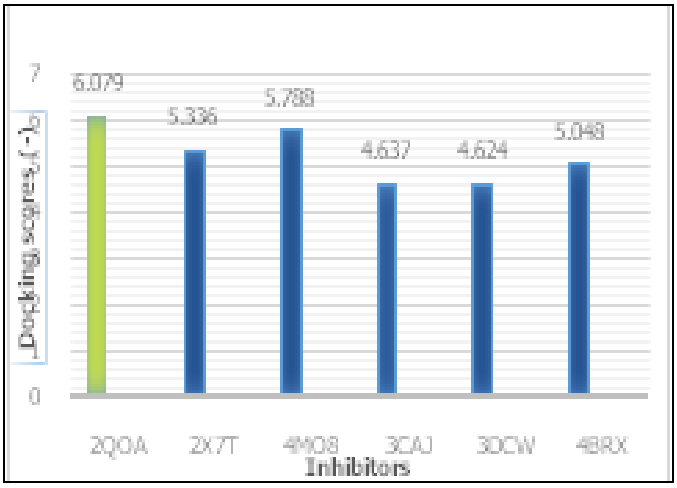

FIG. 19: DOCKING SCORE OF CAVITY 6

CONCLUSION: Docking using Hex and Maestro was done [8]. 2X7T and 2QOA are the suitable inhibitors of cavities 2 and 6 respectively to down regulate the expression of CAIX in the human body. The tumor tissue specific CA IX expression has led researchers to propose several novel treatment strategies [9]. A promising treatment strategy is to use CA selective inhibitors. Tumor cells probably use CAs as key enzymes to adapt to

the hostile environment caused by metabolic stress of cancer cells, and thus, acidification facilitates the spread and invasion of cancer cells. CA IX expression is high in renal cell carcinoma and this has enabled therapeutic trials with high-dose radiolabeled CA IX antibody (cG250) and CA IX loaded dendritic cells [10].

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