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To Study the Effect of Harmine Against DNMT1 for the Treatment of Cervical Cancer Through Molecular Docking Studies

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Abstract

Background: Cervical cancer is one of the most common type of cancer which is found in women and it is the third most death-causing cancer in the world. It mostly occurs due to epigenetic changes. According to previous research, the DNMT1 acts as a factor for enhancing cervical cancer. This study aimed to inhibit the expression of the DNMT1 by secondary metabolites with the help of molecular docking for the treatment of cervical cancer. **Methods:** All studies were based on molecular docking. Docking was carried out between all the ligands and target protein DNMT1 (PDB ID: 4WXX) with the help of docking software. We selected some natural compounds as ligand like *Harmine*, *Alpha spinasterol*, *Myrcene*, *Alloxan*, and *Vasicinone* and DNMT1 (PDB ID: 4WXX) as a target protein. After the protein preparation by Biovia Discovery Studio Visualizer we imported all the ligand in PyRx software for virtual screening. According to the PyRx result and Lipinski's Rule of Five, *Harmine* was the best compound against DNMT1 with its minimum binding energy. **Results:** The Biovia Discovery Studio Client 2020 and AutoDock Vina software were used for the molecular docking between *Harmine* and receptor protein DNMT1 (PDB ID: 4WXX). The result showed 9 poses with different binding affinity, Root means square deviation Lower Bound (RMSD LB) and Root mean square deviation Upper Bound (RMSD UB). The same molecules were further docked through Biovia Discovery Studio Client 2020 and the interaction was visualized under PyMol. **Conclusion:** According to the *in silico* study, *Harmine* was the only compound which can inhibit the activity of DNMT1 (PDB ID: 4WXX). So in the further studies, *Harmine* can be a promising drug for the treatment of cervical cancer after its *in vitro* and *in vivo* studies.

Keywords
Cervical cancer, *Harmine*, Epigenetic changes, DNMT1.

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INTRODUCTION:

Cancer is one of the most occurring diseases in the World. The people throughout the world are infected due to cancer. There are more than 100 types of cancer in which cervical cancer is one of its types. Cancer is the uncontrolled growth of the cell form tumors in the body. Cervical cancer is the third most occurring cancer in women. In the year 2018, there were 570000 reported cases of cervical cancer throughout the world with 311000 numbers of deaths. India also contributes to the global cervical cancer burden, with 97 000 cases and 60 000 deaths in India [1]. Many genetic and epigenetic alterations are responsible for cervical tumorigenesis. Among those alterations, aberrant promoter methylation of tumor-suppressor genes gives rise to its silencing functions and results in the significant carcinogenesis of cervical cancer [2]. Currently, many types of cervical cancer-related repressor genes are known including CCNA 1, CHF, HIT, PAX1, PTEN, SFRP4, and TSC1 [3]. All these genes perform a wide variety of functions to regulate the transcription and expression, with promoter hyper methylation, which leads to the precursor lesions in cervical development and malignant transformation. DNA methylation is catalyzed by DNA methyl transferases (DNMT1). It has been reported that DNMT1 is responsible for precise duplicating and maintaining the pre-existing DNA methylation patterns after replication [4], DNMT1 inhibits the transcription of tumor suppressor genes and facilitated the formation of tumorigenesis which finally develops into cervical cancer. The inhibition activity for DNMT1 could reduce hyper methylation of repressive genes and promote its expression, and reverse phenotype of a malignant tumor. Thus, specific inhibition of DNMT1 could be one strategy for cervical cancer therapy [2].

Despite the progress and advancement in cancer research work, there is need for more discoveries and development in anti-cancer therapeutics. Over few decades, natural products have received extensive attention as potential anti-cancer agents because of their low toxicity and potential efficacy. In present scenario, population is running towards herbal compounds, as there are no side effects of the compound. More than 50% of all the drug forms used in the clinical fields around the world have originated from the compounds extracted from plants [5]. Traditional drugs were time and resource consuming, but now in this technical era, bioinformatics has played an important role in research to save both time and resources. Molecular docking is a technique used to screen the drugs on the basis of structure based drug designing. The interaction of the small molecules with the target protein is analyzed in docking. Structural-Based Drug Designing uses molecular docking method which addresses the ligand binding sites with a protein of known three-dimensional structure [7]. One of the computational approaches, docking, helps with screening a large set of compounds based on their free binding energies and proposes structural hypotheses of how the molecules could inhibit the target [8]. In our study, some different natural compounds from different plant sources were collected such as *Harmine*, *Alpha spinasterol*, *Myrcene*, *Alloxan*, and *Vasicinone*. The aim of the research was to study interaction of selected natural compound against DNMT1 for the treatment of cervical cancer with the help of molecular docking.

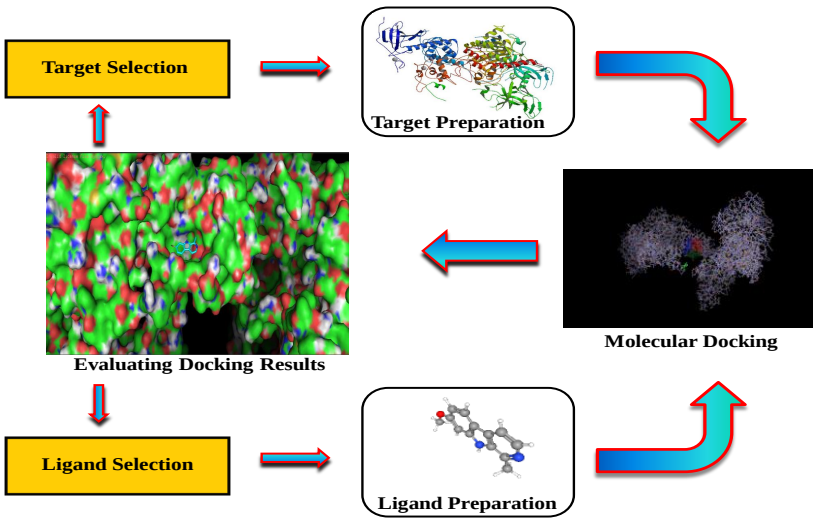


Figure 1: Procedure of Molecular docking

METHODOLOGY:

Identification of protein

DNMT1 (DNA (cytosine-5)-methyl transferase 1) protein was used as a receptor for selected ligand. The protein selected for the study was responsible for propagating the DNA methylation patterns during DNA replication. Abnormal expression of DNMT1 can cause aberrant methylation of some tumor suppressor genes' CpG islands, further resulting in making the tumor suppressor gene inactive and cell carcinogenesis [9]. Highly expressed DNMT1 has not only been detected in a variety of tumor cells, but has also been found to appear earlier than DNA Methylation [10]. With respect to cervical carcinogenesis, an increased expression of DNMT1 protein has been observed during multistage cervical carcinogenesis [11].

The structure of DNMT1 Protein (PDB ID 4WXX) was obtained from Protein Data Bank (PDB) https://www.rcsb.org/ [12]. The PDB file of 4WXX protein uploaded in RAMPAGE tool for identification the validation of protein stability http://mordred.bioc.cam.ac.uk/~rapper/rampage.php. The DNMT1 (PDB ID 4WXX) protein was downloaded in .pdb format.

Retrieval of Ligands

Harmine, Alpha spinasterol, Myrcene, Alloxan, and Vasicinone were the five natural compounds which were used as ligands in the study.

Harmine (HM) is a β-carboline alkaloid that was originally isolated from seeds of *Peganum harmala* and *Banisteriopsis caapi* in 1847[13]. Harmine is widely distributed in various medicinal plants and has long been used in folk medicine in the Middle East and Asia [14]. Studies have demonstrated that HM exhibits significant antitumor activities *in vitro* and *in vivo* [15] including inhibiting proliferation, promoting apoptosis [16] and preventing tumorigenesis.

Alpha -spinasterol is type of a phytosterol isolated from *Ganoderma resinaceum* mushroom. The previous study has been exposed that α-spinasterol elicits good anticancer activity against breast and ovarian cancer cells with minimal effect on normal cells viability or proliferation [17].

Myrcene, or β-myrcene, is an alkene natural hydrocarbon. It is more precisely classified as a monoterpene. Monoterpenes are dimers of isoprenoid precursors, and myrcene is a significant component of the essential oil of several plants, including bay, cannabis, and hops [18,19]. It is produced mainly semi-synthetically from myrcia, from which it gets its name.

Alloxan was first isolated by Brugnatelli in 1818 and initially described by Frederick Wohler and Justin Liebig in 1838[20]. *Alloxan* causes diabetes by a mechanism which basically involves partial degradation of the beta (b) cells of pancreatic islets and subsequent compromise in the quality and quantity of insulin produced by these cells. Its use as a diabetogenic drug in experimental animals was first reported by Dunn and McLetchie in their study in which they successfully induced diabetes in experimental rabbits [21].

Vasicinone are the two most biologically active quinazoline alkaloids found in the leaf extracts of *Adhathoda vasica Nees* (AV) and it has been reported that vasicinone is the main metabolite of vasicine [22]. It has been reported that synthesized vasicinone analogues possesses apoptotic properties in a cell specific manner [23]. In addition, the vasicinone analogues also act as potent inhibitor of the PI3K/Akt/FoxO3a pathway under *in vitro* and tumor regression *in vivo* model.

All the natural compounds were chosen on the basis of literature. These natural compounds were retrieved from PubChem online database https://pubchem.ncbi.nlm.nih.gov/. The compounds were downloaded in SDF format. All these compounds were converted from .sdf format to .pdb format by Online SMILES Translator https://cactus.nci.nih.gov/translate/, the .pdf files of the ligands were downloaded in .pdb format.

Protein preparation

Biovia Discovery Studio Visualizer is software which is used for analysing the protein molecule in different orientations. The docked molecule structure can also be viewed in this software. This software was used to prepare the protein molecule by removing the water molecules and extra ligands if attached to their active sites. Firstly, the protein molecule was loaded in the graphical windows and under view option its hierarchy was analyzed. Water molecules and attached ligand molecules were deleted by selecting the atoms. The crystal structure of the protein molecule was further saved in .pdb format. This protein molecule was used for docking.

Virtual Screening

PyRx software was used for the virtual screening of the ligands. The PyRx software demonstrated the binding affinity and binding energy of each ligand via the virtual screening. The protein molecule was loaded in PyRx window and was converted from .pdb format to. pdbqt format. The ligand molecules were also imported in .sdf format. All the energies from the ligands were minimized and all the ligand compounds were converted from .sdf format to. pdbqt format. The results were analyzed based on their binding affinity.

Drug likeliness property analysis

The natural compounds were selected for final molecular docking studies by screening those ligands which were having drug like properties. Ligands were screened on the basis of Lipinski's rule of five. Lipinski's rule of five states the following [24]: -

- Molecular mass less than 500 Da;
- High lipophilicity (expressed as LogP less than 5);
- Less than 5 hydrogen bond donors;
- Less than 10 hydrogen bond acceptors;
- Not more than more rule should violate.

The Lipinski's rule of five was analyzed using online web server SwissADME http://www.swissadme.ch/. The SMILE notations of the ligands were copied from PubChem and were submitted on SwissADME for the analysis of Lipinski's rule of five.

Molecular docking between Receptor protein (PDB ID: 4WXX) and Harmine

Molecular docking was carried out between the target protein molecule and the ligand. The best screened ligand was analyzed for docking against the protein molecule through Biovia Discovery Studio Client 2020 and AutoDock Vina software.

Biovia Discovery Studio Client 2020

Biovia Discovery Studio Client 2020 was used to dock the protein target with the best ligand molecule. The protein molecule was prepared for the docking followed by the ligand molecule. Both protein and ligand molecules were loaded on the graphical window and after that charges were added. The best docked molecule showed the interaction of amino acids between protein and ligand. The absolute energy, clean energy, Confg Number, MolNumber, Relative energy and pose number were analysed as a result.

AutoDock Vina using MGL tools

The protein target was loaded on the graphical window in .pdb format. The water molecules were deleted from the protein molecule and polar hydrogen atoms and Kollman charges were added to the protein molecule. The protein was further converted into. pdbqt format. The ligand molecule was imported and was converted into. pdbqt format. Both the protein and ligand molecules were loaded on the graphical screen. The boundaries of the grid box were set as shown in **Figure 2**. After preparation of protein and ligand molecule docking was launched from command prompt and the results were analyzed.

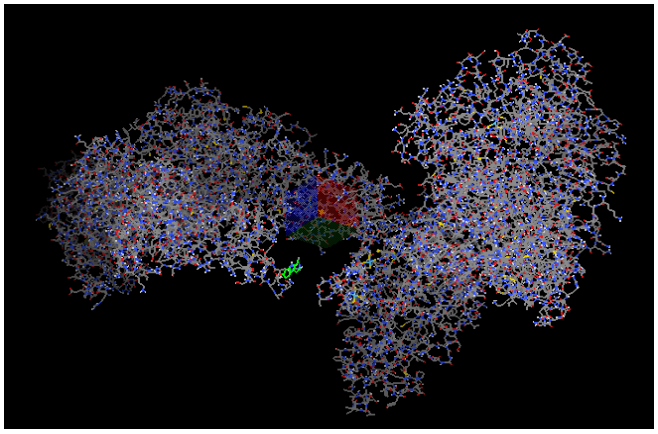


Figure 2: Grid Box Preparation

Structure visualization

PyMol software was used for visualization of protein and ligand interaction. After AutoDock Vina the output file was automatically saved in selected folder with the name output. pdbqt file. The protein. pdbqt and output. pdbqt files were loaded on the graphical screen of PyMol. The interaction between the protein and ligand were visualized and analyzed.

RESULTS AND DISCUSSION:

The DNMT1 (DNA (cytosine-5)-methyl transferase 1) (PDB ID: 4WXX) was retrieved from Protein Data Bank as shown in **Figure 3.** The resolution of the protein was 2.62 Å and belongs to transferase class. The stability of the protein was analyzed through Rampage as shown in **Table 1.** Harmine (CID: 5280953), Alpha spinasterol (CID: 5281331), Myrcene (CID: 31253), Alloxan (CID: 5781), Vasicinone (CID: 442935) were downloaded in 3D structure in .sdf format as shown in **Table 2.**

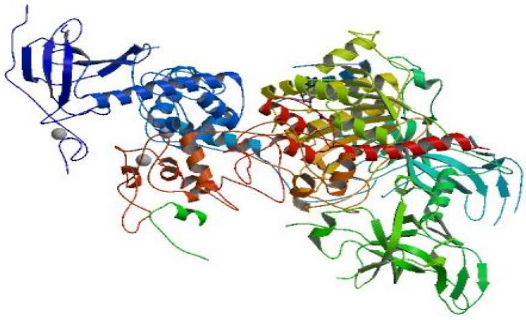
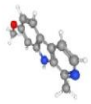
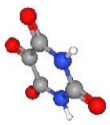
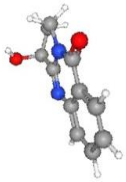


Figure 3: 3D structure of DNMT1 (PDB ID: 4WXX) Protein

Table 1: Stability of DNMT1

Number of residues in favoured region	(~98.0% expected)	2183	94.50%
Number of residues in allowed region	(~2.0% expected)	107	4.60%
Number of residues in outlier region		19	0.80%

Table 2: 3D Structure of Ligands

				
HARMINE CID - 5280953 MF - C13H12N2O MW - 212.25 g/mol	**ALPHA SPINASTEROL** CID - 5281331 MF - C29H48O MW - 412.7g/mol	**MYRCENE** CID - 31253 MF - C10H16 MW - 136.23g/mol	**ALLOXAN** CID - 5781 MF - C4H2N2O4 MW - 142.07g/mol	**VASICINONE** CID - _442935 MF - C11H10N2O2 MW - 1202.21g/mol

The protein molecule was prepared through Biovia Discovery Studio Visualizer, all the ligands and water molecules were deleted as shown in **Figure 4.** Virtual screening of the ligand molecules was done through PyRx software. According to the minimum binding energy ligands were screened. The binding affinity of Harmine was -5.7, Alpha spinasterol was -7.5, Alloxan was -4.9, Myrcene was -4.3 and Vasicinone was -5.8 as shown in **Table 3** and the Binding energies of Harmine was -6.0, Alpha spinasterol was -7.6, Alloxan was -5.1, Myrcene was -4.6 and Vasicinone was -6.4 as shown in **Table 4.** The ligands which were selected after PyRx result were Alpha spinasterol, Vasicinone and Harmine. These ligands were further analyzed for drug likeliness property analysis.

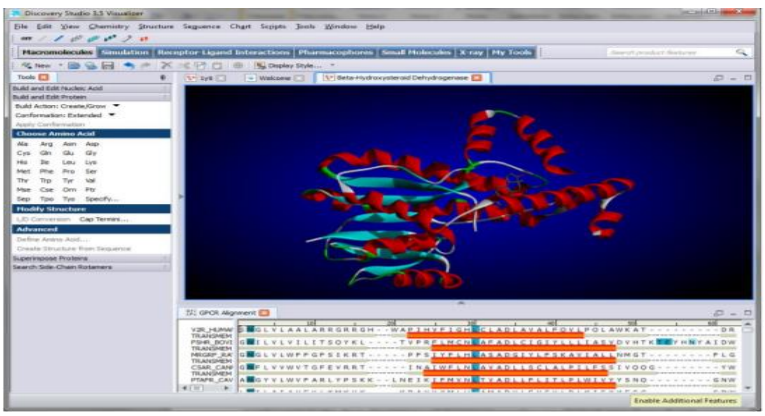


Figure 4: Preparation of Protein Molecule (DNMT1)

Table 3: Binding affinity, RMSD lower bond and RMSD upper bond of Ligands

Compound	Ligand	Binding Afinity	Mode	RMSD lower Bond	RMSD upper Bond
Alloxan	4wxx_5781_uff_E = 50.01	-4.9	1	1.281	2.709
			2	1.28	2.213
Alpha spinasterol	4wxx_5281331_uff_E = 569.98	-7.5	1	1.536	2.85
			2	1.929	4.091
Harmine	4wxx_5280953_uff_E = 370.57	-5.7	1	2.657	5.495
Myrcene	4wxx_31253_uff_E = 90.99	-4.3	1	1.914	5.154
			2	15.295	17.918
Vasicinone	4wxx_442935_uff_E = 288.70	-5.8	1	4.247	6.253

Table 4: Binding Energies of the Ligands

Sr. no.	Compound	CID	Ligand	Binding energy
1	Myrcene	31253	31253_uff_E = 90.99	-4.6
2	Vasicinone	442935	442935_uff_E = 288.70	-6.4
3	Harmine	5280953	5280953_uff_E = 370.57	-6.0
4	Alpha spinasterol	5281331	5281331_uff_E = 569.98	-7.6
5	Alloxan	5781	5781_uff_E = 50.01	-5.1

Drug likeliness property analysis was done through SwissADME and ligands were screened according to Lipinski's Rule of Five as shown in **Table 5.** Harmine was the only molecule qualifying all the properties of Drug.

Table 5: Drug Likeliness Property Analysis

Ligands	Molecular weigt < 500 k da	Number of H-bond acceptors < 10	Number of H-bond donors < 5	Log $P_{o/w}$ (MLOGP) < 5	Violations
Harmine	212.25 g/mol	2	1	1.56	Yes; 0 violation
Vasicinone	202.21 g/mol	12	1	0.78	Yes; 1 violation
Alpha spinasterol	412.69 g/mol	1	1	6.62	Yes; 1 violation

The protein target DNMT1 (DNA (cytosine-5)-methyl transferase 1) (PDB ID: 4WXX) and Harmine (CID: 5280953) were docked through AutoDock Vina software. The result showed 9 poses with different binding affinity, Root mean square deviation Lower Bound (RMSD LB) and Root mean square deviation Upper Bound (RMSD UB) as shown in **Table 6.**

The same molecules were further docked through Biovia Discovery Studio Client 2020 as shown in **Table 7.** Biovia Discovery Studio Client 2020 shows the interaction of the protein molecule with the ligand through Absolute energy, clean energy, Config number, molecule number, relative energy and the pose number. The interaction was further visualized under PyMol as shown in **Figure 5.**

Table 6: AutoDock Vina Results

Mode	Affinity (kcal/mol)	Dist from best mode	
		Rmsd l.B.	Rmsd U.B.
1	-6.5	0	0
2	-6.2	13.44	14.99
3	-6.1	3.106	5.474
4	-6	3.394	4.654
5	-6	1.921	2.984
6	-5.8	3.663	6.117
7	-5.7	2.156	2.891
8	-5.6	28.033	29.589
9	-5.6	2.764	5.935

Table 7: Biovia Discovery Studio Client 2020 Results

Harmine	Absolute energy	Clean energy	Conf Number	Mol Number	Relative energy	Pose Number
1.	-5.7	-6.0	72	1	1.62066	1
2.	-4.8	-5.3	81	1	2.33201	2
3.	-4.5	-4.3	54	1	2.45901	3

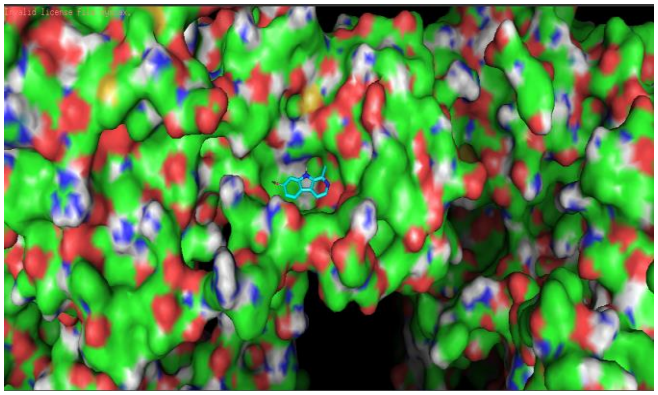


Figure 5: Interaction of DNMT1 (PDB ID: 4WXX) with Harmine (CID: 5280953)

CONCLUSION:

In the *in silico* study, the molecular docking technique was used to investigate the potential of natural compound (ligand) against the selected protein (receptor/target). According to this docking, *in silico* study predicted the best interaction of the ligand (Harmine, Alpha spinasterol, Myrcene, Alloxan, Vasicinone) with the target protein DNMT1 (PDB ID: 4WXX). The analysis of the best-docked ligands permitted us to know the binding mode of compounds involved in this study and confirm the role as an anticancer agent. Harmine was found to be the only compound with best binding energy and it also qualifies the Lipinski's rule of five with zero violations. Harmine may act as a drug for the treatment of cervical cancer by inhibiting DNMT1 protein. The obtained results are useful to understand the structural features required to enhance the inhibitory activities. In future studies, Harmine obtained from natural sources can be a promising drug for the treatment of cervical cancer.

CONFLICT OF INTEREST STATEMENT:

The authors declare that there is no conflict of interest.

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