The Effect on DNMT1 through Compounds for the Treatment of Cancer: An In-Silico Approach

Mishka Tyagi[1], Noopur Khare[2], Abhimanyu Kumar Jha[1]

[1]Department of Biotechnology, Faculty of Life Sciences, Institute of
Applied Medicines and Research, Ghaziabad, Uttar Pradesh, India
[2]Shri Ramswaroop Memorial University, Barabanki, Uttar Pradesh, India

ABSTRACT

Background: Cancer is a disease that involves the abnormal gowth of the cell. After growing uncontrollably in continuation, it influences and grows in other parts of the body as well[1,2]. Benign types of Tumors doesn't invade other body parts whereas Malignant Tumor support spreading and invading other parts of the body[3]. The main objective of this work is to use Molecular Docking method to develop a drug for the treatment of Cancer.

Methods: Docking was used to dock all the selected ligands with target protein i.e., DNMT1. The selected ligands were Thiophene, Sulfonamides, Chalcone, and Nitroimidazole. PyRx Software was used for the purpose of virtual screening to check various properties of these selected ligands like the Binding Affinity, RMSD (Lower Bound), RMSD (Upper Bound) etc. PyRx and SwissADME both softwares were used for finding out the Drug Likeliness of every compound and to find out the best Compound to be docked with the target protein. Chalcone was found to be the best compound for Cancer treatment having least binding affinity.

Results: Molecular Docking of Chalcone with DNMT1 protein was performed with the help of AutoDock Vina Software. Output showed 9 positions of different binding affinities and RMSD values (both Lower Bound and Upper Bound). Furthermore, the visualization of results was done with the help of PyMOL Software.

Conclusion: According to this study, Chalcone was the only compound from selected ligands that may be used to treat Cancer. Chalcone may act as a promising drug for cancer treatment in future perspectives.

KEYWORDS: *AutoDock Vina, DNMT1, Molecular Docking, Thiophene, Chalcone, Sulfonamides, Nitroimidazole*

INTRODUCTION

Cancer is the name given to a collection of related diseases. In all types of cancer, some of the body's cells begin to divide without stopping and spread into surrounding tissues. Cancer can start almost anywhere in the human body, which is made up of trillions of cells. Normally, human cells grow and divide to form new cells as the body needs them. When cells grow old or become damaged, they die, and new cells take their place. When cancer develops, however, this orderly process breaks down. As cells become more and more abnormal, old or damaged cells survive when they should die, and new cells form when they are not needed. These extra cells can divide without stopping and may form growths called tumors.

Tumors are groups of abnormal cells that form lumps or growths. They can start in any one of the trillions of cells in our bodies. Tumors grow and behave differently, depending on whether they are cancerous (malignant), non-cancerous (benign) or precancerous (Premalignant) [4].

There are three main types of tumor:

Benign Tumor: These are not cancerous. They either cannot spread or grow, or they do so very slowly. If a doctor removes them, they do not generally return.

Premalignant Tumor: In these tumors, the cells are not cancerous, but they have the potential to become malignant.

Malignant Tumor: Malignant tumors are cancerous. The cells can grow and spread to other parts of the body.

Cancer is a genetic disease—that is, caused by changes in our genes that controls our cells function, especially how they grow and divide.

Genetic and Epigenetic changes that cause cancer can be inherited from our parents. These changes develop as a result of errors that occur during cell division or because of damage to DNA caused by certain environmental exposures. Cancer-causing environmental exposures include substances, such as the chemicals in tobacco smoke, and radiation, such as ultraviolet rays from the sun.

Cancer is the name given to a collection of related diseases. In all types of cancer, some of the body's cells begin to divide without stopping and spread into surrounding tissues. Cancer can start almost anywhere in the human body, which is made up of trillions of cells. Normally, human cells grow and divide to form new cells as the body needs them. According to the International Agency for Research on Cancer, in 2002, cancer killed > 6.7 million people around the world and another 10.9 million new cases were diagnosed. If the results are extrapolated, at the same rate, an estimated 15 million people will have cancer, annually, by 2020. A large portion of Cancer disease is caused due to the Genetic mutations[3]. Other small portion includes the use of Tobacco.

In Computer Assissted Drug Designing(CADD), Molecular Docking is an important tool playing a major role for docking procedure. The main objective of this docking is to search out the major modes of binding ligand to target protein. The Successful docking procedure follows up the good outpit making research work successful as well.

Molecular docking is an attractive scaffold to understand drug bio-molecular interactions for the rational drug design and discovery, as well as in the mechanistic study by placing a molecule (ligand) into the preferred binding site of the target specific region of the DNA/protein (receptor) mainly in a non-covalent fashion to form a stable complex of potential efficacy and more specificity. The method predicts the best binding energy with the ligand molecule against protein.

The Aim for this experiment is to examine the active and domain sites of DNMT1, to carry out auto docking of target protein with the selected ligand and to determine the their active sites and compound docking so that we can analyze the therpeutic effect of compound against the target protein DNMT1.

MATERIALS AND METHODS
Step -1: Identification of proteins
By studying various published literatures and research work, disease causing protein can be retrieved through various database. Disease causing protein was retrieved from Uniprot. Structure of the disease causing protein molecule was retrieved in '.pdb' format from RCSB Protein Data Bank (PDB)[5].

Step - 2: Identification of Ligands
After studying the literature,4 compounds/ligands were selected for the study of Molecular Docking. The 4 compounds/ligands were:
1. Thiophenes
2. Sulfonamides
3. Chalcones
4. Nitroimidazole.

All these ligands were retrieved from PubChem[6]. After searching the name of compound/ligand, they were downloaded in '.sdf' format in a 3D structure form. Thereafter, Conversion of all of the downloaded compounds/ligands were downloaded in '.pdb' format with the help of online SMILES translator[7,8].

Step -3: Virtual Screening through PyRx
PyRx is a software that is basically used to screen ligands with the target protein and find out their binding affinity with the target protein. This software runs only on '.pdbqt' format. At first step of PyRx procedure, Protein molecule was loaded & then converted to a macromolecule. Furthermore, all the selected ligands of '.pdb' format were loaded and then converted to '.pdbqt' format. After running of programming, screen displays resultant tables giving details about ligands properties. Minimum binding energy was displayed on the screen of the software and some other details as well. Docking was performed between the target protein and the ligand molecule after considering the ligand with minimum binding energy.

Step - 4: Swiss ADME
Swiss ADME is an online web server that uses SMILE notations of ligands that were copied and pasted from Pub Chem. With the help of this online web server, analysis of selected ligands were done through their drug likeliness property. In other words,

drug likeliness property analysis was done through SwissADME. Basically, drugs were inspected for Lipinski's rule of five. Lipinski's rule of five are as follows:

1. H-bond donors must be <5.
2. H-bond acceptors must be <10.
3. The molecular weight must be <500 Dalton.
4. Partition co-efficient LogP must be <5.
5. Only 1 rule is authorized to be violated.

The ligand that follows all 5 rules of lipinski (as mentioned above) is selected for the final process of Autodocking.

Step - 5: Autodock

At first, the target protein in '.pdb' format was loaded on the homepage of Autodock. The target protein was prepared for autodocking by following steps of AutoDock followed by removing water molecules, then adding hydrogens polar atoms thereafter by adding kollman charges. After this, the protein was selected and was saved in '.pdbqt' format. Then, the selected ligand was loaded on homepage of AutoDock. Following the further process of AutoDocking, Grid box was selected for the region where docking was to be performed. Thereafter, Command Prompt, also known as cmd, was used for executing the results of performed AutoDock.

Step - 6: Visualization of results through PyMOL

PyMOL is a tool which is used to visualize the molecular surface of the docked structure. In other words, structural visualization was done with the help of this tool. The output in '.pdbqt' format was runned in PyMOL. Molecular surface of the output was seen on the screen.

RESULTS AND DISCUSSION

The target protein DNMT1 was downloaded from Uniprot in '.pdb' format as shown in Figure 1 with additional details of protein in figure(b). DNMT1 belongs to transferase category. Protein resolution was 2.31Å and the method for this protein was X-ray diffraction. The other selected ligands were also retrieved from the PubChem as 2D or 3D conformer in '.sdf' format which were further converted to '.pdb' format by online SIMILIE .sdf to .pdb convertor as shown in Figure 3, Figure 4, Figure 5 and Figure 6.
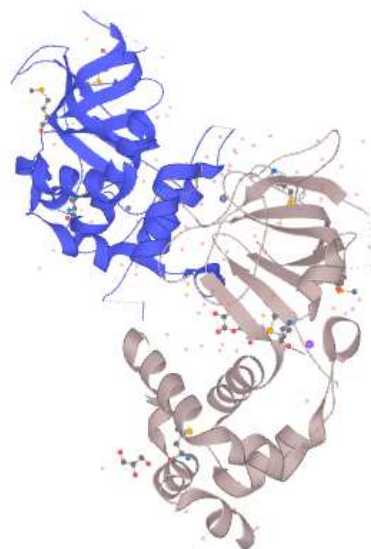

Figure 1: Structure of DNMT1

➢ **Protein Name:** DNA-(cytosine-5)-methyltransferase

➢ **Gene:** DNMT1

➢ **Protein database no:** 3epz

➢ **Classification:** Transferase

➢ **Organism:** Homo Sapiens

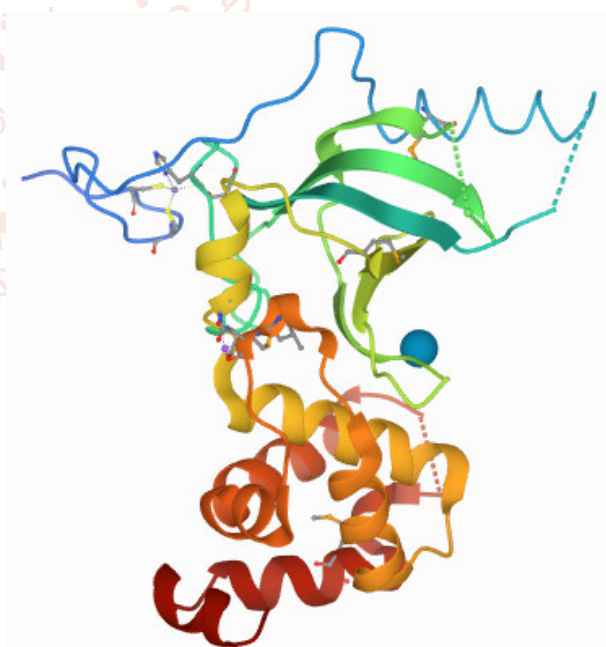➢ **Expression system:** Escherichia coli

➢ **Mutation:** No

Figure 2: Biological assembly 1

Figure 3: Biological assembly 2

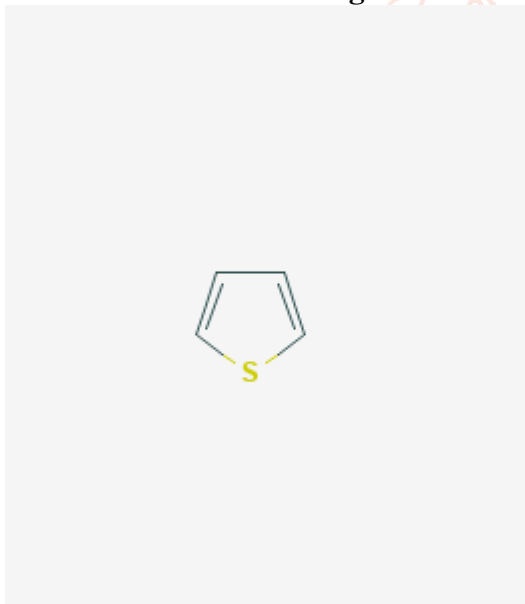## 2D Structure of the selected Ligands
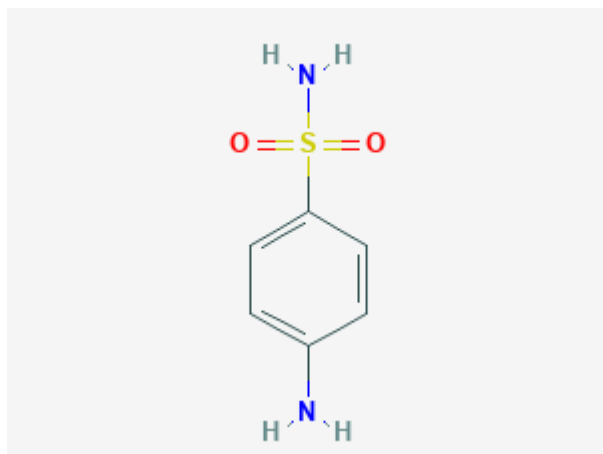

Figure 4: 2D structure of Thiophene
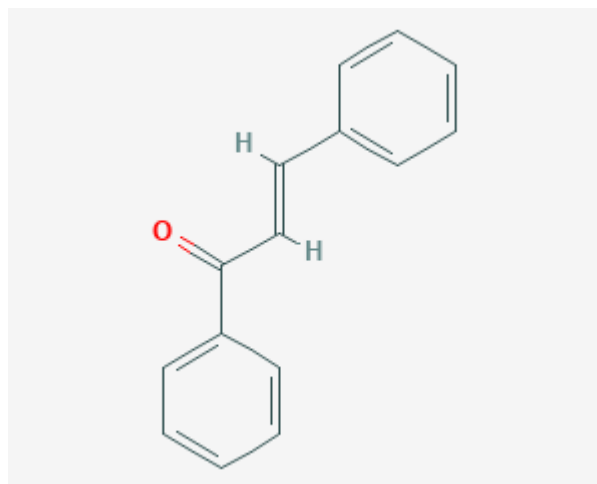

Figure 5: 2D structure of Sulfonamide

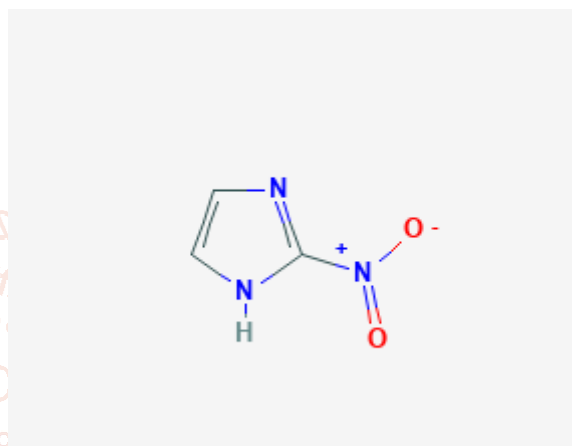**Figure 6: 2D structure of Chalcone**

Figure 7: 2D structure of Nitroimidazole
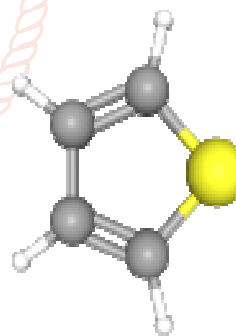
3D structure of Ligands

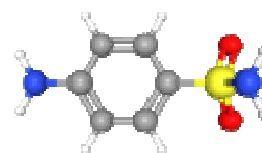**Figure 8: 3D structure of Thiophene**

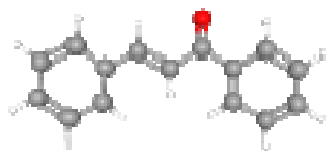**Figure 9: 3D structure of Sulfonamides**
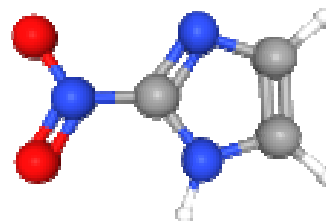
Figure 10: 3D structure of Chalcone

Figure 11: 3D structure of Nitroimidazole

Table 1: Details of the Ligand molecules were retrieved from PubChem

S. No	Ligand Name	PubChem ID	Molecular weight	Molecular Formula	Hydrogen Bond Donor	Hydrogen Bond Acceptor
1.	Thiophene	8030	84.14 g/mol	C4H4S	0	1
2.	Sulfonamide	5333	172.1 g/mol	C6H8N2O2S	2	4
3.	Chalcone	637760	208.25 g/mol	C15H12O	0	1
4.	Nitroimidazole	10701	113.08 g/mol	C3H3N3O2	1	3

All of these selected ligands were runned and their screening was done on PyRx software. The results were displayed on PyRx and are mentioned in Table 2 and Table 3. The binding affinity of every ligand was displayed on the screen. The results were taken in considerations carefully and then the ligand with suitable binding affinity was choosen for further process of auto docking. But before that, Swiss ADME was used for drug likeliness property analysis. A compound was selected after analyzing the drug likeliness through PyRx and Swiss ADME.

Table 2: PyRx Results showing properties of different ligands in order to dock suitable Ligand with the Target Protein.

Selected Ligands	Binding Affinity	RMSD (LB)	RMSD (UB)
Thiophene	-2.5	17.928	18.649
Sulfonamide	-4.7	4.335	5.652
Chalcone	-5.2	3.879	8.528
Nitroimidazole	-4.3	0.049	1.673

Table 3: Binding energy of different ligands with target protein

Ligands	Binding Energy
Thiophene	-2.5
Sulfoamide	-4.7
Chalcone	-5.2
Nitroimidazole	-4.3

Here, After studying the PyRx results, binding energy of selected ligands were concluded and noted down in a tabular form.

Then after, these molecules were run in Swiss ADME for inspection of drug likeliness.

Table 4: Swiss ADME Result Analysis

Name of Ligand	Mol. Wt.	H-Bond Donor	H-Bond Acceptor	Lipinski
Thiophene	84.14 g/mol	0	0	Yes; 0 violation
Sulfonamide	172.20 g/mol	2	3	Yes; 0 violation
Chalcone	208.26 g/mol	0	1	Yes; 0 violation
Nitroimidazole	113.07 g/mol	1	3	Yes; 0 violation

In SwissADME, ligands were observed on the basis of Lipinski's rule of five, its molecular weight, Hydrogen bond donor, and Hydrogen bond acceptor. According to the SwissADME results, it was concluded that chalcone was the most qualified ligand for Autodocking step with the target protein. It follows the lipinski's rule of 5 as well as showing the 0 violation.

After SwissADME, the last step was docking through AutoDock Vina. As observed from the above results, Chalcone revealed itself as the most suitable out of these 4 selected ligands for docking with the target protein. In Auto Docking, the target protein was docked with the selected ligand and then the output was in '.pdbqt' format and was runned in command prompt.
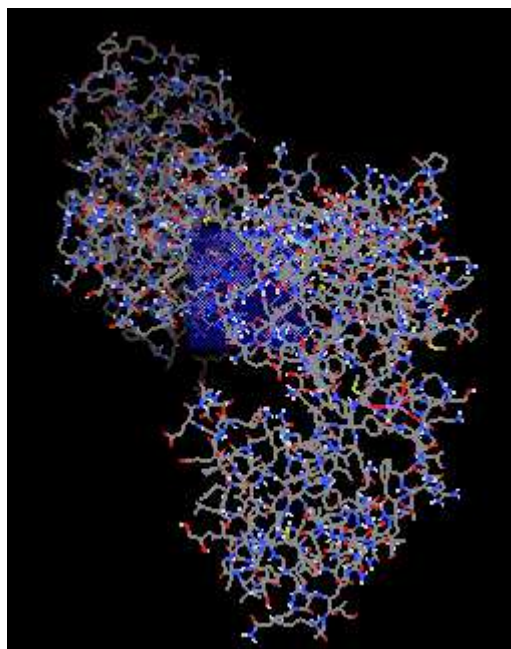
Figure 12: Preparation of Grid Box

AutoDock Vina software was used to dock the ligand Chalcone with the target protein DNMT1. Table-5 reveals Chalcone to be the best ligand to bind against DNMT1 target protein by the help of docking using Autodock Vina.

Table 5: Results of AutoDock Vina

Mode	Binding Affinity (kcal/mol)	RMSD lower bound	RMSD upper bound
1.	-7.6	0	0
2.	-7.3	1.766	6.559
3.	-7.1	1.374	2.321
4.	-6.6	2.964	6.693
5.	-6.3	2.010	6.578
6.	-6.3	24.647	25.858
7.	-6.2	24.877	26.170
8.	-6.1	24.199	26.751
9.	-6.0	4.618	7.656

PyMOL was used to visualize the output in '.pdbqt' format.

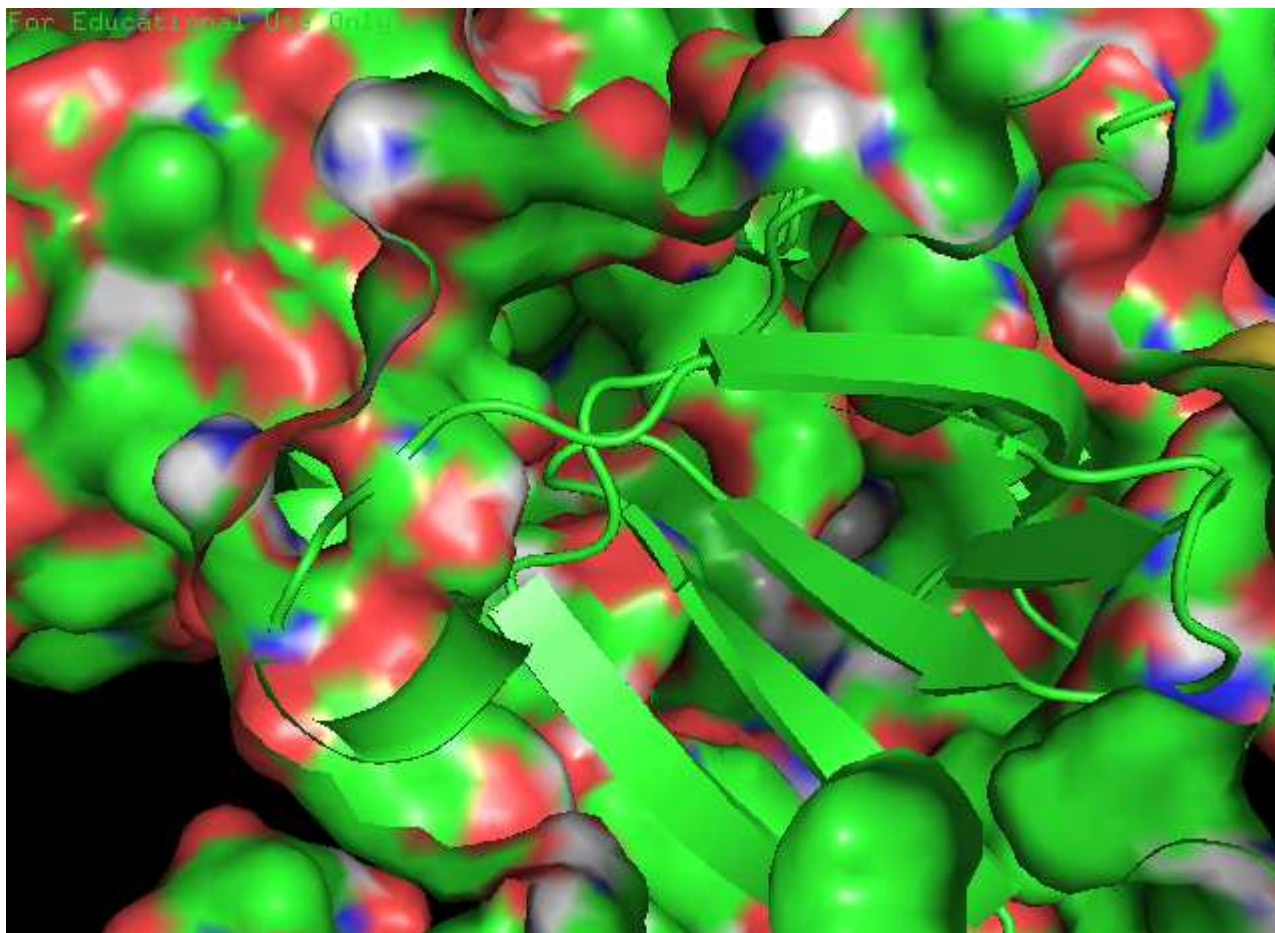**Figure 13: Result Visualization in PyMOL**

Figure 14: Interaction of DNMT1 with Chalcone through PyMOL visualizer

CONCLUSION

In this *in silico* analysis, Chalcone might act as an inhibitor and can be utilized to act as a drug in treatment of Cancer. Therefore, this drug may prevent cancer and may play an effective role for cancer treatment. Studies of docking showed that there was the strong affinity of Chalcone towards the target protein of cancer. It may be used in a form of drug after *in vitro* and *in vivo* studies which may act as a promising anti-cancer agent. This Drug may prevent cancer by forming effective drug against cancer.

CONFLICT OF INTEREST

The Author declare that there is no conflict of interest

ACKNOWLEDGEMENT

REFERENCES

[1] "Cancer". *World Health Organization*. 12 September 2018. Retrieved 19 December 2018.

[2] "Defining Cancer". National Cancer Institute. 17 September 2007. Retrieved 28 March 2018.

[3] "Defining Cancer". National Cancer Institute. 17 September 2007. Retrieved 28 March 2018.

[4] Yvette Brazier, 2019, medically reviewed, Medical news today, https://www.medicalnewstoday.com/articles/249141.

[5] www.rcsb.org.

[6] https://pubchem.ncbi.nlm.nih.gov/.

[7] https://cactus.nci.nih/translate/.

[8] https://cactusa.nci.nih.gov/cgi-bin/translate.tcl.